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Investigating the potential of Hibiscus seed species as alternative water treatment material to the traditional chemicals

ALFRED NDAHI JONES

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Abstract

Developing countries pay a high price for water treatment due to importation of water treatment chemicals. Today, more than 663 million people lack access to a clean water supply which results in many deaths. Hibiscus plant seeds, namely Okra, Sabdariffa and Kenaf were investigated to identify their suitability as alternative water treatment materials to provide clean water supply to people in developing countries. Coagulation and disinfection ability of the extracts were assessed using a jar tester and Collilert-18 Quanti-Tray methods whereas dissolved organic carbon (DOC) test was performed using Shimadzu TOC analyser. The results of this work revealed that all the seed samples possess an anionic coagulant protein with a low molecular weight of 39 kDa. The potential of the seeds in crude form was clearly demonstrated, albeit with some issues regarding organic nutrient addition to the clarified water. However, this challenge was overcome by purifying the seed proteins in an ion exchange column where the impact of DOC addition was significantly reduced in the treated water, as demonstrated via fluorescence excitation-emission matrices. Additionally, the coagulant proteins identified in the region of tryptophan-like fluorescence were found to be stable after heat treatment. Furthermore, sludge production using seed extracts was found to be 5 times lower than that of aluminium sulphate (AS) and the pH of the treated water remained largely unaffected after treatment. Floc strength tests, undertaken using a laser diffraction instrument Mastersizer 2000, showed that the use of seeds as coagulant aids in combination with AS improved floc properties, leading to faster floc growth and shorter coagulation time. Bacterial inactivation was also notable, achieving more than 2-log removal in E-coli and faecal coliform using the purified protein with a zero record of bacterial regrowth after lengthy storage.

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Dedication

This thesis is dedicated in memory of my late mother, Mrs Fatima J. P. Ndahi.

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Abbreviations

ANOVA	Analysis of variance
APS	Ammonium persulphate
AS	Aluminium sulphate
BC	Buffer capacity
BOD	Biochemical oxygen demand
Cd	Cadmium
COD	Chemical oxygen demand
COOH	Carboxylic acid
Cr	Chromium
CSE	Crude salt extract
Cu	Copper
CV	Column volume
DBP	Disinfection by-product
DI	De-ionised
DOC	Dissolved organic carbon
E-coli	Escherichia-coli
EEM	Excitation-emission matrix
Fc	Faecal coliform
GDP	Gross domestic product
G.R	Growth rate
H	Hydrogen
HAA	Haloacetic acid

HCL	Hydrochloric acid
FITR	Fourier transform infrared
IEC	Ion exchange column
IEP	Isoelectric point
IEX	Ion exchange
IWA	International water association
JMP	Joint monitoring program
kDa	Kilo dalton
KCE	Kenaf crude extract
KE	Kenaf
KFAC	Kenaf fibre activated carbon
L	Litre
L	Linn
LRV	Log removal value
M	Molar
MDG	Millennium development goal
MIC	Minimum inhibitory concentration
Ml	Mill
MO	Moringa oleifera
MPN	Most probable number
MW	Molecular weight
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NH_2	Amine
NOM	Natural organic matter

NPOC	Non purgeable organic carbon
NTU	Nephelometric Turbidity Units
OCE	Okra crude extract
OH	Hydroxyl
OK	Okra
OM	Organic matter
Pb	Lead
PKP	Purified kenaf protein
POP	Purified okra protein
POU	Point-of-use
PSP	Purified sabdariffa protein
PVC	Polyvinyl chloride
RIP	Ribosome-inactivation protein
RPM	Revolution per minute
SB	Sabdariffa
SCE	Sabdariffa crude extract
SDG	Sustainable Development Goal
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SSA	Sub-Saharan Africa
TDS	Total dissolved solid
TETfund	Tertiary education fund
THM	Trihalomethane
TOC	Total organic carbon
UNICEF	United Nations International Children's Emergency Fund
UV	Ultraviolet

WHO	World health organisation
WSP	Water soluble protein
W/V	Weight volume ratio

Notations

d_{50}	Median diameter flocs
R_f	Floc recovery factor
S_f	Floc strength factor
K_{zp}	Zeta potential of kenaf
O_{zp}	Zeta potential of okra
S_{zp}	Zeta potential of sabdariffa

CHAPTER 1 - INTRODUCTION

1.1 Background

The growing concern about clean water supply in the least developed countries has triggered innovative research to find potential alternatives to chemicals used in traditional water treatment. Water treatment in any part of the world seeks to provide the population with clean and safe drinking water, devoid of pathogens. Access to safe drinking water improves productivity, health and wealth. However, this life sustaining commodity is inaccessible in many communities across the globe. To tackle this challenge, the World Health Organisation (WHO) formulated a programme to alleviate drinking water supply problems worldwide through the millennium development goals (MDGs), which stipulated the halving of the population without access to clean drinking water by the year 2015 (WHO, 2000). To date, over 663 million inhabitants in third world countries, most of whom live in rural areas still lack access to safe and improved drinking water (JMP, 2013, WHO, 2015). Furthermore, the major problems associated with poor access to safe water are exacerbated by economic deprivation and social incapacitation of many of the affected countries, rendering them unable to afford conventional methods of water treatment. Figure 1.1 shows the proportion of the population in developing countries using improved clean drinking water in 2015 (WHO, 2015).

While substantial progress has been achieved regarding MDGs in water and sanitations, achievements were uneven across countries. In 2015, 91% of the world population, approximately 6.6 billion used improved drinking water compared with 82% in 2000

(UN, 2016). However, despite the progress made in water in all region of the world, coverage was low in SSA and Oceania (UN, 2016). Moreover, not all improved drinking water were properly managed in this regard because at least 1.8 billion people were exposed to drinking water contaminated with faecal bacteria (UN, 2016). However, the recent SDG report by the UN (2016) shows that, only 56% and 68% of Oceanian and SSA population respectively used improved drinking water by 2015.

To address this challenge, the united Nation came up with 17 goals, tagged Sustainable Development Goals (SDG) in order to improve upon and sustain the progress made by the MDGs by 2030. One of the key aspects of the SDG is the implementation of the Integrated Water Resource Management (IWRM) following the MDG goal 6 to half the population without clean drinking water by 2015. The 2030 SDG agenda (for goal 6) recognizes the importance of clean drinking water in bringing development in other areas such as health, education and poverty reduction (UN, 2016). Additionally, the SDG is to address holistically the water management cycle with more countries facing the challenge of being below the 25% threshold, which is the first stages of water stress (UN, 2016).

1.2 Water supply situation in developing countries

Developing countries are the ones worst hit by the menace of water scarcity and lack of access to clean drinking water. In many developing countries, households have access to less than 20 litres per capita per day (l/c/d) (WHO, 2012, Chenoweth, 2008). Women and children bear the burden as they spend most of their time searching for water. Children are kept out of school to fetch water far away from homes at the expense of their education. Moreover, engaging children in the water collection process often

render the water vulnerable to contamination before it gets to the point-of-use (POU) at home. Consequently, local people ignorantly drink contaminated water resulting in outbreaks of disease, leading to many deaths. Water-related disease claims over 1.8 million lives annually, with most of those affected being children below five years old (Haller et al., 2007). The absence of conventional sanitation facilities, causing people to defecate in the open, and poor hygiene practices exacerbate the issue further.

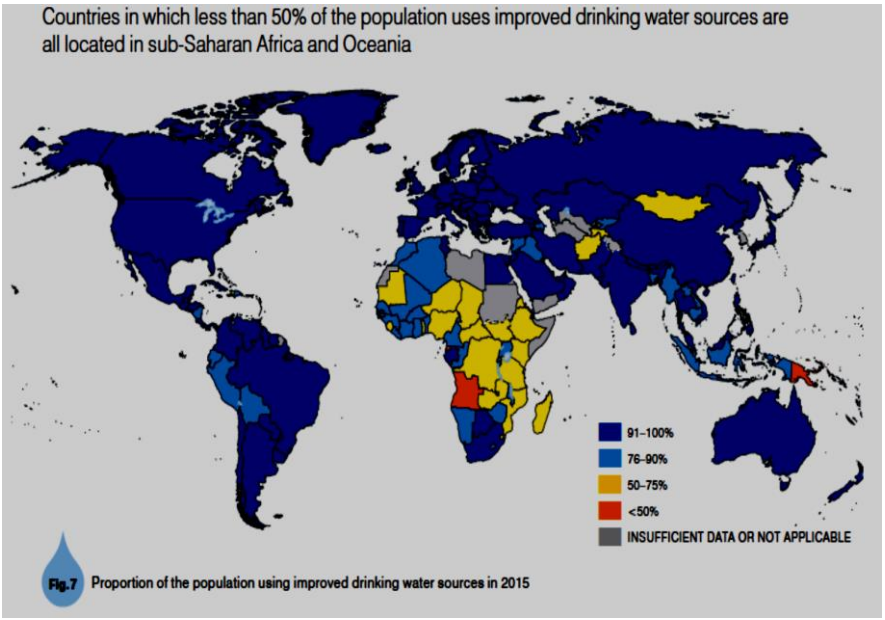

Figure 1.1 Global population using improved drinking water (WHO, 2015).

In areas with poor sanitation practices, surface water runoff could easily be polluted. However, one of the biggest challenges with surface water treatment in the sub-tropical regions of the world is the huge seasonal variation in turbidity (McConnachie et al., 1999) caused by heavy rain fall events. Lack of proper drainage system exacerbates the issue of turbidity further. Turbidity can harbour or carry pathogens which, if not treated, can result in several, severe waterborne diseases (Mac Kenzie et al., 1994). Diarrhoeal disease, being the worst child killer disease known to man, is often waterborne and has an effect greater than the impact of acquired immune deficiency syndrome (AIDS),

tuberculosis and malaria combined (Bartram and Cairncross, 2010). However, it has been reported that diarrhoea outbreaks may be reduced significantly with a focused intervention targeting improved water quality (Fewtrell et al., 2005). Separate studies by Chidavaenzi et al. (1998) and Mazengia et al. (2002) showed 50% improvement in bacterial quality of water which had been stored at home for 24 hours. Conversely, Lindskog and Lindskog (1988) showed that the microbial quality of water deteriorated at home than at the source. The later was supported by Wright et al. (2004) and Mellor et al. (2013) who revealed that water becomes contaminated during the process of collection, transportation, storage, and drawing. Thus, in developing countries, the POU water treatment option could be the best choice to reduce diarrhoea infection (Fewtrell et al., 2005, Clasen et al., 2006, Sobsey et al., 2008). Hence there is a need for innovative POU water treatment materials in developing countries.

1.3 Problems of water supply in Sub-Saharan Africa

Clean water and good sanitation practice are two key indicators that could bring about positive change in human development. After the global consensus to half the number of people without access to safe drinking water by 2015(WHO, 2000), no meaningful achievement was recorded in the early implimentation period in many Sub-Saharan countries and Asia. A snapshot of previous progress in the continent indicated that water supply coverage improved by only 6% between 1990 and 2010, and the estimated population without access to water increased between 1990 and 2010, from 279 to 344 million (WHO, 2012, Abia et al., 2015). People in this region suffer markedly from water-borne infection due to lack of clean water supply and improper hygiene services. In 2013, in Africa alone, more than 325 million of the 748 million inhabitants did not have access to clean drinking water (WHO, 2013).

Three years before the MDG target date, in Sierra Leone, only one in ten of the poorest in rural areas of the country has clean drinking water (WHO, 2012). Similarly, only 12 percent of the rural Kenyan population have access to clean and safe drinking water (Were et al., 2008). Additionally, in Somalia, only 29 percent of the population have access to an improved drinking water source (Wax, 2006). Persistent drought cycles since the early years of this decade have led to communal conflicts over potable drinking water, including violence and killing in some cases (Wax, 2006). However, the recent SDG report by the UN (2016) shows that, only 68% of SSA population used improved drinking water by 2015 while 56% in Oceania have access to good drinking water.

The cost of providing clean water to meet the MDG was prohibitive from the onset. Spending on the water sector together is one-quarter of what is required. However, a significant proportion of the finances available to the industry is currently being wasted due to inefficiencies, estimated at $2.7 billion (Foster and Briceño-Garmendia, 2010). Operation and maintenance inefficiency of water utilities cost the region $900 million a year and impede service expansion (Foster and Briceño-Garmendia, 2010). Eliminating this gross inefficiency would still leave an overall financial gap for the water sector at 1.2 percent GDP (Foster and Briceño-Garmendia, 2010). In rural areas, another major challenge is related to the high breakdown rate from poor maintenance practices. Overall, this has undermined the sustainability of the available water infrastructure. The 2015 target and its daunting challenges highlight the importance of understanding the performance of the water sector in the region. Its achievement and shortcomings and the factors most critical to expanding the coverage are crucial to the entire region.

Another challenge is the increase in population. It is expected that the population of Africa will double by the year 2050 to 2 billion inhabitants (Bongaarts, 2009), at a rate of 2.5 percent annually. Universal water coverage is not keeping pace with the population growth, especially in a country like Libya (Abughlelesha and Lateh, 2013), thus, reducing the per capita available for the inhabitants. Several countries in the region are already water stressed. An increase in population requires more food, which requires land and water (Pech and Sunada, 2008). The negative difference between water supply and demand in some countries creates a severe water deficit (Abughlelesha and Lateh, 2013), so crippling the entire struggle for sustainable drinking water for both domestic and industrial application.

To address the current challenge, people in some communities have used natural plant extracts to clarify their drinking water supply for several decades (Jahn and Dirar, 1979, Marobhe et al., 2007b). While rural people have relied for so long on locally available natural materials in water treatment, they originally lacked knowledge of the principles behind the application due to poor methodologies; but to some extent, the process achieved a substantial water treatment standard. Currently, several natural materials such as *Moringa oleifer* (MO) Jahn and Dirar (1979), Hibiscus *esculentus* (Okra) mucilage Al—Samawi and Shokralla (1996), Cactus (*Cactus latifaria*) Diaz et al. (1999), Common bean (*Phaseolus vulgaris*) Kukić et al. (2011), Mustard (*Brassica juncea L*. or *Czern*) seeds Bodlund et al. (2014) have passed through experimental investigation stages, and the outcome of such studies have so far provided encouraging results for people in developing countries (da Conceicao et al., 2015, Rezende et al., 2016). The WHO has recently approved moringa oleifera (MO) as a water treatment material for people in low-income countries. However, despite the encouraging results

obtained from recent works, the mechanism of action of these extracts is still not well understood by researchers worldwide. While many believe the coagulant compounds is a protein or polysaccharide (Gassenschmidt et al., 1995, Ndabigengesere and Narasiah, 1998a), Okuda et al. (2001b) argued it to be a polyelectrolyte. Nevertheless, many more potential natural plant and animal products such as Hibiscus seeds, animal bones and tissues are yet to be evaluated for use in drinking water treatment as coagulants or as disinfectants. The work reported in this thesis extends the global knowledge on low cost effective water treatment.

1.4 Hibiscus plant seed yield and its prospect in water treatment

The use of natural extract in water treatment can only be appreciated if the mass of seed production by the plant can support large-scale water treatment. In order to understand the sustainability of using OK, SB or KE seeds as a cheaper alternative in water treatment, the study investigated the seed yield potential of each tree/plant and calculated the volume of water production per plant as a coagulant.

- An average OK-tree yield potential:

An average OK pod contains 113 (8 g) seeds, and the average yield/plant is in a range of 55 pods which produce an equivalent of 440 g. The results show that 2 g of OK seed can treat up to 20 litres while one OK pod can treat an average of 80 litres. In addition, the gross OK plant seed yield could treat as large as 4400 litres or an equivalent of 4.4 m^3 of water.

- An average SB-tree yield potential:

An average SB capsule contains 26 (0.9 g) seeds, and under normal planting condition, each plant yields an average of 130 capsules which produce an average of 117 g.

However, every 2 g of SB seed can treat 16 litres of water whereas each plant is capable of treating 936 litres or an equivalent of 0.936 m^3 of water.

- An average KE plant yield potential:

When KE plant was investigated, the results show that each KE capsule contains 22 (0.7 g) seeds, and the yield per plant is in the range of 145 capsules. Therefore, one KE plant is capable of producing 101.5 g of seeds. In this case, 2 g of KE seed could treat 12.5 litres of water while each plant is capable of producing seeds that could treat up to 634.375 litres or 0.634 m^3 of water.

Furthermore, the benefits analysis of using the extracts for people in developing countries is enormous where access to water is as low as 20 L/c/d (Chenoweth, 2008). Hence, the application of Hibiscus seeds can sufficiently increase access to potable water supply.

1.5 Cost analysis of importing water treatment chemical verses Hibiscus seeds

It is important in this work to compare the cost of using each of the three Hibiscus seed species with one of the synthetic water treatment sachets to understand the viability of the extracts in water treatment. Oxfam GB Supply Operation is one of the NGOs currently producing sachet water treatment chemical in many developing countries to make water accessible to the people in emergency situations. FCF/3: Sachet water treatment – Flocculent and Disinfectant – 6000 pce which contains aluminium sulphate and chlorine is currently being used in water treatment.

1.5.1 Description of cost of using synthetic water treatment sachet

Each pack contains 6000 sachets of powder (5g) for household water treatment where one sachet can treat 20 litres of water. The cost of one pack is £312.00, not including

shipping/transport, distribution, education and community motivation. The United Nations standard products and service code for this product is 47.10.16.05.

- 6000 sachets of powder (5g), gross weight is 42 kg and cost £312.00
- 1 sachet cost (31200/6000) = 5.2 pence.
- 1 sachet can treat 15 -20 litres of water.

Therefore, the cost of treating approximately 20 litres of water is 5.2p, equivalent to the minimum per capita of 20 L/c/d in developing countries to meet domestic needs.

1.5.2 Description of cost of using Hibiscus seeds in water treatment

Hibiscus seeds used in the study were purchased in a 2.5 kg (2500g) measured container.

For example, 2500g of Okra cost 60 pence and from the above calculation:

- 2g of Okra seed can treat 20 litres of water.
- The cost of 2g is 0.05p; therefore, 0.05p is required to treat 20 L of water.

The other sample is Russel (*Hibiscus sabdariffa)*, 2500g of the seed cost 60 pence and from the above calculation:

- 2g of *Hibiscus sabdariffa* can treat 16 litres of water; therefore, 2.5 g can treat 20 litres of water.
- The cost of 2.5g is 0.06p; therefore, 0.06p is required to treat 20 L of water.

The third seed sample is Kenaf (*Hibiscus cannabinus*), 2500g of the seed cost 40 pence and from the above calculation:

- 2g of Kenaf seeds can treat 12.5 litres of water, thus 3.2g of the seed is required to treat 20 litres of water.
- The cost of 3.2g is 0.05p; therefore, 0.05p is required to treat 20 L of water with Kenaf seeds.

Overall, the cost of treating water using any of the Hibiscus specie seed is 99% cheaper than the use of synthetic water treatment sachet used in many developing countries by Oxfam.

1.6 Thesis outline

All chapters in this thesis are presented in the following format:

Chapter 2 – Literature review: This chapter provides information about the current knowledge on the subject of water supply and its associated challenges especially for people in developing countries. It presents a review and highlights progress made in improving access to water supply by local communities through innovative research across the globe on the use of natural materials as water treatment coagulants.

Chapter 3 – Knowledge gap: This chapter presents information about the existing knowledge gap that is yet to be filled regarding knowledge on the application of natural extracts in drinking water treatment. The chapter explores areas that need to be studied to close these gaps.

Chapter 4 – Materials and methods: In this chapter, a clear step-by-step procedure adopted in conducting all the experimental runs is presented. The chapter states clearly how the work of this research was undertaken.

Chapter 5 – Coagulation potential of Hibiscus plant extracts results: This chapter presents the results obtained from all the laboratory works which cover the first objective of the study. It provides information on the potential of using Hibiscus plant extracts as a coagulant in home water treatment.

Chapter 6 – Characterisation of coagulated flocs using Hibiscus seed extracts results: This chapter presents the results of the properties of the coagulated flocs regarding floc size, strength, growth rate, and regrowth ability.

Chapter 7 – Effect of natural organic matter (NOM) in water coagulated with natural extracts results: In this chapter, the impacts of organic compounds in water coagulated using Hibiscus seed extracts and purified protein in terms of DOC are presented. The chapter also presents results of the characterisation of NOM in water, before and after treatment using fluorescence spectroscopy to obtain the fluorescence excitation emission matrices (EEMs) peaks.

Chapter 8 – Antimicrobial potential of Hibiscus seed extracts in water purification: This chapter presents the antimicrobial potential of the crude and purified proteins in inactivating bacteria in the water. Specifically, performance evaluation was conducted against total coliform, E-coli, and faecal coliforms.

Chapter 9 – Discussion: This chapter presents a discussion of all the results in Chapters, 5, 6, 7 and 8 together and compares its relevance to the existing knowledge of the subject undertaken by researchers across the globe.

Chapter 10 – Conclusions and recommendations for further studies: This chapter summes up all the findings of this work in connection to the aim of the research and make positive recommendations for future investigations.

1.7 Published papers arising from this research

The novelty of this research study led to the following publications in peer-reviewed journals and international conferences.

Journal papers

1. **Jones, A. N. and Bridgeman**, **J.,** 2016. Investigating the characteristic strength of flocs formed from crude and purified Hibiscus extracts in water treatment. Water Research. 103, 21-29. DOI:**10.1016/j.watres.2016.07.019**.
2. **Jones, A. N. and Bridgeman**, **J.,** 2016. An assessment of the use of native and denatured forms of okra seed proteins as coagulants in drinking water treatment. Journal of Water and Health. 14 (5), 768-779. DOI: **10.2166/wh.2016.015.**
3. **Jones, A. N. and Bridgeman, J.,** Evaluating the disinfection potential of crude and purified Kenaf and Sabdariffa seed proteins in water treatment. (Submitted to ICE - Water Management Journal).
4. **Jones, A. N. and Bridgeman, J.,** A fluorescence-based assessment of the fate of organic matter in water treated using crude and purified seed proteins in drinking water treatment. (To be re-submitted to Water Research).
5. **Jones, A. N. and Bridgeman, J.,** Addressing drinking water problems in developing countries using natural plant-based material: A review (Manuscript to be submitted to Environmental Technology).

Conferences

1. Natural organic matter (NOM) in drinking water, 6th IWA Specialist Conference. Malmo, Sweden 7–10 September, 2015. Platform presentation. **Jones, A. N. and Bridgeman**, **J.,** 2015. Characterisation of natural organic matter (NOM) in water treatment using seed extracts. VATTEN 71, 239-245.
2. Particle Separation: Advances in particle science and separation: meeting tomorrow's challenges. 22 – 24 June 2016, Oslo/Norway. Platform and poster presentations.

CHAPTER 2 – LITERATURE REVIEW

2.1 Background

This chapter presents an in-depth overview of current knowledge on the research subject. It highlights some of the challenge confronting many parts of the world in terms of access to clean water supply. Emphasis is placed on progress being made in tackling this issue through the use of natural materials in drinking water purification. The chapter also presents some potential natural extracts that have undergone a series of investigation to identify their suitability for drinking water treatment.

2.2 Natural materials of plant-based origin in folk medicine

It is important to appreciate the fact that even in ancient civilization, people could relate the link between drug production and disease because they were relying on nature to maintain healthy living and treatment of ailments (Kim, 2005). Additionally, in those days, people used natural animal and plant products strictly based on taste to treat certain illness, while others employ these products in the purification of muddy waters to control the spread of water borne diseases (Kim, 2005, Kansal and Kumari, 2014).

Natural plant-based materials have been used for decades in the treatment of numerous human ailments across the globe where modern primary medical healthcare is lacking (Chitme et al., 2004, Palombo, 2006). Traditional or folk medicine is a well-established medical practice which comprised of knowledge acquired over generations within ancient societies before the advent of modern medicine (Palombo, 2006). Some modern medicines derived their primary compounds from natural plants. Solecki (1975) reported that six of the Achillea genus used more than 60,000 years ago by the

Neanderthals is still useful as herbal plants across the world. In addition, Yongabi (2010) had shown that there are more than 300,000 medicinal plants in Sub-Saharan region alone. In assessing the use of traditional medicine base on WHO guideline, Kim (2005) developed the rational use of natural materials in primary health care. The study observed that over 80% of people in developing countries still employ traditional medicines in health care services due to lack of modern healthcare facilities.

To explore other potentials in natural extracts as far back in the 70s, research showed that crude extract from Nirmali (*Strychnos potatorum)* seed achieved a reduction of between 50 and 52 percent of bacterial colonies in water after 30 min contact time (Tripathi et al., 1976). In addition, Madsen et al. (1987), Olsen (1987) and Ndabigengesere and Narasiah (1998b) investigated the antimicrobial ability of *Moringa oleifera* (MO) in water and wastewater treatment where an average of between 90 and 99% bacterial colony removal was observed using MO crude water extract. Similarly, is has been acknowledged in a study by Shaheed et al. (2009) and Matthews et al. (2009) that the application of crude extracts of Sponge gourd (*Luffa cylindrical)* and Neem oil could achieve significant inactivation of total and *faecal coliform,* achieving a maximum of 86% inactivation of bacteria and viruses to reduce the spread of infectious disease in drinking water. Also, Nwaiwu et al. (2012) shows excellent bacterial inhibition in *Hibiscus sabdariffa* (SB) and Okra (OK) (*Hibiscus esculentus*) distilled water extracts in treating pond water with numerous bacterial colonies such as *shigella* and *salmonella.*

Beside this, several natural extracts such as MO, Cactus (*opuntia* and *latifera* species), and Mustard seeds have been studied in literature as coagulants in water treatment (Jahn

and Dirar, 1979, Diaz et al., 1999, Bodlund et al., 2014). However, despite the recent advances in the use of natural occurring materials in water treatment as coagulants, comparatively the application of these natural extracts has not received the needed attention as disinfectants even though many have long been used in traditional or folk medicine to treat numerous diseases and as a pesticide in developing countries (Shaheed et al., 2009, Dubreuil, 2013), these natural products could as well be tested as a potential disinfectants.

In conventional water treatment, however, the most widely used disinfectant chemical is chlorine (Koivunen and Heinonen-Tanski, 2005). Water is chlorinated to kill and eliminate bacteria and viruses present in the source and to maintain an acceptable level of chlorine residual in the final water to protect consumers against the effect of bacterial ingress and growth in pipes. Furthermore, disinfection is the most critical step employed in conventional water treatment to make water safe to consumers. However, because of cost associated with this chemical, developing countries would benefit more if alternative disinfectant materials are found locally.

2.3 Application of natural extracts in water treatment

Due to lack of access to potable water supply in developing countries, the most cost-effective and straightforward point-of-use (POU) water treatment technology adopted in ancient civilisation was to use natural extracts. The use of natural extracts in water treatment dates back over 4000 years (Kansal and Kumari, 2014) and as far back to Sanskrit writings where certain natural derivatives were used in water treatment. It is also on record in the Old Testament and Roman archives that the use of natural extracts dates back to 77 AD (Dorea, 2006). Naturally-occurring materials consist of both

animal and plant-based resources. For instance roasted seed powders of maize (*Zee-mays)* and Nirmali were used to treat water by Peruvian soldiers as well as in some Indian communities (Kansal and Kumari, 2014). In Sudan, rural women used MO seed extract to treat their drinking water supply (Jahn and Dirar, 1979, Jahn, 1986) while in Tanzania, Marobhe et al. (2007b) carried out a survey on traditional water purification practice by local women. The study observed that majority of people in rural Tanzania use leguminous plants such as Jerusalem thorn (*Parkinsonia aculeate),* cowpea *(Vigna unguiculata)* and *Voandzeia subterranean* for their water treatment (Marobhe et al., 2007b).

Furthermore, several animal products have been widely used in water purification. Kawamura (1991) investigated the application of chitosan, a cationic compound, and sodium alginate, a natural polysaccharide (anionic compound) of animal origin, as coagulants in water treatment which resulted in improved water quality comparable to that obtained with AS at a similar dose. Rural people have used animal-based products such as isinglass and chitosan obtained from shredded sturgeon fish and crustaceans respectively to treat contaminated water (Bratby, 2006, Choy et al., 2015). Hu et al. (2013) also used low doses of chitosan in combination with AS to produce successful turbidity removal with low sludge volume compared to sludge generated by AS as a primary coagulant. However, animal-based materials are limited compared with natural plant-based resources. Hence, the latter is the most widely studied because of its availability and ease of obtaining the products.

However, in assessing their potential, difficulty arises due to lack of information regarding the chemical composition of the tissue responsible for the coagulation activity

which makes them less attractive as water treatment materials. These setbacks have rendered such materials ineffective to compete favourably with traditional chemicals in water treatment (Ndabigengesere and Narasiah, 1998a). Most notably, some natural extracts such as MO and Common Beans (*Phaseolus vulgaris*) contains cationic compounds according to Ndabigengesere et al. (1995) and Sciban et al. (2006) whereas in others, the coagulation compounds are anionic as in Cactus and OK mucilage (Zhang et al., 2006, Miller et al., 2008, Patale et al., 2012). Similarly, other products have been reported to be non-anionic polymers and are also available (Bolto and Gregory, 2007). This wide variation in composition requires critical investigations regarding their suitability. Thus, with the current understanding, a lot more information is needed to make such materials viable in drinking water treatment. Additionally, it is noteworthy that the application of natural extracts in drinking water treatment has no reported health hazard associated with the consumption of the treated water because many of the materials used are primary sources of proteins.

Below are some of the natural extracts that have passed through some investigation stages. Notable among them are; MO seed extract (Jahn, 1986, Ndabigengesere and Narasiah, 1998a, Sanchez-Martin et al., 2012, Petersen et al., 2016), Cactus *latifaria spp* (Diaz et al., 1999), Cactus *Opuntia spp* (Zhang et al., 2006, Miller et al., 2008). Also, the coagulation potential of Chestnut and acorn (*Fagaceae*) has been reported (ciban et al., 2009). Common bean (*Phaseolus vulgaris*) seed, a primary source of proteins in Tanzania and many other countries, have also been evaluated as a potential coagulant (Antov et al., 2010, Marobhe et al., 2007b). Others which have undergone similar studies include Nirmali seeds (*Strychnos potatorum*) (Babu and Chaudhuri, 2005), and different Mustard seeds species (Bodlund et al., 2014). Some researchers also

investigated the efficacy of *Enteromorpha* extracts (Zhao et al., 2015b), fava beans (*Vicia faba* L.) (Kuki et al., 2015) and Tannins from *Turkish Acorns* (Özacar and Şengil, 2002, Özacar and Şengil, 2003). Similarly, Al—Samawi and Shokralla (1996) investigated mucilage obtained from powdered OK seed pod as a coagulant material together with AS. Investigations also revealed that other natural materials such as Kenaf (KE) (*Hibiscus cannabinus*) plant fibre could be use in the same way as synthetic activated carbon in water treatment Chowdhury et al. (2012) for the removal of heavy metals in water.

Therefore, this review is based on the application of natural plant-based materials in water treatment. The main reason for excluding animal-based materials is, however, based on their applicability, since they are unlikely to be used for mass production due to limited availability. However, it is noteworthy that apart from MO, most of those plants presented have limited information but have been tested in water treatment in one form or the other. Some of the few natural plant-based extracts that have been used in water treatment are at this moment presented:

2.3.1 *Moringa oleifera* seeds

MO is a tropical plant species which belongs to the family known as *Moringaceae*. MO is referred to as a multipurpose or multifunctional plant because most of its parts were found to be very useful, for instance, it is used in folk medicine and as a primary source of food (Fuglie, 2001). MO leaves, stem, roots, shell, seed oil and protein are used in a variety of applications such as a vegetable in soup, for cure of diabetes and lowering of blood pressure in patients, cooking oil and in the preparation of morning coffee. As such, it is often referred to as being a 'miracle tree' (Fuglie, 2001). MO contains high

mineral and protein content and is recommended to be used to fight malnutrition in developing countries (Ghebremichael, 2004). It is resistant to drought and is found in abundance in most regions of Africa and Asia. It is thought of being native to Asian countries and Africa and grows within one year of planting. Many people have used MO leaves as a source of vegetable in Asia and Africa because of its nutritional values (Morton, 1991). MO has been used as machine lubricant and in the cosmetic industry (Ghebremichael, 2004).

The MO plant is the most studied natural material in water treatment to date. Jahn and Dirar (1979) conducted the first study using MO water extract in Sudan as was being prepared by the people for the removal of turbidity from the Nile River. Significant turbidity removal was reported compared to that obtain with ash which was also used in many villages in Sudan. In addition, having seen the potential of MO as a coagulant in water treatment, Jahn (1986) further studied the quality of water produced using MO water extract. The study observed a change in taste and colour of the water treated using MO extract and also noted an odour is emanating from the water after 48 hour post treatment. To address the issue of deterioration in water quality, Jahn (1986) recommended that water treated using MO extract to be consumed within 24 hour treatment. Following these research studies, Ndabigengesere et al. (1995) characterized the active coagulant compound in MO seed using different techniques such as lyophilisation, dialysis, ultrafiltration, chemical precipitation and electrophoresis. Interestingly, it was observed that the active agents in MO extract are proteins with a molecular weight (MW) of 13kDa and isoelectric point (IEP) of 10 and 11.

Furthermore, the result of zeta potential measurement of MO extract suspension shows that it is a cationic polyelectrolyte with surface charge of (+6 mV) and its coagulation

mechanism was reported to be by adsorption and charge neutralization. In contrast, Okuda et al. (1999), Okuda et al. (2001a) and Okuda et al. (2001b) improved the extraction process using NaCl concentration and purified the seeds by dialysis, de-lipidation and ion exchange. Overall, the studies show improved coagulation performance of MO salt extract than in water extract, and the coagulation mechanism was said to be neither by double layer compression, inter-particle bridging no by charge neutralization but by enmeshment of particles. Okuda et al. (2001b) also argued that protein analysis by Lowry and Bio-Rad methods after dialysis showed negative results and the polysaccharide concentration analysis also showed zero concentration. Similarly, it was clear that there was no more lipid after the extraction step, thus it is arguable that MO salt extract is neither a protein, polysaccharide nor lipid, but a polyelectrolyte having single MW band as small as 3 kDa. Conversely, Gassenschmidt et al. (1995) identified the MW of the purified MO protein in IEC as also consisting of dimeric MW bands of 6.5 and 14 kDa with IEP of 11 whereas Ghebremichael et al. (2005) found the MW of the purified MO protein on a cationic exchanger to be less than 6.5 kDa with IEP greater than 9.6. The slight variation in the protein band with MW around 6.5 kDa and IEP of (10 and 11, and >9.6) may be due to the different processing methods adopted to obtain MO protein. Interestingly, Ali et al. (2010) used a high performance liquid chromatography method to identify the bioactive MW of the coagulant protein in MO seed. The result shows that MO protein has MW between 1 and 6.5kDa. Although many studies have accepted that the active agent in MO is a natural cationic protein showing charge neutralization as the main coagulation mechanism, except that of Okuda et al. (2001b). Thus, the investigation results reported by Okuda et al. (2001b) should be given fair consideration and not be disregarded

because there might be a coagulation agent in MO with such property. Such difference in the analysis portrays the difficulty in natural understanding extract or polymer studies which requires complete and thorough investigations.

After assessing the quality of water treated using MO extract by Ndabigengesere and Narasiah (1998a) using both dry shelled and non-shelled MO in a jar test experiment. The study shows that the concentration of organic load in treated water increased significantly at higher dosage with no change in water pH, alkalinity and conductivity in treated water with small sludge volume production. These results were considered to be an advantage compared with the application of AS in water treatment. It is noteworthy that all the purified protein reported show high coagulation performance in a jar test experiments compared with the crude samples under different laboratory conditions due to increased adsorption (Okuda et al., 2001b, Ghebremichael et al., 2005, Ghebremichael et al., 2006, Sanchez-Martin et al., 2010, Ali et al., 2010). The studies also indicated that the issue of organic nutrients addition in treated water was eliminated and showing DOC removal in water and the pH remain unaffected. Similarly, both Muyibi et al. (2002) and Pritchard et al. (2009) used MO seed obtain from Nigeria and Malawi in treating turbidity in pond water in Kano-Nigeria and in a shallow well in Malawi which achieved between 92−99% turbidity removals. The findings from these studies show no significance variation in MO extracts performance irrespective of region.

Muyibi and Evison (1995b) and Muyibi and Evison (1995a) optimised physical parameters in kaolin water and also investigated the softening potential of hardness in waters using MO extracted with DI in synthetic water spiked with calcium chloride,

naturally hard surface water and groundwater in Newcastle Upon Tyne. The results observed higher coagulant dose demand in high turbidity water with increased rapid and slow mix velocity gradient and time compared with optimization of low turbidity water. Similarly, in the case of hard water, it was observed that calcium hardness reduced faster and in the two natural water samples to zero with an increase in MO dosage while pH showed no effect on hardness removal. In addition, a reduction of over 27% in alkalinity of the groundwater was observed, thus presenting another advantage in using MO over AS as a coagulant. In synthetic wastewater treatment, Ndabigengesere and Narasiah (1998b) showed excellent removal of lead from contaminated synthetic water using shelled and unshelled MO extracts due to the formation of NOM complexes with lead. The performance of the stored MO extracts and raw seed in low medium and high turbidity water (50, 100-200 and 200-300 NTU) were assessed by Katayon et al. (2004) who stored MO extract suspension at room temperature of 3 and 28 °C for 1, 3, 5 and 7 days and Katayon et al. (2006) who again stored the raw seeds at 3 and 28 °C for between 3 and 5 months respectively. Maximum turbidity removal of 92.3% was observed in 300 NTU compared with between 73-86% in the (50-100 NTU) water using sample which had been stored for one day while maximum performance of 14 and 3% was recorded with crude extract stored for 5 and 7 days respectively. In addition, performance decreased with storage where seeds which had been stored for 1 month outperformed those stored for up to 5 month in high turbidity water. Interestingly, the efficiency of MO seed was found to be independent of temperature condition.

Aside its turbidity removal potential, Madsen et al. (1987) investigated the traditional method of water purification in Sudanese village at 30 °C using MO extract to remove turbidity, *faecal coliform* and other enteric bacteria. A turbidity reduction of between

80-99% was observed with a 1-4 Log removal of bacteria. After 24-hr post treatment, a regrowth of secondary bacteria such as *Salmonella typhimurium*, *Shigella sonnei* and *E-coli were* witnessed whereas *Vibrio cholerae*, *Streptococcus faecalis* and *Clostridium perfringens* remain totally inhibited. Later, Ndabigengesere and Narasiah (1998b) investigated the efficacy of shelled and non-shelled MO seed, and AS in treating two samples each from municipal and industrial wastewater from Magog Canada. Both shelled, non-shelled and AS application resulted in considerable reduction in *total coliform* count from 35000 to 3000 for *total coliform* and 21000 to 1800 for *faecal coliform* per 100 ml. However, after resuspension of the sludge, a regrowth of both *total* and *faecal coliform* was witnessed, suggesting that the microorganisms were not inactivation but lived in the sludge. Hence, the claim that MO extract could inactivate pathogen in water by some authors is not in agreement with this study. Conversely, Ghebremichael et al. (2005) reported an antimicrobial activity of 1.1-4Log removal of *E-coli* (D31 and K12) and B. *thuringiensis* (Bt7, Bt75) and P. *aeruginosa* when purified MO protein was obtain on an IEC. Furthermore, some studies have shown that the coagulation compound in MO extract is a water-soluble protein capable of removing *total coliform* and *E-coli* in natural pond water (Anwar et al., 2007, Pritchard et al., 2010). Recently, da Conceicao et al. (2015) used MO DI extract to obtain a result that was compliant with Brazilian drinking water standards in fluoride-polluted groundwater. The importance of MO was further demonstrated by Petersen et al. (2016) who shows the potential of MO extract to successfully eliminate *Cryptosporidium parvum oocysts* in wastewater intended for irrigation farming.

As reported in the preceding sections, the main setback of using MO extracts is the continuous increase in organic compounds into the treated water (Okuda et al., 2001b,

Ghebremichael et al., 2006, Marobhe et al., 2007a) leading to a change in colour, taste and odour. Following this setback, purification of crude MO seed to address this change yielded positive results where only the coagulant MO protein was obtained (Ghebremichael et al., 2006, Ali et al., 2010, Sanchez-Martin et al., 2010). Interestingly, the two-step purification of MO proteins using 0.3 and 0.6 M NaCl concentrations significantly reduced residual DOC in treated water (Sanchez-Martin et al., 2010). This is advantageous because any issue related to DBP formation would have been taken care of if the final water is recommended to be disinfected using chlorine. In contrast, there are limited studies on the application of MO extract in wastewater treatment and sludge dewatering ability. Assessment of possible DBP formation should be done to understand if MO could release natural organic compounds that could exacerbate this challenge.

2.3.2 Cactus mucilage

Cactus is an indigenous plant grown in many arid and semiarid regions of the world Sáenz et al. (2004) and is a member of *Cactaceae* family with many species in Central America (Shilpaa et al., 2012). Some studies found *Cactus* spp. as constituting a huge threat to agricultural production in many parts of the world (Bright, 1998, Zimmermann et al., 2000). The *Opuntia* species is called prickly pear in the USA and is well-known as a primary source of mucilage substances similar to that of OK (Sáenz et al., 2004, Miller et al., 2008). The mucilage contains compounds such as L-rhamnose, D-galactose, L-arabinose, D-galactose and some other compounds such as galacturonic acid (Sáenz et al., 2004, Yin, 2010). Cactus is also medicinal and has been used in the treatment of several ailments by native herbalist (Yin, 2010).

Because of the presence of water-soluble mucilage in *Cactus latifaria*, Diaz et al. (1999) prepared 5% w/v suspension in water which was initially extracted with methanol to remove the polar substances in the pad. The jar test results conducted on 20−200 NTU synthetic water show that in all the turbid water tested, between 10–20 mg/l dose was the optimum to achieve residual turbidity of less than 10 NTU. Furthermore, Diaz et al. (1999) compared and observed the performance of *Cactus latifaria* in water treatment with that of AS and MO to be similar although the required dose of AS was higher, yet turbidity removal efficiency was the same. In addition, the methanol-treated solid from *Cactus latifaria* was tested for coagulation which did not show any difference with the non-treated solids, thus indicating the use of solvent to remove polar substances could only incur additional cost and offer no advantage in terms of performance. *Cactus latifaria* contain about 11.6% protein (Diaz et al., 1999). Furthermore, Zhang et al. (2006) used water extract of *Cactus Opuntia* species in a jar test experiment to test for turbidity, alkalinity and effect of change in pH of the water on its performance. Turbidity removal was 94% in 200 NTU water using optimum dose of 50 mg/l higher than that reported by (Diaz et al., 1999). However, at optimum pH of 10, Zhang et al. (2006) demonstrated low performance of *Cactus Opuntia* in 10°C than in 35°C water although the difference was not much. The effect of turbidity removal on alkalinity in water treated with *Cactus Opuntia* also show increases in turbidity removal resulted in increased final water alkalinity and Zhang et al. (2006) suggests that the presence of some ions in the sample affected the coagulation process. However, this study also concluded that the performance of *Cactus Opuntia* species to be similar to that of AS and MO extract in water treatment (Zhang et al., 2006) but the impact of ions in the sample need serious evaluation.

Further study was conducted by Miller et al. (2008) using *Cactus Opuntia* species in kaolin suspension at pH of 10. Turbidity removal was reported to be 98% in 162−200 NTU water ranges (Miller et al., 2008). Although *Opuntia* spp. is an anionic compound with IEP of 2, the coagulation mechanism is mainly by adsorption and bridging as observed from the microscopic image of flocs formed and zeta potential measurement (Miller et al., 2008). Furthermore, these studies indicated that the mucilaginous substance obtains from e.g. *Cactus* (*Opuntia* and *latifaria*) is a strong water treatment candidate (Diaz et al., 1999, Miller et al., 2008, Yin, 2010, Zhang et al., 2006). The presence of galacturonic acid and protein in the pad clearly presents its coagulation ability, achieving good turbidity removal in water (Diaz et al., 1999, Miller et al., 2008). In the tropics, Cactus plant could be a useful alternative to the traditional coagulant currently in use. Shilpaa et al. (2012) also evaluated the potential of powder *Opuntia ficus indica* in a jar test experiment using synthetic water. At 20 mg/l, *Cactus Opuntia* mucilage reduces the turbidity from 500 to 1.3 NTU, approximately 99.8% reduction. In wastewater treatment, Nharingo et al. (2015) also found that *Cactus fiscus indica* water extract as a bio-flocculent capable of removing 100 % Pb and 85.74 % of zinc (Zn) in water. In addition, Cactus extract achieved 84.16 % removal of Cd and 93.02 % of copper (Cu) under optimum conditions in Zimbabwean River (Nharingo et al., 2015).

Overall, all the studies indicated the water treatment potential of Cactus plant species at smaller coagulant dosage compared with AS and other natural coagulants. Investigation of its toxic effect has not been conducted as it is not a primary source of food. Thus its application in drinking water needs to be carefully evaluated. Also, there has not been any detailed study regarding the effect of NOM addition and the application of its purified form in water treatment processes, this also needs further evaluation.

2.3.3 Common bean seed

Common bean belongs to the genus and species of (*Phaseolus vulgaris*). It is a good source of protein and carbohydrate and highly consumed in Latin American and African countries (de Almeida Costa et al., 2006). The protein contents in raw and freeze-dried cooked Common bean sample was reported to be 20.9 and 22.1% whereas the carbohydrate component is 54.3 and 59.9% respectively (de Almeida Costa et al., 2006). Similarly, Sciban et al. (2006) had shown that the protein content in Common bean seed is between 20 and 30% whereas Gunaratna et al. (2007) and Marobhe et al. (2007b) identified the protein compounds to be cationic after purification on cationic IEC, which is capable of removing turbidity in water. Common bean extract have low sugar contents, phenolic and phytic acid and the IEP was obtain by measuring the zeta potential at varying pH and the point with zero zeta potential (i.e. pH 3.61) regarded as its IEP (Kukić et al., 2011). An important analysis by SDS-PAGE was conducted by Montoya et al. (2008) on the coagulant protein from Common bean to identify its MW. The MW was then identified as being a dimeric protein with MW of approximately 20 and 50 kDa. In contrast, Morales-de León et al. (2007) observed in a separate SDS-PAGE analysis that the IEP is 4.5 and two MW proteins present in Common bean are 26 and 49 kDa. It is notable, however, that both the IEP and MW of Common bean seed are different from that of MO seed and may likely present some unique activities. However, similar assessment was made on the concentration of protein in Common bean seed by Antov et al. (2010) who observed an equivalent concentration of 0.51 mg/ml in the seed, and this support the fact that Common bean is a popular food amongst the Brazilian population for a long time (de Almeida Costa et al., 2006). The presence of high protein contents in Common bean has resulted in innovative researches

into the coagulation potential of its extract. This is because several studies in the literature have shown that the coagulant compound in natural extracts to be protein (Ndabigengesere et al., 1995, Ghebremichael et al., 2006, Bodlund et al., 2014).

Common been has undergone some investigations into its suitability in water treatment in a study by Sciban et al. (2005) who reported higher performance of the extract at pH above 10 and in low turbidity water of 18 NTU. The findings reported here is not in agreement with previous studies who observed poor performance of natural extracts in low turbidity water ((Ndabigengesere et al., 1995, Katayon et al., 2006) which is a useful observation that gives it a unique advantage over other extracts. However, considering the impact of NOM in treated water, Sciban et al. (2006) and Antov et al. (2010) performed partial purification of the protein using ammonium sulphate, desalting, dialysis and IEX and elution with NaCl. The results show good turbidity removal efficiency of 72.3% in 35 NTU water, 22 times higher than the performance of the crude DI water extract at a dose of 0.73 mg/l. Similarly, the purified sample produced organic matter concentration of 0.35 compared with 5.9 mg of crude extract treated water from 2.3 mg/l. This finding presented similar observation by some authors who used MO extracts (Gassenschmidt et al., 1995, Ghebremichael et al., 2005, Sanchez-Martin et al., 2012) who observed greater adsorption ability and reduced NOM in treated water. In addition, the purification of Common bean did not require lipid extraction because it does not have lipid which is advantageous. The challenge of possibility of floc formation being inhibited through surface coating as indicated with other extracts is also eliminated in Common bean (Ali et al., 2010). Sciban et al. (2006) further observed that, precipitation and desalting by dialysis of the samples eluted with 0.5, 1.0, 1.5 and 2.0 M NaCl solutions presented different coagulation mechanisms.

Such observation suggests that there may be different protein in the various fractions with varying coagulation potential. Identification and characterisation of these proteins would give a clearer understanding of fraction with higher activity. With an average of 50 grams of the extract, 20 L of turbid water was treated, achieving 90% turbidity removal. However, coagulant protein from common bean obtained by ultrafiltration of the crudes extract produced lower turbidity removal of 49% compared with 51% achieved with the crude sample (Antov et al., 2012). In terms of extraction time, Kukić et al. (2011) reported that 10 min is sufficient to extract coagulant protein from Common bean compared with 3hrs using a magnetic stirrer which would save time used in processing the extract.

Beside the coagulation ability of the Common bean species, the antimicrobial compounds in the seed has been reported elsewhere (Amarowicz et al., 2008). Tannin extracted from Common bean was compared with a conventional drug, streptomycin in bacterial inhibition. The minimum inhibitory concentration (MIC) of bacteria with tannin were 125 μg/ml for *Listeria monocytogenes*, *Salmonella Typhimurium* and *Lactobacillus plantarum* while streptomycin achieved only 31.3 and 7.8 μg/ml MIC for *Salmonella Typhimurium and Lactobacillus plantarum* respectively (Amarowicz et al., 2008).

2.3.4 Luffa cylindrica extract

Luffa cylindrica is a member of (*Cucurbitaceouse*) family which is prevalent in tropical countries, such as in many parts of Asia, Africa and the United State (Partap et al., 2012). It is spongy and commonly called sponge gourd, mainly used by local people in washing of a body and cleaning of cooking utensils. Luffa cylindrical has a cellulose

structure which is negatively charged when in contact with water (Shahidi et al., 2015a). The immature fruit is eaten raw as it contains compounds such as lavonoids and RIP used in traditional medicine (Anbukarasi and Kalaiselvam, 2015). Earlier, Bhattarai (1989) has reported that one of the most used natural extract among Nepal population is the seed of luffa cylindrical. In addition, the importance of luffa cylindrical has been reported in a study by Ruiz-Marín et al. (2009) who investigated the removal of nutrients and organic matter in a bioreactor supported with Luffa cylindrical fibre. The results show that Luffa cylindrical improves biofilm development as a support material. It recorded a significant removal of biochemical oxygen demand (BOD) of 92.5% compared with 80% obtained with PVC as a support material (Ruiz-Marín et al., 2009). The study further observed that, in the final effluent the mean ammonia nitrogen concentration was 17 mg/l for Luffa cylindrical and 19 mg/l for PVC support respectively (Ruiz-Marín et al., 2009). Though the study of luffa cylindrical is new, one of the first known optimization study using a jar test apparatus was conducted by Sowmeyan et al. (2011) on different natural extracts including luffa cylindrical and AS as primary coagulants. While several authors followed through aqueous extracting processes by washing using tap water, Sowmeyan et al. (2011) used formaldehyde for the washing of the seeds. The reason for using this chemical instead of water is because it is efficient in the washing and cleaning of dust and debris on a substance. Interestingly, Luffa cylindrical achieved maximum turbidity and hardness removal of approximately 86 and 40% respectively. However, a reaction between the extract and chloride in water causes a reduction of up to 7.6% in chloride concentration at optimum dose of 8g/l of the extract (Sowmeyan et al., 2011). Thus, the application of luffa cylindrica in water treatment may pose some challenges if chlorine is used as

disinfectant because it will consume the available residual chlorine in the treated water. Additionally, as with the other natural coagulants, Luffa cylindrical also achieved 60% removal of total dissolved solid (TDS) in water treatment.

The anionic nature of Luffa cylindrical made it an active extract for the removal of cationic metals in wastewater (Anbukarasi and Kalaiselvam, 2015) in the form of Luffa fibre. Anbukarasi and Kalaiselvam (2015) investigated the water adsorption potential of composite particle fibre and showed a significant performance than the non-treated sample which can be used in damp site. In a more important test in water purification, Shahidi et al. (2015b) performed some batch adsorption tests using Luffa cylindrica fibre as a natural adsorbent for the removal of Cadmium (Cd) (II). The results from the study show high possibility of using Luffa for the removal of Cd (II) in water with adsorption capacity of 6.7 mg/g. However, cadmium removal was observed to be highly dependent on solution pH, dosage and Cd (II) concentration.

Because of the presence of antioxidants such as saponin, RIP, phenolic compounds etc. direct application of Luffa cylindrical extracts obtained against *E-coli* and many other bacteria is reported elsewhere (Oyetayo et al., 2007). Shaheed et al. (2009) evaluate the potential luffa cylindrica fruit and seed between 40 and 333g/l as a disinfectant against *total* and *faecal coliform* in water treatment and contact time of 15-180 min. The results show that the inhibition of the two indicator organisms was variable and dose dependent with a maximum performance of 86% at 180 min. Although the overall results show some disinfection ability in luffa plant, the performance did not achieve the WHO standard for drinking water quality. Similar, the limited results conducted using the

extract as a coagulant is not sufficient to judge its potential in water treatment which requires more studies.

2.3.5 Nirmali extract

The plant Nirmali (*Strychnos potatorum)* Linn, is a plant belonging to the *Loganiaceae* family (Jayaram et al., 2009). Nirmali extract is used extensively in folk medicine in many parts of India and Sri Lanka (Jayaram et al., 2009). Literature has shown that the first water treatment potential of the ground seed powder was investigated by Sen and Bulusu (1962) who attributed the removal of turbidity due to the presence of anionic polyelectrolytes and protein. Similarly, Tripathi et al. (1976) observed that the coagulation ability of Nirmali extract was also due to the presence of carbohydrates and alkaloids having a −COOH− group with free OH^- which improves its performance. Adinolfi et al. (1994) also investigated the combination of polysaccharide and galactan extracts from the ground powder of Nirmali seed to achieve a turbidity removal of 80% in synthetic kaolin water. In addition, Raghuwanshi et al. (2002) conducted some analysis on 20 agro-based coagulants and observed that with 3 mg/l dose of Nirmali as a coagulant aid in combination with AS, the dosage of AS was reduced from 45 mg/l to 25 mg/L, achieving residual turbidity of less than 0.2 NTU. The volume of sludge produced after the experimental runs was noted to be 40% lower compared with the volume of sludge generated by AS as a primary coagulant. Similarly, Sowmeyan et al. (2011) had shown over 80% removal in turbidity in 32 NTU water. Comparison of its performance in high and medium water need to be assess to understand its potential in water treatment further. It is clear from these results that Nirmali seed possess some water treatment potential even though the number of studies conducted so far is limited to turbidity removal and sludge volume production.

Further study was conducted by Jayaram et al. (2009) who evaluated the use of the Nirmali seed powder in heavy metal removal using batch mode adsorption experiments at varying pH and contact time. The study observed the removal of Pb to be highly affected by pH, contact time, biomass dose and metal concentration and its adsorption ability was 16.4 mg/g. The Fourier transform infrared (FTIR) spectroscopy analysis shows the adsorption process of the extract to be due to the presence of many functional groups (Jayaram et al., 2009). More research are required to look at other areas of water treatment especially with regard to NOM addition and the effect of OH groups along galactomannan and that of galactan chains which was said to ultimately lead to abundant adsorption sites for interparticle bridging (Yin, 2010).

2.3.6 Tannin extracts of plants

Tannin is a water-soluble polyphenol compound extracted from bark and wood of several natural plant products Chung et al. (1998) including *Acacia* and *Castanea* trees (Graham et al., 2008, Beltrán-Heredia et al., 2009, Yin, 2010). Tannin is reported as having MW in a range of hundreds to thousands and mainly used in transforming animal hide and skin into leather products and thus primarily utilized in the leather industry (Yin, 2010). However, other natural plants where tannin has been extracted includes; Luffa cylindrical, OK, KE, SB, MO, faba beans, millets, barley and many leguminous plants for their antimicrobial potential (Price et al., 1980, Chung et al., 1998). Tannin reacts and forms complexes with compounds such as starch, protein and some digestive enzymes to reduce their nutritional values as food (Chung et al., 1998). An epidemiological study conducted by Morton (1972) in Italy showed that natural plant base tannin are mainly used in shoe leather making and is associated with human cancer. A survey in Italy by Battista et al. (1995) where leather making is a big business

found that tannin was responsible for nasal cancer among shoe-making workers. As such, the adverse effect of tannin to human health may have been the reason behind the limited research works on tannin as coagulant in drinking water treatment. Nevertheless, Özacar and Şengil (2002) and Özacar and Şengil (2003) used tannin obtain from *Turkish Acorns* and reported that tannin is anionic compound which was more effective compared with anionic polyelectrolyte (AN913) as a coagulant aid in combination with AS, producing residual turbidity of 0.02 from 20 NTU. The study also observed a significant reduction of AS volume in tannin +AS than in AN913 +AS treated water. In contrast to this observation, Graham et al. (2008) assessed the properties of commercial tannin containing amine and phenolic groups in water purification and found that commercial tannin extracts are cationic. In this case the coagulation mechanism of tannin would be complex to understand under this condition.

Sludge filterability investigated using specific resistance measurement by Özacar and Şengil (2003) also show greater performance as coagulant aid than when AS was used alone. However, despite this advantage, its application in drinking water treatment may present some danger to human health because of its toxicity especially in developing countries where access to water is a big challenge. The coagulation potential of tannin in water treatment was seen to be influenced by its modified chemical structure as reported in a study by Özacar (2000). Comparatively, Özacar (2000) and Özacar and Şengil (2002) observed that tannin extracted from Valonia could be used as a sludge conditioner as well. Furthermore, the application of tannin as sludge conditioner would significantly reduce cost associated with sludge handling in water treatment works.

2.3.7 Cowpea *(Vigna unguiculata)* seed

Cowpea *(Vigna unguiculata)* is a tropical plant grown in many African regions and in some Asian countries which is considered a major source of protein (Popelka et al., 2004, Marobhe et al., 2007a). *V. unguiculata* is tolerant to poor dry soil with most of its parts being used in the preparation of local food (Popelka et al., 2004). Beside its being used as a main source of food, the high protein contents in the seed prompted recent study on its coagulation ability. Marobhe et al. (2007a) compared the performance of both the crude extract and two-step purified proteins eluted with 0.3 and 0.6 M NaCl concentration. The investigation reported an increase in coagulation performance of 92% at a dose which was 5 times lower, compared with 87% turbidity removal achieved with the crude extract. Similarly, Marobhe et al. (2007a) observed that after purification where 96% of the organic compounds in the seed was eliminated, the MW of the coagulant protein conducted using SDS PAGE analysis was 6 kDa similar to that of MO. Choy et al. (2015) showed that *V. unguiculata* seed is recognised in some rural communities of Africa in folk medical. To obtain a better understanding of the potential of *V. unguiculata* in water treatment, Sotheeswaran et al. (2011) showed that the seed extract is capable of removing 22% of hardness, and over 30% of Pd and Chromium in water. However, despite these findings, information about its applicability in drinking water treatment is still limited, further investigations are highly recommended in order to explore its potentials.

2.3.8 Fava beans

Fava beans (also called *Vicia fava* Linn L.), is native to the Mediterranean region and used as a primary staple food but was later replaced by another member of the specie,

phaselous beans in the Balkans (Mihailović et al., 2010). It is largely grown in European regions and parts of South America as a major food plant for Nile River populations (Duc, 1997). There are reports on the extensive production and cultivation of fava bean for export in Serbia Mihailović et al. (2010) which contain approximately 22.4–36% protein and 57.8–61% carbohydrates respectively (Hedley, 2000). But, Kuki et al. (2015) reported the protein content to be higher in fava bean, approximately 29.5%. To obtain the coagulant protein in fava seed, Okada and Okada (1998) purified the water soluble protein (WSP) by precipitation using ammonium sulphate followed by chromatography and observed two peaks of scavenging activity with MW of 70 and 28 kDa. Furthermore, after conducting SDS-PAGE analysis of the 28 kDa peak, a single protein band of about 14 kDa was also visible, thus indicating that this peak consists of dimeric proteins having MW of 28 and 14 kDa. Interestingly, since the active coagulation compound in most natural extracts is said to be protein Ndabigengesere et al. (1995), the presence of high protein concentration prompted Kuki et al. (2015) to assess the coagulation potential of fava bean in water treatment. Coagulant protein in fava bean was extracted with both distilled water and different NaCl solution. Kuki et al. (2015) observed that other than protein, other chemical compounds in fava bean seed such as phenol, tannin and phytic acid might be active coagulation agents. This is evident as Özacar (2000) and Özacar and Şengil (2002) used tannin extract of Turkey Valonia as primary and as coagulant aid in drinking water treatment. Fava bean extracted with distilled water and high ionic strength (1 M NaCl) produced similar results in terms of turbidity reduction compared with 25% achieved with 0.5 M NaCl (Kuki et al., 2015). Conversely, this result is not in agreement with previous studies where the extraction of coagulant protein was performed with NaCl solution and

increased performance was reported due to salting in effect (Ndabigengesere and Narasiah, 1998a, Okuda et al., 2001b, Ghebremichael et al., 2005). Similarly, pH was reported to have no impact on coagulation of fava bean extract (Kuki et al., 2015) which did not agree with previous studies (Ndabigengesere et al., 1995, Katayon et al., 2006). Although very little is known about the applicability of fava bean in water treatment, its coagulation compound behaviour is different from other natural extracts reported and this may require considerable investigations.

2.3.9 Maize (*zee mays*) seeds

Maize (zee *may* Linn L.) is widely planted and grown in Western hemisphere (Hallauer and Carena, 2009) in North Dakota in the 1600s. Maize seeds contains numerous proteins primarily used to store nitrogen for the growth of the seedling (Watson and Ramstad, 1987). The seed kernels contains some low MW basic polypeptides, which could be resolved on cationic IEX using lower pH level (Duvick et al., 1992). The SDS-PAGE analysis of one of the peaks in maize seed extract revealed a protein band with MW of 3.7 kDa having significant antimicrobial potential (Duvick et al., 1992). It has been reported elsewhere that Maize kernels are not only rich in oil but also have considerable starch and protein contents (Chander et al., 2008). Interestingly, these are compounds that have been investigated in water treatment. Several studies have used the extract obtain from Maize seed as a coagulant in drinking water treatment (Raghuwanshi et al., 2002, Mandloi et al., 2004, Unnisa et al., 2010). Raghuwanshi et al. (2002) and Mandloi et al. (2004) revealed that the main coagulation compound in Maize seed extract was a non-ionic starch, whereas in most natural extracts, the coagulation compounds reported were either protein or mucilage (Ndabigengesere et al., 1995, Diaz et al., 1999, Bodlund et al., 2014). Thus, the argument of presenting

starch as another coagulating compound in natural extract requires further evaluation which could broaden our understanding of the use of natural extracts in water treatment. To buttress this claim, river water from Bilaoli with high bacterial counts was treated with Maize extract at optimum dose of 200 mg/l, turbidity removal was approximately 75%, and the combined effect of the extract and solar radiation resulted in 100% bacterial inactivation (Unnisa et al., 2010). Additionally, there was no evidence of regrowth of either *E-coli* or *coliform bacteria* after 24-hour post treatment. The results suggest the antimicrobial potential of maize extract as a disinfectant in water purification. Similarly, Bilaoli lake being one of the most difficult river water to treat in India because of its characteristic low turbidity and low ionic strength was treated with maize seed extract and the head loss across the filter bed and bacterial removal evaluated (Mandloi et al., 2004). Total *coliform* count in the filtered water was reduced by 95.4% compared with 7.7% achieved with AS. However, in all cases, residual turbidity was 1 NTU which was WHO compliant (Mandloi et al., 2004). Furthermore, despite the limited research work conducted on Maize in water treatment, the conclusion made by some authors that the coagulant is a starch could be a positive development in this regard. However, more research needs to be done to assess the impact of maize extracts in water treatment regarding NOM addition, pH, temperature and coagulant dose. Also, its performance in wastewater treatment need to be investigated to evaluate maize extract further.

2.3.10 Mustard seed

Mustard seed (*Brassica juncea* L. or *Czern*) is a member of *Brassicaceae* family (Bodlund et al., 2014). It is a terrestrial plant grown largely in India with different colorations and used as spice in folk medicine, and also as a source of oil (Ildikó et al.,

2006). Mustard seed contains high protein content of between 28 and 36% (Ildikó et al., 2006).

Mustard plant have been studied by Dushenkov et al. (1995) and Dushenkov et al. (1997) for the removal of Uranium in water and rhizo-filtration of heavy metals showed significant decrease in Uranium concentration from 874 to <20 µg/l, which met EPA standards for wastewater discharge through bio-accumulation in plant roots. Although little is known about this mechanism where natural plants could accumulate metal intracellularly and transport it to the shoot, there are reports on the use of melon husk and wool fibre to remediate metal ion in this regard (Balköse and Baltacioğlu, 1992). In addition, Dushenkov et al. (1995) showed high removal of Pb concentration by Mustard plant, technically due to tissue adsorption with the roots indicating the presence of concentrated insoluble inorganic precipitate, primarily of Pb phosphate. However, the treatment process adopted in these studies cannot be a feasible water treatment alternative to people in developing countries because of the process involved. Moreover, Uranium contamination is not common to many region of the world but turbidity and pathogens are the main issues of concern in water treatment. Again, such an alternative cannot be employed as POU water treatment process to make water easily accessible even where there is threat of Uranium contamination in water.

An investigation of the potential in different Mustard seed species was conducted by Bodlund et al. (2014) who investigated the effectiveness of coagulant compounds in Mustard (small, yellow and large) in water treatment. Three Mustard seed species were extracted and then heated to a temperature of 95°C for 5 hrs to assess the impact of denaturation on the extract. Similarly, SDS-PAGE analysis was performed on the crude

and the heated sample. Bodlund et al. (2014) showed the coagulant compound in Mustard seed extract to be thermos-tolerant with MW sizes of approximately 6.5 and 9 kDa similar to that of MO seed extract. Among the species, Mustard large showed greater coagulation potential, achieving 70% turbidity removal compared with 60 and 45% achieved by Mustard small and Mustard yellow respectively after 90 min sedimentation. The protein sequence analysis of Mustard also show 19 amino acid protein peptides having about 37% flocculent properties (Bodlund et al., 2014). This is particularly an important property that could help provide information regarding the peptide linkage and carbon chain in the extract. However, due to limited information on Mustard seeds in water treatment, study by Bodlund et al. (2014) should be the bases for further investigations since there is similarity in its compound to that of MO seed protein.

2.4 *Hibiscus Malvaceae* families

The plant Hibiscus linn (L) belongs to the *Malvaceae* family. The family comprises of about eighty-eight (88) genera and 2300 species distributed across the world (Burkill, 1997). Members of the family are tolerant to most climates (except in very cold regions) and can grow favourably in temperate, warm and tropical climates (Hickey and King, 1997). The fruits of most plant species are used in food preparation and their calyx utilised in the production of locally flavoured soft drinks (Bolade et al., 2009, Foline et al., 2011). It is noteworthy that many Hibiscus plants are used in folk medicine because of their biological activities, especially as it relates to the following; antihypertension, antioxidant, antipyretic, antimutagenic, antifungal and antibacterial chemopreventive (Faraji and Tarkhani, 1999, Navarro García et al., 2006). In Turkey, the seeds of several Hibiscus plants are roasted, ground and used as a coffee substitute (Çalışır et al., 2005a,

Çalışır et al., 2005b). Many Hibiscus species are primarily planted for ornamental purposes and also have proved to promote hair growth and aid in the healing of body and duodenal ulcer (Adhirajan et al., 2003). Kılıç et al. (2011) investigated the linoleic acid content of one of the species grown in Turkey and reported that the linoleic content was found to be higher than that obtained from China with 67.5 percent. A transesterification evaluation of oil extracted from Hibiscus seeds could be a good material for biodiesel production comparble to ASTM D 6751 and EN 14214 (Anwar et al., 2010, Kılıç et al., 2011). It is also considered as a low-cost absorbent for use in the treatment of liquid waste containing metal ion and oil (Othman et al., 2008, Kılıç et al., 2011). Some important economic applications of Hibiscus plant are in the fashion industry, as a source of fibres for paper making it one of the most beneficial plant known to man Burkill (1997) and Akpan (2000) and for the remedying of susceptible and polluted land.

2.4.1 *Hibiscus Esculentus* (Okra)

OK is a member of the Hibiscus family called *Abelmoschus esculentus,* which is a genus of about 15 or more species of flowering plants in the mallow family (*Malvaceae*). It is one of the most widely known and utilized species of the genus (Naveed et al., 2009). OK plant is identified by its green coloration, tapering and elongated seedpod. It was regarded originally as an Indian plant but is now planted and grown in many tropical countries of the world (Sengkhamparn et al., 2010). OK is planted in the Middle East, Africa, and South America (András et al., 2005, Gulsen et al., 2007, Benchasri, 2012). It has a massive area under cultivations in many African countries and Asia with huge commercial and other economic potentials. Figure 2.1 shows an immature OK seed pods and some mature dried seed kernels.

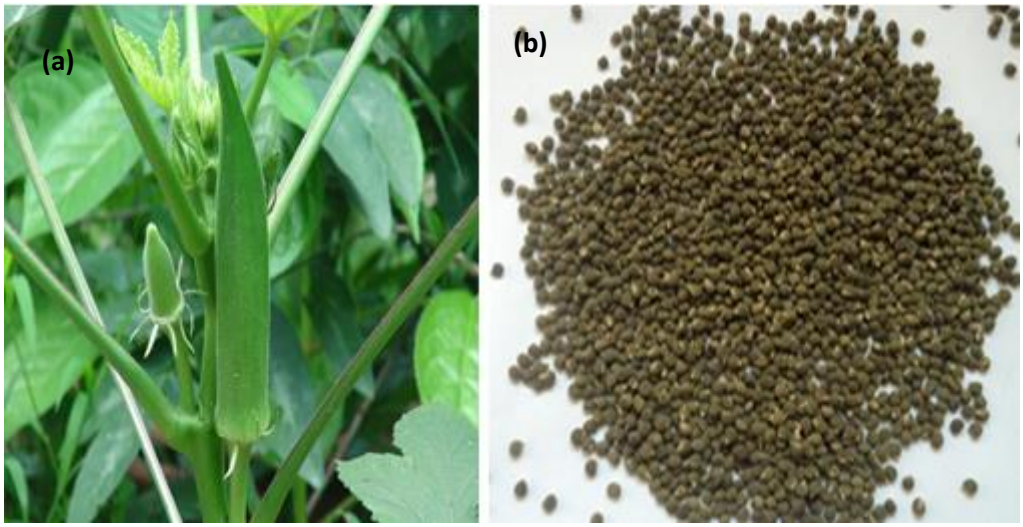

Figure 2.1 Okra plant with (a) some immature seed pods and (b) seed kernels (Etsy, 2013).

In English-speaking countries, OK is commonly known as "ladies finger" which is frequently consumed as a vegetable. It is fried or boiled, mixed with salads, soups, and stews (Whit, 1999, Opie, 2010). Among the species, OK has been reported to have a very high protein content of between 40-55% in a defatted sample obtained by Kjeldahl method where percentage nitrogen is multiplied by a factor of 6.25 compared to between 10-20% in crude seed coat extract (Oyelade et al., 2003). In other studies where proximate and amino acid analysis was also performed by Kjeldahl and IEC, the protein content was found to be between 21.8 and 24.9% and total essential amino acid of 35.4 and 38.1 g/100g of crude protein or 40.6 and 47.8% total amino acid (Ndangui et al., 2010, Aremu et al., 2014)

The fruit of OK is a capsule-liked structure, filled with viscous white mucus; the seeds kennels are arranged in the seedpods and have good nutritional value. It is a complete source of fibres, calcium, minerals and acts as a source of most of the vitamins the body needs. The plant of OK is typically different from most other common vegetables because it contains a high content of mucilage substances in the pod. The stem, leaves,

and the seedpods of this plant have mucilage used in the production of soup and gravies (Yayock et al., 1988, Ogaji and Nnoli, 2010).

OK mucilage refers to the thick and slimy substance found in the plant and is water soluble which produces a slippery aqueous colloidal suspension with slightly longer macromolecule chain than the ones found in other plants (Sengkhamparn et al., 2010). Additionally, the drag reduction performances of mucilaginous suspension obtained from OK have been investigated in a closed-looped circulation system to improve flow in water mains (Hayder and Rosli, 2009, Bari et al., 2010, Mohd Azimie, 2012, Abdulbari et al., 2014). These studies observed highest drag reduction efficiency in water to be up to 60% in the presence of 400 ppm of DRA. Both the immature and matured fresh seedpods have high mucilage content with limited contents observed in dried seed pods. Mature OK pods were prepared and extracted using 0.05 mole sodium acetate buffer and 0.05M sodium oxalate and then both sugar and uronic acid compounds were identified by automated colorimetric orcino method. The results show that OK is rich in carbohydrate that is mainly present in the form of polysaccharide and galactan side chains (Sengkhamparn et al., 2009, Sengkhamparn et al., 2010). Interestingly, it was observed that there are also two classical polysaccharide components in OK mucilage. One of the elements is chemically acidic polysaccharide while the other is a neutral polysaccharide. The former predominate and is associated with proteins and minerals as the active compounds (Dev and Quensel, 1988, Jideani and Bello, 2009).

Mishra et al. (2008) synthesized OK mucilage by grafting acryl-amide using radical polymerization method with ceric nitric acid. The polysaccharide compounds found in a mucilage solution of OK pod consists mainly of D-galactose, L-rhamnose, and D-

galacturonic acid and the crosslinked polymeric swelling behaviour was found to be useful in water purification (Mishra et al., 2008). However, analysis of the percentage components indicates that the galactose is (25%), rhamnose (22%), galacturonic acid (27%) and amino acid (11%) (Sengkhamparn et al., 2010). Miller et al. (2008) studied the independent use of galacturonic acid in water and observed a high level of flocculating activity, thus, making it a potential candidate in water purification. Some of the physical and chemical properties of OK mucilage also includes high water solubility, plasticity, elasticity and viscosity (BeMiller et al., 1993). These important properties are dependent on one or more of the following factors; temperature, pH, sugar and salts content and storage time as earlier found by Bhat and Tharanathan (1987). As such, its quality contributes to the improvement in functionality, especially water binding and emulsifying properties and the viscosity of its protein content is excellent (Jideani and Bello, 2009).

Natural polymers such as mucilage are water-soluble polysaccharides and are found widely in natural plants. Mucilage is easy to isolate, purify, and they are non-toxic, biocompatible, and biodegradable and will not cause environmental pollution. Similarly, the mucilage compound has also found extensive application as a tablet binder for pharmaceuticals (Ofoefule et al., 2001). Kumar et al. (2009) and Gasendo et al. (2012) revealed that the mucilage is a powerful suspending and binding agent in the production of a drug such as paracetamol.

Anwar et al. (2010) investigated the extraction of biodiesel from OK seed oil by methanol-induced transesterification with an alkali catalyst at optimum conditions of 7:1methanol to oil ratio and temperature of 65°C and rotor speed of 600 rpm. The study shows that the fuel properties of OK oil methanol extract such as viscosity, density,

lubricity, kinematic, cetane number acid value, flash point etc. are comparable to those standards of ASTM D 6751 and EN 14214. Again, because of its medicinal values, Okwu et al. (2007) and Okasha et al. (2014) studied the chemical composition of OK seed and found some active antimicrobial agents in the seed which consists of flavonoid, alkaloids, saponin, tannin and phenol. These compounds are found in abundance in the seeds which is used in traditional medicine. Kondo and Yoshikawa (2007) purified OK mature seed by precipitation, IEX and size exclusion chromatography using ammonium sulphate. The study identified the presence of ribosome-inactivation protein (RIP) in OK seed as one of the primary antibacterial agents capable of inactivating bacteria having protein MW of 30 kDa. RIP active agents have an allergenic and cytotoxic effect on several microbes because once inside a cell, it has been estimated that a molecule of RIP was sufficiently enough to kill a cell (Stirpe and Barbieri, 1986).

The polymeric compounds in OK seed pods are effective as synthetic flocculants in the treatment of tannery effluent and wastewater (Agarwal et al., 2001, Agarwal et al., 2003b). The flocculating potential of mucilage from fresh stems mucilage extracted from 300 g OK plant in 150 ml water by Yao et al. (2005) achieved more than 35% removal of turbidity in synthetic kaolin water of 35 NTU in Cote d'Ivoire . Furthermore, it was observed that by varying the mucilage dosage and pH influences the test results. Interestingly, the application of fresh OK stems in turbidity removal present the likely flocculating compounds in most of its parts including the seeds. In addition, in all the mucilage extract tested from the immature pods, the immature seeds were not removed; hence it may likely contribute to the flocculating activity in the process. Removing the seed kernels from the pods and conducting similar test runs

would give a better understanding of its potential. In textile wastewater, however, the mucilage had potential in removing suspended solids, total dissolved solids and colour at an optimum dose of 0.8mg/l (Srinivasan and Mishra, 2008). Additionally, Anastasakis et al. (2009) performed an assessment of Greek OK seedpods mucilage extracted with DI water on synthetic wastewater by adding 100 mg of kaolin to 10 mg of humic acid in 1 L and biologically-treated effluent from extended aeration and sedimentation. This was the only study that investigated the effect of NOM addition in the treatment process using OK mucilage. The study reported a reduction of 97% in turbidity of synthetic wastewater using OK mucilage at 5 mg/l dose while DOC increased from 1.1 TO 3.2 MG/L with optimum pH being 6. Similarly, turbidity removal in effluent water was 74% at higher dose of 2.5 mg/l than that used in the synthetic wastewater. In addition, DOC in effluent water increased from 13.3 to 18.6 mg/l at same dosage of 2.5 mg/l. Anastasakis et al. (2009) observed that the increase in DOC was due to the presence of organic molecules in OK seedpod. Furthermore, de Jesus et al. (2013) ground the dried immature seedpod and washed it with acetone and observed that it is comprised of hydrophilic colloids, low molecular weight acid, proteins and polysaccharides. A trial of this compound on kaolin water with high iron contents using a jar tester achieved 81% removal of iron at optimum pH of 8 and turbidity removal was 75% after only 1 min flocculation time (de Jesus et al., 2013). Freitas et al. (2015) performed an experiment to investigate performance of OK mucilage on chemical oxygen demand, turbidity and colour removal in textile wastewater in a jar tester. 1g OK seedpod was soaked in 100 ml solution of NaCl, potassium chloride and sodium nitrate after h hr stirring to swell. Mucilage was separated and fibres by filtration in a 500 micron steel filter and viscous mucilage used

as a coagulant. The study also observed the presence of polysaccharide in OK mucilage as coagulant aid resulted in higher removal of COD, turbidity and colour than when ferric chloride was used alone in the wastewater treatment. Percentage removal of turbidity was 97% compared with 93% when ferric chloride was used. Similarly, COD and colour removals were 85 and 93% compared with 50% recorded with the inorganic coagulant respectively (Freitas et al., 2015). The increase in COD removal by more than 35% and the reduction of inorganic dosage by 72.5% are enough evidence to adjudge that OK mucilage is an excellent coagulant aid in wastewater treatment. A bio-flocculant of OK mucilage extracted with water has shown a successful dewatering of sludge which resulted in more than 98% and 68% solid removal and water recovery similar to the performance of commercial polyacrylamide flocculants, at an optimum dose (Lee et al., 2015).

Unfortunately, as with most local vegetable plants, the seed of OK plant has received a little research attention despite the wide water treatment potential (presence of protein, galactronic and mucilage) identified in its seed in the preceding parts.

2.4.2 Kenaf (*Hibiscus Cannabinus*)

Kenaf (KE) *(Hibiscus Cannabinus* Linn) is a member of the *Malvaceae*. It is an annual herbaceous plant that grows under a range of weather and climatic conditions (Meints and Smith, 2003). It grows to more than 3m within three months of planting, often tall and woody with single stem and minute prickles on the stems and stalks. It is widespread in most tropics and is found in Ethiopia, Zimbabwe, Mozambique and Uganda (Katende et al., 1999). It is also widely distributed in Cameroon (Agbor et al., 2005) and in northern Nigeria where both the leaves and the seed are consumed as a

vegetable soup (Meera and Agamuthu, 2012). The seed capsules contain fine loosed hairy structures, which is very irritating when in contact with human skin. Each capsule contains five segments with a total of 20 to 26 seeds per capsule (Dempsey, 1975). Figure 2.2 shows KE plant and some mature dried seed kernels. KE seed has an approximate length of 6 mm and its width is approximately 4 mm. The weight is approximately 35,000 to 40,000 seeds per kilogram. Under normal planting condition, the plant takes between 4 to 5 weeks to reach maturity. KE represents a multipurpose and versatile crop for energy and natural fibre for industrial applications (Meera and Agamuthu, 2012). It has rich fibre content that serves as a perfect material for cordage and rope making, and in the paper industry (Falasca et al., 2014).

Figure 2.2 Kenaf flowering plant (a) with fruits and (b) dried seed kernels (Etsy, 2013).

Furthermore, the natural fibre content of KE poses no risk to human health if the ground particle is accidentally inhaled during processing (Joseph et al., 2002, Edeerozey et al., 2007). Additionally, the plant has appreciable protein contents which is processed and used by village women to improve breast milk. Mariod et al. (2010) characterized the

concentration of protein in defatted KE seed using petroleum ether to remove the oil and the powder suspended in distilled water in 1:100 ratio. Protein concentration was obtained by Kjeldahl method using a factor of 6.25, and multiplying by the nitrogen content. The percentage of protein in the seed was observed to be 13.04% Mariod et al. (2010), and can be used in the preparation of coffee and other local food (Falasca et al., 2014). In addition, Alexopoulou et al. (2015) reported in a review on how to improve KE yield in Europe and China where KE is being assessed for sustainable bio-based products with new variety released in the US and China. The new variety is expected to improve yield and high biomass productivity. Agbor et al. (2004) reported the evidence of phenol, tannin, saponin, alkaloids and steroids in KE seed. Similarly, the chemical composition of KE seed has been reviewed by Cheng et al. (2016) who observed that the seed possess some active antimicrobial agents, such as saponin, alkaloids, tannin, essential oil, fatty acids and steroids. The presence of protein and other compounds present KE seed as a potential material to be evaluated in water treatment as a disinfectant. Some properties such as saponin and RIP can inactivate microbes as they foam when in contact with water (Shaheed et al., 2009).

Abe et al. (1999) compared wastewater treatment efficiency of 20 different plants by designing plant-bed filter ditches where terrestrial and aquatic plants could be used to remove nitrogen and phosphorus from wastewater. The study showed that KE plant has some vital compounds capable of treating pond wastewater with low nitrogen and phosphorus concentrations. Accordingly, such properties give KE the ability to absorb nitrogen and phosphorus in the soil and can be used to remedy water and soil contaminated by oil as indicated in a review by Adebajo et al. (2003). KE products have been utilized in the production of a low-cost absorbent for use in the treatment of liquid

waste containing iron and oil. Othman et al. (2008) investigated the potential of KE inner fibre for the treatment of oil and metal ion contaminated water using crushed chips of inner core KE fibre processed by carbonization where 2 grades of diesel and cooking oil were used. It was observed that the carbonaceous matter achieved maximum sorption capacities of 35 and 30 g/l for diesel and cooking oil respectively more than the performance of commercial adsorbent. Meera and Agamuthu (2012) investigated the phytoremediation of landfill leachate with a high concentration of arsenic (As) and Fe using KE. In the study, KE demonstrated considerable uptake of between 87-91% As and Fe accumulation in plant roots with insignificant amount found in plant leaves and other parts. Furthermore, the work observed the height of KE grown on leachate to be 11% taller than those grown with inorganic fertilizer (Meera and Agamuthu, 2012).

Furthermore, Annie et al. (2011) performed batch adsorption test using an activated carbon derived from KE bast fibre (KFAC) rinforced with chitosan biocomposite and characterized by FTIR. The study showed intraparticle diffusion of copper diffuses was faster at the beginning of the adsorption process, and then slowed down and stabilizes and recommended that chitosan/KE composite as an effective adsorbent for copper ions removal in wastewater. Similarly, Norzita (2012) investigated the removal of arsenic with hybridised polyacrylamide and cellulose derivative from KE fibre using microwave-assisted grafting method, which indicated improved performance than when polyacrylamide was used alone. KE fibre is also effective in wastewater treatment as an absorbent for Pd (II), Cr (III) and Cr (VI) which could remove up to 90% of the chemicals within 5 minutes contact time (Marcus Jopony and Hun, 2013). Similarly, Gharehchahi et al. (2014) showed capillary effect of 1.2 and 1.9 m lengths of KE fibre in water softening with hardness between 352 and 612 mg/l as calcium carbonate. The

performance of KE was found to be dependent on the length with an average hardness reduction of 108.43 and 163.74mg/l, thus showing good potential in softening hardness in water.

However, there has not been any report of the use of this plant seed in water treatment, and not much is reported about the potential of the seed in other ventures. Therefore, if this plant is harnessed properly, it could be an effective water treatment material, possessing the dual function of coagulation and disinfection because of the presence of protein and antimicrobial agents in the seed. The high performance of KE fibre may provide a good base to investigate other parts of KE plant such as seed in water treatment.

2.4.3 Russel (*Hibiscus Sabdariffa*) Linn

Hibiscus SB is a shrub belonging to the family of *Malvaceae* plants. It became popular because of its growth speed, quicker than other natural plants. The plant SB is believed to be native to Asia (India to Malaysia) or tropical Africa. It is about 3.5m tall and has deep penetrating taproots. It has cylindrical typically dark green to red stems. Hibiscus SB is used in various applications including food. It has been used extensively in the making of a common local soft drink, popularly known as Zobo in Nigeria and many other tropical countries (Dalziel and Elliot, 1973). In English-speaking regions, it is called Roselle, Sorrel or Guinea Sorrel. The calyx is rich in acid, pectin, protein and minerals; such as iron, calcium, and aluminium. Figure 2.3 shows a Sabdariffa plant with some flowering calyx and mature dried seed kernels. The stem and leaf have an acid flavour that closely resemble that of cranberry (Morton, 1987). The antioxidant property derived from the anthocyanins compound is another useful acid from SB (Ajiboye et al., 2011). The calyx is particularly used in folk medicine for the treatment

of hypertensive patient (Abu-Irmaileh and Afifi, 2003, Lin et al., 2007, Wahabi et al., 2010). The seeds of SB are bigger than the pearl millet varieties with average dimensions of 2.98-3.36 mm, others are 1.86-2.24mm while some are from 1.70-2.01mm (Jain and Bal, 1997). Omobuwajo et al. (2000) showed that an average diameter of SB seeds is 5.53 mm with the oval capsule fruits containing between 22 and 34 seeds. In Nigeria, a digested SB seed is used and given to augment low and poor milk production; poor let down and maternal mortality (Okasha et al., 2008). Phytochemical analysis of the seeds, stems and leaves of Hibiscus SB was conducted by Mungole and Chaturvedi (2011) who hamogenized them into fine powder and then extracted with petroleum ether on sohxlet apparatus. The screening was performed using thin layer chromatogrphy. The results show the presence of concentration of alkaloids, saponins, steroid ring, deoxy sugar, tannin, phenolic and flavonoids in all the samples. The observed compounds may likely inhibit microbial growth in water since they are the main agents that make them viable in folk medicine.

Figure 2.3 *Sabdariffa* plant showing (a) flowering capsule and (b) dried seed kernels (Etsy, 2013).

Rao (1996) performed proximate analysis of the whole mature seeds of two SB species. In each case, the protein content in the seed was found to be between 18.8 and 22.3% while the lipid content was between 19.1 and 22.8%. In addition, the analysis reported abundant phosphorus, magnesium and calcium compounds whereas high presence of lysine and tryptophan proteins was also noted. In recognition of this, there has been tremendous focus on the cultivation of this crop to provide the needed cheaper and cost-effective protein in developing countries. The proteins could also be useful in water treatment. SB seeds can be roasted because of the high protein content and is consumed as a substitute for coffee and sauces in soup preparations (Morton, 1987, Qi et al., 2005, Da-Costa-Rocha et al., 2014). It is noteworthy that SB seeds is recognised also in China as a potential protein (El-Adawy and Khalil, 1994, McCaleb, 1996).

The phyto-disinfectant ability of the calyx was invested by Yongabi et al. (2011) who used the water extract to treat turbid water and achieved 60 to 90% reduction in *E-coli* and *total coliform* count compared with AS treated water. The study suggests that the calyx possessed both the coagulating and disinfecting agents. In most natural plant materials evaluated in the literature for their possible application in water treatment, the coagulant compound is usually targeting the protein and in most cases are cationic (Ndabigengesere et al., 1995, Ghebremichael et al., 2005, Broin et al., 2002) while others are anionic. It is noteworthy that there is no existing information on the use of SB seed in water treatment which requires intense investigation due to its protein.

2.5 Coagulation and flocculation mechanisms

Coagulation and flocculation are considered to be the principle behind the treatment of both water and wastewater in most conventional plants (Shammas, 2005). Coagulation

is simply the process of destabilizing the particles by introducing metal salts to chemically change the particles, such that the charge maintaining their stability is overcome or by charge neutralization where the aggregate are smaller and dense (Gregory, 2005). Coagulation mechanism is measured by the rate of destabilization of the particles. The process is achieved through destabilization and precipitation mechanisms, such as surface charge modification, double layer compression, adsorption and also particle bridging (Duan and Gregory, 2003, Gregory, 2009). Flocculation on the other hand is a slow mixing process (i.e. orthokinetic aggregation) used after the particles are coagulated. Flocculation process brings together (bridging) the destabilized smaller, micro-flocs resulting from coagulation into contact with one another to form larger macro-flocs (Klimpel and Hogg, 1986). Flocculation step is crucial and is measured by the rate of collision efficiency between particles which do not settle and are thus difficult to remove by sedimentation. Floc formation depends on the collision efficiency (α) between the particles (Gregory, 2005). The intensity of mixing is reduced as the water proceeds through the flocculation process to achieve optimum performance.

2.5.1 Zeta potential and charge measurement

Zeta potential is the potential difference between the external thin surface layer of ionized particle and that of the surrounded oppositely charged ions from the liquid as a whole. In a simpler term, it is a measure of surface charge on particles in suspension. It indicates the behaviour of particles in a system and is widely related to the stability of the suspended materials under given set of water characteristics. Thus, any zeta potential near zero reveals the minimal electrical repulsive force and so increases the

tendency of capture (Gregory, 2005). All particles in water are invariably negatively charged (Dentel, 1991, Sharp, 2005). However, changes in general water characteristics and environment effects which generate process compliance issues, have resulted to increased application of charge measurement in the water industry. Most natural colloids in water exhibit a negative zeta potential of between – 5 and − 40 mV (Sharp, 2005). Zeta potential is a property of an electrically charged interface which is measurable and is determine from any of following methods; electrophoresis, electro-osmosis, stream potential and sedimentation (Branko, 1992, Delgado et al., 2005). On the other hand, the state of a charged surface is characterized by spatial distribution of ions around the particle whereas the zeta potential is the characteristic of the charged surface. Therefore, both zeta potential and charge are obtain from the same measurement (ionic measurement) using the same methods (Delgado et al., 2005). Measurement by electrophoresis is the simplest, quickest and the most widely used method because there are varieties of devices such as zetameters available in the market.

It is noteworthy that the use of anionic compounds to flocculate negatively charged particle is debatable. However, in contradicting the theory of double layer compression when dealing with anionic coagulant, La Mer (1966) used potato starch which is an anionic compound to flocculate kaolin particles in water. Similarly, Diaz et al. (1999), Zhang et al. (2006) and Miller et al. (2008) flocculated kaolin water with an anionic compound extracted from C*actus latifaria* and *Cactus opuntia* and observe a turbidity removal performance of > 90%. La Mer (1966) argued that the anionic compound hydrolysed in solution where the COOH group replaces the amino groups and increases the adsorption effect of the anion at higher dosage. Under high dosage, the adsorption ability increases and outweighs the electrostatic repulsions between the coagulant and

the particle which later transformed to bridging action. Thus it is not out of place to say an anionic extract could flocculate negatively charged particles in water through adsorption and bridging action, and not by charge neutralization because of electrostatic repulsion between the coagulating compounds and the negatively charged particles.

2.5.2 Sodium dodecyl sulphate Polyacrylamide gel electrophoresis

SDS-PAGE is a detergent with an anionic head-group and a tail which binds proteins non-covalently where the SDS causes the denaturation of protein (Laemmli, 1970). The analysis is the most widely used technique to separate and characterize proteins by electrophoresis to denature and dissociate the proteins from each other, using polyacrylamide gel as a support medium. Under this condition the charge of a protein is masked and protein migrates towards the anode (Laemmli, 1970). When proteins are treated with SDS, they attain a similar shape and charge-to-mass ratio which is then determine by molecular weight during the PAGE. SDS-PAGE analysis was conducted in order to understand the various molecular weight and protein sizes that are present in the extracts, and also to obtain information on protein (s) with coagulation activity in both crude and purified proteins.

2.6 Summary

Most of the natural plant materials presented in this chapter have exhibited water treatment potentials, even though many of them are yet to be investigated fully. MO seed is the most studied natural extract to date in water treatment. it is also the most effective of all the materials studied in water treatment. As such, more intensive studies are required to identify other potential water treatment materials to make clean water accessible to people in developing countries to improve quality of life, and

subsequently, sustain human development. In areas where natural extracts have been tested and used as point-of use (POU) water treatment, large water production using these products is yet to take off by the people.

Developing countries imports water treatment chemicals overseas in foreign currency. Thus, any effort to provide an alternative natural material of either plant or animal origin as a coagulant or disinfectant could avail developing countries with considerable savings. Al—Samawi and Shokralla (1996) and Muyibi and Okuofu (1995) have shown that more than 50 percent of AS requirement could be saved if OK mucilage, MO extract or tannin extract is used as a primary coagulant or as a coagulant aid in water treatment . Natural materials with coagulating potential are widespread, affordable, and less expensive and are easily biodegradable and produce much lower sludge volume compared to the inorganic chemicals. The use of natural coagulants of plant-based represents a vital development of grassroots sustainable environmental technology since it focuses on the improvement of the quality of life for rural people.

The use of natural extracts in water treatment can be integrated into household water purification, considering its enormous advantage. However, the main challenge of using natural extract in water treatment is the increase in organic compounds in the treated water. NOM in water could cause a change in taste, colour and odour in the clarified water and can also be a precursor of disinfection by-products (DBPs) formation if the water is disaffection with chlorine based chemical. Several works have successfully purified the target compounds to address this challenge. The success of this process has brightened the future application of natural material in water treatment.

CHAPTER 3 – KNOWLEDGE GAP

3.1 Background

While several natural plant extracts such as MO seeds, *okra* pod-mucilage, *faba bean* (*Vicia faba* L) seeds, *Strychnos potatorum, Phaeolus vulgaris* etc. have been investigated for their coagulating potential in water treatment (Jahn, 1986, Agarwal et al., 2003a, Muthuraman and Sasikala, 2013, Kuki et al., 2015), there is little information on the use of *Hibiscus* plant seed as a coagulant. It can be seen from the preceding chapters that, despite all the work undertaken to date, significant gaps are still present in our knowledge of the action of plant seed species when used as coagulants and disinfectants. Specifically, there exists a lack of detailed understanding in the areas concerning the coagulated floc characteristics, such as floc growth, breakage and re-growth and strength. Again, there is also a lack of information on the character and composition of organic matter presence in water treated with natural extracts, as most of the studies only highlight the presence of organic matter in terms of TOC or DOC addition in the treated water (Okuda et al., 2001b, Ghebremichael et al., 2006). Furthermore, a lack of information, in particular on the zeta potential of natural coagulant presents difficulties in understanding the coagulation mechanisms of natural plant extracts in water treatment (Choy et al., 2015). Here, it is critical to look at the zeta potential of the extract in order to appreciate the potential of natural coagulants in drinking water purification and the stability of the colloids in the water.

3.2 Aim and objectives of the study

This study aims to close the gaps identified by conducting research into the suitability of hibiscus plant seeds for POU water treatment in order to enhance our understanding

of issues that have not been addressed in previous studies using naturally-occurring plant extracts. Similarly, the work reported will explore the potential and effectiveness of the rich biodiversity of plants using Hibiscus plant seed species as novel coagulants in drinking water treatment. The study will assist people in rural areas of developing countries to have access to a clean source of drinking water avoiding the difficulties associated with conventional methods. The primary aim will be achieved through the set of underpinning objectives outlined below:

- To investigate the potential of using Hibiscus plant seed species as coagulants.
- To develop a simple method of improving the efficiency of the extracts and compare the results with that obtained after purification using IEX.
- To investigate floc growth, breakage, re-growth and strength of water coagulated using hibiscus seed extracts and purified proteins.
- To apply fluorescence excitation-emission matrices to monitor, evaluate and characterise the composition of natural organic matter (NOM) in treated water using crude extract and purified seed proteins as coagulants.
- To study the antimicrobial potential of seeds using the crude and purified coagulant samples.

CHAPTER 4 – MATERIALS AND METHODS

4.1 Background

This chapter presents descriptions of procedures used in obtaining the Hibiscus plant seeds and provides information regarding the reagents and chemicals, machinery and equipment used in experimental runs. All experiments were conducted in triplicate.

Two forms of natural coagulants (crude and purified proteins) were used throughout the study. The crude extracts were obtained after the first two processing steps following Ndabigengesere et al. (1995) with modifications using 1.0 M NaCl solution and centrifuging at 4500 rpm for 10 min to obtain the coagulants as in Figure 4.1. The purified proteins were obtained after the first three steps following Ghebremichael et al. (2005) with some modifications where lipid extraction and purification was performed using sohxlet extractor and HiTrap anion exchange column, and elution using 0.3, 0.5 and 1.0 M NaCl as in Figure 4.1.

The first objective of the study was achieved by conducting laboratory experiments using the crude seed extracts in a jar test apparatus to assess their coagulation performance in synthetic turbid water of 50, 100 and 200 NTU which is similar to turbid water obtain from Nigerian rivers and ponds after rainfall events and subsequent settlement. Similarly, the effects of varying pH ranges on the coagulation efficiency of the crude extracts were performed according to Zhang et al. (2006) and their impact on final water pH also assessed following Diaz et al. (1999). Evaluation of sludge volume production by the extracts was also conducted following Ndabigengesere and Narasiah (1998a) with modification using syringe and the results compared with that of AS in water treatment. The SDS-PAGE analysis of both the crude extracts and the purified

proteins was compared in terms of MW according to Ndabigengesere et al. (1995) and Ghebremichael et al. (2006) methods. The SDS-PAGE analysis was performed in order to obtain a better understanding of the size of the protein in the seeds with coagulation potential. Coagulation mechanism of the crude extracts and the purified samples were also investigated by performing a zeta potential measurement in river and synthetic water samples according to (Ndabigengesere et al., 1995).

The second objective of the study was achieved by investigating the performance of denatured samples by temperature and storage time in water treatment. The main reason for conducting such a test is to assess whether denaturation of protein could improve the coagulation performance of the extracts and then compare the results with the results obtain after purification in IEC. Denaturation was conducted using the crude extract samples according to Ghebremichael et al. (2005) and Bodlund et al. (2014) with modifications after heating at 60, 97 and 140 °C temperature and crude extract and unprocessed seeds were also stored following Katayon et al. (2004) and Katayon et al. (2006) with some modifications after 1, 3, 7, 10 and 14 days respectively, and also by storing the seeds after 24 months to assess their performance.

The third objective of the study was conducted to investigate the properties of the coagulated flocs using a laser diffraction instrument, Mastersizer 2000, particle analyser as reported elsewhere (Jarvis et al., 2005a, Jarvis et al., 2005b, Zhao et al., 2013a). Floc properties such as floc strength, size, breakage and recovery ability were investigated. Similarly, the importance of coagulation time and rate of floc growth were evaluated to determine their importance as coagulant aid in water treatment. In these tests, both crude and purified proteins were investigated as primary and as coagulant aids and the performance of the crude extracts at pH 4 also evaluated.

The fourth objective of this research was achieved by conducting an investigation to assess the impact of NOM addition in treated water using both crude and purified proteins following (Okuda et al., 2001b, Ghebremichael et al., 2006, Sanchez-Martin et al., 2010, Antov et al., 2010). In the study, DOC concentration in water before and after treatment was performed using Shimadzu TOC analyser and fluorescence fingerprints with fluorescence spectroscopy following (Bieroza et al., 2009). These experiments were conducted to investigate the relationship between fluorescence peak and DOC concentration in water and to observe if peak T intensity could be a useful tool to assess OM in water.

The fifth objective of the study was achieved by investigating the bacterial inhibition potential of both crude and purified proteins in water treatment using a jar tester according to Ndabigengesere and Narasiah (1998b) and Shaheed et al. (2009) with modifications using Collilert-18 Quanti-Tray methods. A 72-hr post treatment was also assessed to obtain better understand of the impact of residual crude extracts and purified proteins in treated water. The impact of the samples was evaluated on *E-coli*, *faecal coliform* and *total coliform* count in water.

4.2 Coagulant processing steps

Researchers across the globe have used different methods in various studies to process natural organic based extracts. However, the literature advocates the use of three major processing steps; i.e. primary, secondary and tertiary steps (Yin, 2010) (Figure 4.1), the simplest of these steps being the primary steps which have been adopted in several research activities to extract different parts of natural plant such as the seeds, leaves, stems, shoots, fruits, pads etc. as water treatment coagulants. Similarly, rural women in

third world cities have embraced this method to process their natural extracts as coagulants for POU water treatment. The primary step is employed due to its simplicity and can be used even at village level without the use of any advanced technology to process the materials. In the secondary method, the process involves the extraction of specific compounds in the material such as lipid/fats, ash, carbohydrate, etc. to obtain the bioactive compounds using chemical solvents. This method offers significant advantages as it removes compounds which hitherto could be a problem in the treatment process. It has been established in literature that the presence of compounds such as lipids can limit inter-particle bonding during water treatment (Ali et al., 2010). Additionally, a further merit of this method is that the extracted lipids can be channelled into other viable alternatives such as in household edible oil and in industrial application as lubricant. The final, third step is the tertiary step which is the most expensive stage, and may even add to the overall treatment cost because of the technologies involved. Several studies have used one or more of these processes successfully, and the success of many academic research works could be attributed to this method (Ndabigengesere et al., 1995, Ghebremichael et al., 2006). It has been reported elsewhere that protein purification can be accomplished using any of the three most popular methods depending on equipment availability, for example, lyophilisation by (Ndabigengesere et al., 1995) and ion exchange chromatography (IEC) as reported by (Ghebremichael et al., 2006, Kansal and Kumari, 2014). Similarly, Okuda et al. (2001b) and Antov et al. (2010) adopted a dialysis method to isolate the coagulant protein compounds. Protein purification resulted in the tertiary step achieved greater purity, the proteins are generally obtain free of any other organic compounds (Sanchez-Martin et al., 2010).

The work reported in this thesis employed all three main processing steps with some modifications. However, in the tertiary processing steps, the study used only the ion exchange chromatography method of protein purification in order to compare its performance with that obtain from simple denaturation processes (heating and storage).

Furthermore, several simpler options were also investigated in order to identify cheaper, friendly and more cost-effective methods for people in developing countries, such as heating across range of temperature or denaturing via storing of the suspension for some days. Following that the unprocessed seed was also kept for 24 months after seed harvesting and its efficiency also tested.

The three major coagulant processing steps used in the study are presented schematically in Figure 4.1.

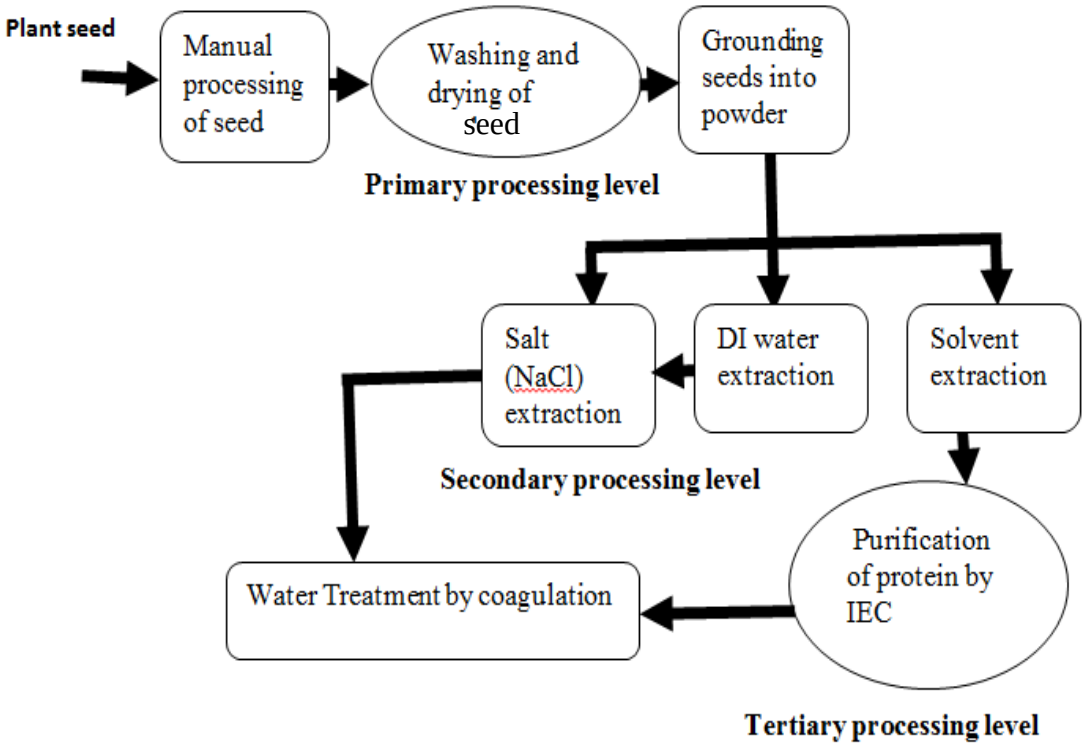

Figure 4.1 Flowchart showing natural coagulant processing steps in obtaining the extracts (Yin, 2010).

4.3 Purchasing, collection and preparation of the seed samples

All the high-quality seeds used in this study, *Okra*, *Kenaf* and *Sabdariffa*, were obtained from a local market in Borno State, Nigeria. The seeds were harvested from the matured and completely dried capsules and pods on the plants because the immature seeds may not possess compounds for effective coagulation activities. The seeds were manually prepared by removing them from the capsules and pods to access the kernels. The kernels were then sorted, packaged and labelled appropriately for ease of identification, and transported to the UK for laboratory processing, preparation and analysis. The seeds were washed with laboratory tap water to remove contaminants such as stones, plant debris and dust that might affect their integrity and quality. The cleaned seeds were then oven dried using (Memmert oven type, UF 30 115 VOLT, Germany), at 60 ºC for six hours before grinding into fine powder.

4.4 Chemical and reagents used in the study

Analytical grade chemicals and reagents [sodium chloride (NaCl), sodium hydroxide (NaOH), aluminium sulphate (AS), methanol, Hydrochloric (HCl) acid, ethanol and hexane, and sodium phosphate dibasic] were obtained from Fisher Scientific, UK and [kaolin Fluka-60609, sodium phosphate monobasic monohydrate] were obtained from Sigma-Aldrich, Germany. Blue pre-stained protein standard P7706s and Ponceaus S solution and Coomassie Brilliant Blue R-250 were purchased from New England Bio-labs, UK. Others (1 M Tris, pH 6.8, 205 SDS, 40% Acrylamide, 10% ammonium per sulphate (APS) and TEMED) were also obtained from New England Bio-labs, UK. Deionized (DI) water was used to prepare all the suspensions and stock solutions in this study.

4.5 Preparation and extraction of active compound in the seeds

The dried seeds were ground into a fine powder for 2 minutes using a laboratory disc miller (Tema mill, Germany). The ground seed powders were then sieved through a set of stainless steel sieves (600 to 212μm) and the finest powders retained in the 212 and 300 μm sieve sizes were thoroughly mixed and used in the experiments as shown in (Figure 4.2). 1.0 M sodium chloride (NaCl) solution was prepared by dissolving 58.44 g in 1000 ml of DI water because it is aggressive enough to extract the protein from plant tissue, to obtain the required concentration. The crude seed extracts (CSEs) were prepared from the ground seed powders by adding 1.0 M NaCl solution to the seed powder to make 2% (w/v) suspension. The suspension was stirred vigorously using a magnetic stirrer (Stuart scientific, UK) for 15 minutes at room temperature of (19±2 ºC). The suspension was then centrifuged at 4500 rpm for 10 minutes using a Heraeus Megafuge16 (Thermo Scientific, Germany).

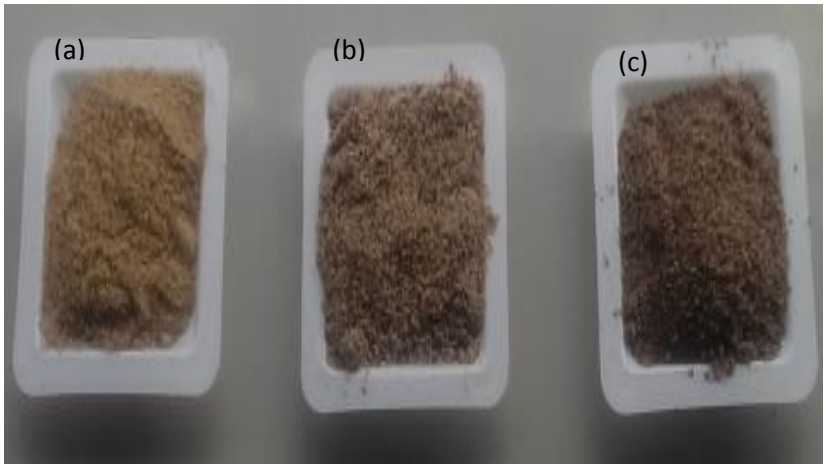

Figure 4.2 Ground seed powders of (a) Okra (b) Sabdariffa and (c) Kenaf.

The suspension was decanted and the residual solids (pellets) dried in an oven (Memmert type, Germany), at 50ºC overnight. The weight of the dried solid material was measured to ascertain the amount of seed powder used in making the suspension.

The decanted suspension was then filtered through a Whatman No. 42 filter paper. The filtrates were termed CSEs and were then used as primary coagulants or as coagulant aids in a series of jar test experiments. OK crude extract (OCE), KE crude extract (KCE) and SB crude extract (SCE) were used throughout the study. The zeta potential of the CSEs was measured using Zetasizer Nano ZSP (Malvern instrument, UK).

4.5.1 Denaturation of the crude seed extracts

A protein is said to become denatured when its folding structure is altered as a result of exposure to certain elements of physical factors (e.g. heat, storage, and change in pH etc.), causing the protein to become biologically inactive. However, this process was adopted to investigate the impact of denaturation on coagulation performance of the Hibiscus seeds protein after its conformation structure is changed by either heating or storage.

Protein denaturation was performed to investigate it effect on the coagulation potential of the extracts. The process of denaturation of the seed extracts was adopted from other studies (Ghebremichael et al., 2006, Bodlund et al., 2014) with some modifications. Varying temperature ranges 60-140°C were considered to observe the denaturation process due to temperature. This was done to ensure people in developing countries where the main source of energy is firewood can adopt the heating process. The CSEs were heated to different temperatures of 60, 97 and 140 °C for 6, 4 and 2 hours respectively, using a hot plate. The wider temperature ranges were randomly chosen to understand the thermostability of the extracts and the effect of each temperature to help people in rural areas where the main source of energy is firewood. The thermally treated samples were then centrifuged at 4500 rpm for 10 minutes and filtered through a

Whatman no. 42 filter paper to obtain the coagulant. Similarly, the extracts were stored for 1, 3, 7, 10 and 14 days at room temperature of 19 ± 2 ºC to denature the extracts. The storage was performed to assess its denaturation effect on the performance of the extract and the different days were chosen to observe its coagulation activity after prolong time. Finally, the molecular weight of the extracts and the purified protein samples (see protein purification) were determined on 12% Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) according to (Fling and Gregerson, 1986, Bodlund et al., 2014). Before running the gel, both the stacking and the running gel were prepared according to Table 4.1.

4.6 SDS-PAGE analysis

SDS-PAGE analysis was conducted in order to understand the various molecular weight and protein sizes that are present in the seed, and also to obtain information on protein (s) with coagulation activity. The analysis is the most widely used technique to separate and characterize proteins by electrophoresis to denature and dissociate the proteins from each other, using polyacrylamide gel as a support medium.

Table 4.1 SDS-PAGE for gel preparation used in the molecular weight analysis.

S/ No	Running Gel		Stacking Gel	
1	54µl	20% SDS	20µl	20% SDS
2	3.0ML	29:I 40% Acrylamide	0.3ml	29:1 40% Acrylamide
3	2.9ml	dH_20	2.85ml	dH_20
4	54ul	10% APS	38ul	10% APS
5	4.0ml	1M Tris, pH 8.8	0.57ml	1M Tris, pH 6.8
6	10µl	TEMED	5µl	TEMED

The protein sample was resolved by SDS-PAGE and transferred to a nitrocellulose membrane (Protran BA-85, Pierce Protein Biology). The membrane was blocked in 5% milk/1X TBST (Tris-buffered Saline-TWEEN 20) and incubated on a rocker at room temperature for 30 min. After the blocking; the membranes were incubated with primary antibody polyclonal goat anti-GFP (AbD Serotec) 1:2000 of antibody in TBST overnight at 4°C. At the end of the incubation, the membranes were washed three times with TBST at 5 min interval each and then incubated with secondary antibody (polyclonal anti-Goat HRP) 1:1000 of antibody in TBST for 1 hr at room temperature on a rocker. The membranes were washed again as completed at the end of the primary antibody incubation. The bots were then incubated with West Pico Chemiluminescent Substrate (Pierce) and then visualized with Gene Snap Software (SynGene) and the proteins stained with either Ponceau S solution or Coomassie dye Brilliant Blue R-250.

4.7 Lipid extraction from the seeds and purification of bioactive ingredients

Lipid extraction and purification of the bioactive compounds in the seeds was performed in order to eliminate the non-coagulant compounds such as lipid, ash, minerals, carbohydrates and other proteins that may be added into the treated water. Excessive NOM loading have been reported by Ndabigengesere and Narasiah (1998a) and Ghebremichael et al. (2006) as the main setback of using natural extract in water treatment. Thus, the purification processes is intended to address this challenge regarding deterioration in treated water quality using natural extracts.

The ground seed powders, sized between (212 and 300 µm) were defatted using high-grade hexane in an electro-thermal Soxhlet extractor. 20 grams of the seed powder was

used during the extraction. For efficient extraction, 1% w/v of seed sample in 2 litres of solvent volume (hexane) was used and heated to 60 °C.

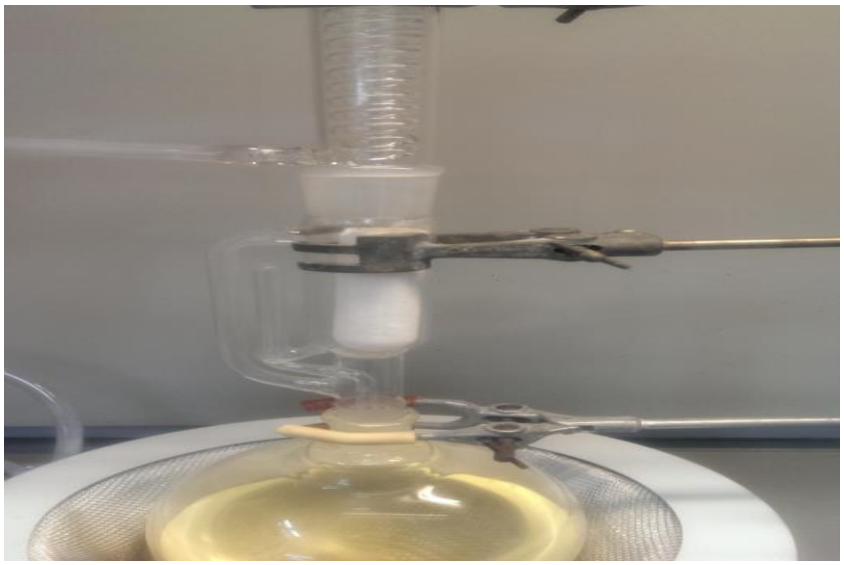

Figure 4.3 Lipid extractions with hexane using thermal Soxhlet extractor.

Figure 4.4 Defatted seed powder of (a) Okra (b) Sabdariffa and (c) Kenaf.

The process was run continually for 8 hours with each complete cycle taking approximately 2-3 minutes. The residues obtained from the extraction thimble were

removed and dried overnight at room temperature of 19 ± 2 °C. The dried residue was ground again into a fine powder using a pestle and mortar and was used in the subsequent purification processes. Each of these samples was kept in a plastic container at 4 °C in a refrigerator until use. The mixture of solvent and the lipid content in the heating flask was then evaporated using Rotary evaporator (Buchi RE121, Switzerland). The lipid remaining in the evaporating flask after successful completion of the solvent evaporation process was weighed and its percentage by weight determined.

4.7.1 Purification by Ion Exchange Column Chromatography

Isolation of the bio-active protein compounds responsible for coagulation activity was performed using IEC. IEC is a widely used technique which is effective and efficient in protein purification and separation in biochemical, chemistry and molecular biology (Ghebremichael, 2004).

4.7.2 Phosphate buffer preparation

Two buffers, A and B were prepared and used in the purification process. Buffer A was obtained by dissolving 27.6 g of sodium phosphate monobasic monohydrate in 1L of de-ionised water to produce 0.2 M stock solution of buffer A. Buffer B was obtained by dissolving 28.4 g of sodium phosphate dibasic in 1L of de-ionised water to produce 0.2 M stock solution of buffer B. To prepare 0.1 M phosphate buffer with a pH value of 6.0 for the purification, 61.5 ml of 0.2 M of buffer A was mixed with 438.5 ml of 0.2 M of buffer B and the resulting solution was diluted with 500 ml of de-ionised water to make 1000 ml of 0.1 M sodium phosphate buffer at pH 6.0.

Finally, the eluting buffers were prepared by dissolving an appropriate volume by weight of NaCl in the phosphate buffer to obtain 0.3, 0.5 and 1.0 M NaCl of eluting buffers.

4.7.3 Preparation and purification of the protein using ion exchange

A Hi-Trap Q HP (1 ml) anion column, (GE Healthcare, Sweden) was used for the purification of the protein of interest from the seeds of the Hibiscus plants. The column was connected to a pump (Watson-Marlow Breeder pump 323, UK) and the pump head adjusted to a flow rate of 1 ml per minute. The preservatives were washed with 10 ml of DI water, followed by 10 column volume of 1 M of NaCl dissolved in the phosphate buffer. The column was then equilibrated with the phosphate buffer 10 CV before loading the protein in order to bring the column to the required pH. 5g of oil-free powder was dissolved in 0.1 M phosphate buffer and mixed thoroughly for one hour using a magnetic stirrer (Stuart Scientific, UK). The suspension was centrifuged at 20,000 rpm at 4 °C for 40 minutes before decanting the supernatant. The supernatant was filtered through a 0.45 µm membrane filter before injection onto the IEX column. The filtered supernatant was injected using the peristaltic pump onto the ion exchange column to separate the protein of interest from the contaminants.

The sample was loaded onto the Column at a flow rate of 1 ml per minute, where the protein of interest was bound to the Column matrix throughout the loading process. The weakly-bound contaminants were washed away with the starting buffer using 10 CV. The bound proteins of interest were eluted, beginning with 0.3, 0.5 and 1.0 M of NaCl-phosphate buffers and the various fractions collected. The Column was washed with another 10 CV of starting buffer before loading another sample to prevent Column

blockage and contamination of subsequent sample to be purified. The collected fractions were analysed for absorbance using a spectrophotometer (Varian Carey 50 probe UV-visible, Australia) and coagulation activity of the purified proteins using a standard jar tester (Phipps and Bird, 7790-900B USA).

Sludge volume was measured in order to determine sludge volume production when Hibiscus seed extracts were used in water treatment. After the jar test experiment, the contents of the beaker with the minimum residual turbidity (optimum coagulant dose) were carefully decanted. 50 ml of the settled sludge was carefully transferred into a syringe and allowed to settle further for one hour. The volume occupied by the sludge was subsequently determined for each sample and the results compared with the volume of sludge produced after treating the same water sample with alum.

4.7.4 Coagulation assay using small sample volume

The coagulation assay was conducted because optimum coagulant dose is widely estimated with a jar test apparatus using between 1 and 6 L beakers. However, large sample volumes are required to optimize the process which may not be convenient if many different samples are to be tested. This process is rapid since it eliminates the preparation of a large sample volume and sample can be screen easily and quickly against the use of a jar tester. The coagulation compound in the purified Hibiscus seeds was assessed using a 2.5 ml cuvette in a spectrophotometer (Varian Cary 50 probe UV-visible, Australia). A 2.40 ml synthetic kaolin water sample was injected in a 2.5 ml SM plastic cuvette UV grade with 0.1 ml of the purified sample to make 2.5 ml mixture. The content was shaken and allowed to stand undisturbed for 45 minutes and then sample absorbance was measured at 600 nm using the spectrophotometer before and

after the test. The difference between the initial and the final absorbance measurements gave an indication of whether active coagulation compounds are present in the protein sample.

4.8 Preparation of aluminium sulphate as a coagulant

Aluminium sulphate as a coagulant was prepared and used in all the experiments as a control coagulant and also as primary coagulant where the extracts are used as coagulant aids.

Two g of AS was prepared by dissolving 2% w/v in 100 ml of DI water. The suspension was mixed thoroughly using a magnetic stirrer (Stuart scientific, UK) for 10 minutes to obtain the coagulant. The AS was applied in preliminary jar test experiments to determine the optimum coagulant dose required for subsequent tests. The optimum dose obtained here was used in the floc strength test in the study.

4.9 Collection and preparation of water samples

Two different water samples were used in the experimental runs; these are river and synthetic waters. The reason for using river and synthetic water samples is to give a better understanding of the performance of the extracts because river water may contain numerous organic and inorganic compounds which might affect the coagulation behaviour of natural extracts.

4.9.1 River water samples from Bourn Brook

All the river water used in this research study was collected from Bourn Brook canal adjacent to the University of Birmingham train station. Water samples were collected in a set of one-litre (1 L) plastic containers and allowed to settle naturally before

conducting any test. Water samples were kept in a refrigerator at 4 °C before the tests. Prior to any test, water samples were filtered through a 0.45 µm Millipore cellulose membrane filter using a vacuum pump whereupon the filtered samples were subjected to DOC and fluorescence spectroscopy analysis using excitation-emission matrices (EEMs). Similarly, water samples used for zeta potential measurements and the determination of the effect of bacterial inactivation potential of the seed extracts were collected from the same location.

4.9.2 Preparation of synthetic turbid water

Synthetic water used in this study was prepared with China clay (Kaolin Fluka, Sigma-Aldrich, Germany) with a refractive index of 1.555 and absorption index of 0.1.

Turbid water samples for the jar test experiments were prepared by adding kaolin particles to tap water. 40 grams of laboratory grade kaolin (Fluka and high grade, Sigma-Aldrich) was added to 400 ml of tap water and the suspension stirred for 30 minutes using a magnetic stirrer (Stuart Scientific, UK). The suspension was made up to 1L by adding 600 ml of tap water and then stirred for further 30 minutes. The suspension was then allowed to stand for 24 hours for the kaolin to hydrate. The suspension was then vigorously mixed for five minutes and the contents mixed with 30 litres of tap water and allowed to stand undisturbed overnight for particle settlement. The supernatant was decanted and its turbidity measured. Depending on the level of turbidity required, the supernatant was either diluted with tap water or concentrated with kaolin suspension. However, it is important to acknowledge the effect of residual chlorine in tap water because one of the main challenges of using natural extract in water treatment is regarding NOM addition in treated water (Ndabigengesere and

Narasiah, 1998a, Okuda et al., 2001b). It is established that NOM in water can react with disinfectant chemicals such as chlorine to produce DBPs such as trihalomethanes (THMs) and haloacetic acid (HAA) (Liu et al., 2014). DBPs in drinking water could be of great challenge to water quality and are carcinogenic (Parsons et al., 2004). Hence the use of tap water to produce synthetic turbid water where natural extract is used should be noted for possible DBP formation. Turbidity and pH were determined as initial values using standard methods before and after conducting the jar test experiments.

4.10 Running the coagulation and flocculation test

4.10.1 Jar tests

Jar tests were conducted using a standard apparatus comprising 6, 1L beakers (Phipps and Bird, 7790-900B USA) to evaluate the optimum coagulant dose for the coagulation tests. For effective dispersion of the coagulant, the water was rapidly mixed at 200 rpm for 1 min during which various doses of the coagulant were added to the beakers. The mixing speed was reduced to 30 rpm for a further 30 minutes to simulate the flocculation stage. The suspension was then allowed to stand undisturbed to facilitate settlement for 1 hr. A final 10 ml treated water sample was drawn 2cm from the top surface of the water in the beakers using a syringe and the turbidity and pH of the water were then measured using a turbidity meter (HI 93703, Hanna) a pH meter (Mettler Toledo SevenGO, Switzerland). All experiments were conducted at room temperature ($19\pm2^{o}C$) for a minimum of three repeated tests. Turbidity measurements were conducted with ±0.3 NTU accuracy throughout the whole measurement range.

The percentage removal of turbidity of the water was calculated from equation 4.1 below:

$$= \frac{\text{initial water turbidity} - \text{final residual turbidity}}{\text{initial water turbidity}} \times 100 \qquad \text{Eq. 4.1}$$

The buffer capacity (BC) of the proteins during coagulation was calculated according to Morr et al. (1973) from equation 4.3:

$$\text{BC} = \text{titrant} / \text{wt of protein (g)} \times \Delta\text{pH} \qquad \text{Eq. 4.3}$$

where,

The titrant is the protein used in the treatment while ΔpH is the change in water pH before and after treatment.

4.11 Antimicrobial experiment using Colilert -18/Quanti-Tray method

River water from Bourn Brook River was used in the study to conduct antimicrobial effects of the extracts and the purified protein samples. The experiment was conducted using the Colilert-18/Quanti-Tray (IDEX Inc., UK) for total coliform, faecal count and E-coli detection because of its ease of operation, flexibility, accuracy and speed. After the jar test experiments, Colilert 18 powder was added to both the raw and the treated water samples in a 100 ml sterilized vessel. The mixture was thoroughly shaken to completely dissolve the powder. The content was then poured into a tray, sealed and incubated at 35 °C for 18 hours to check for coliform and E-coli counts and 44.5$\pm$ 0.2 °C for 18 hours for the assessment of faecal count. After the incubation period, samples were taken for the positive wells count. A yellow colouration was an indication of the presence of coliform. To observe for the presence of either the E-coli or faecal count,

the tray was transferred to a Longwave Ultraviolet 365 nm lamp spectrolite 160 (Spectronics Cooperation, USA). The number of positive wells that fluoresces was counted. Fluorescence well indicates the presence of either E-coli or faecal coliform, this was differentiated based on the temperature used during the incubation period. The number of all the small and large positive wells was then read from the most probable number (MPN) comparative chart provided by the manufacturer, and the results presented as MPN/100ml. To determine the re-growth of bacteria after treatment, final treated water was tested for bacterial re-growth after 72 hours in terms of coliform count, E-coli, and faecal coliform to assess total antimicrobial inactivation potential of the extracts in water treatment. The test was repeated on the treated water which was kept in a disinfected environment to investigate bacterial regrowth and the effect of residual extracts and protein over the 72 hour period.

The removal ability of the treatment process in terms of microorganisms number reduction was calculated from the Log removal values (LRV), using equation 4.4:

$$\text{LRV} = \log_{10} \frac{\textbf{Initial pathogen in raw water}}{\textbf{Final pathogen in treated water}} \qquad \text{Eq. 4.4}$$

The LRV is an established standard used to assess the efficiency of pathogen removal of a treatment process in water and wastewater treatment plants (Water, 2014). The result obtained was compared with the one obtained from calculated values.

The 4−Log bacterial treatment was adopted as:

1−Log removal equal to 9 out of 10 = 90% reduction

2−Log removal equal to 99 out of 100 = 99% reduction

3−Log removal equal to 999 out of 1,000 = 99.9% reduction

4−Log removal equal to 9,999 out of 10,000 = 99.99% reduction

4.12 Fluorescence excitation-emission (EEMs)

Fluorescence spectroscopy investigation was conducted on a raw water sample before and after treatment with the seed extracts samples. Fluorescence analysis is reported in many studies aimed at the characterization of natural organic matter in water (Baker and Inverarity, 2004, Bieroza et al., 2009, Sanchez et al., 2013). Fluorescence-EEMs were produced in this study following Bieroza et al. (2009) using a Varian Cary Eclipse spectrofluorometer at detector scanning wavelength ranges from 200-400 nm (excitation wavelength) and 280-500 nm (emission wavelength), at increments of 5 nm and 2 nm for excitation and emission respectively, with slits width of 5 nm. Instrument stability was checked by recording the Raman values (excitation wavelength 348 nm, emission wavelength 395 nm) before each set of measurements. The Raman value was compared with the most recent measurement on the instrument. Water samples were stored in 1 litre plastic containers at 4°C in a refrigerator. Before conducting any test, samples were filtered using a 0.45 µm membrane filter and the filtrate kept at room temperature to bring the sample to the required instrument temperature of 20 °C. After each test, the cuvette was rinsed thoroughly ten times with de-ionised water and rinsed again with the next sample to be measured at least twice to avoid contamination.

4.13 DOC measurement

DOC measurement was conducted following Bieroza et al. (2009), using a Shimadzu (TOC-V-CSH TOC analyser with auto-sampler TOC-ASI-V), where the study adopted the non-purgeable organic carbon (NPOC) method of DOC determination. Prior to

combustion, water samples were sparged with 2 M hydrochloric acid to eliminate inorganic carbon. The mean of three NPOC results was computed, analysed and the typical error being < 10%. The instrument ran three measurements for each sample and its mean value computed. Laboratory results were recorded and analysed for both crude extracts and the purified protein samples. All experimental measurements were conducted at room temperature (19 ± 2ºC).

4.14 Growth, breakage and re-growth of purified and crude Hibiscus proteins

The evaluation of floc growth, breakage and re-growth was monitored as the coagulation process proceeded with the crude and purified proteins using a laser diffraction instrument (Mastersizer 2000, Malvern, UK). The instrument is capable of measuring particle diameter in the range of between 0.02 and 2000 µm. The method was adopted following similar studies conducted by (Jarvis et al., 2005b, Li et al., 2007, Yu et al., 2012). In the floc strength test reported in this work, the rapid mix was conducted at 200 rpm for 1.5 min, while the flocculation stage was run at 30 rpm for 25 minutes. Water samples for the measurement were prepared by taking 0.3 ml of the kaolin stock solution and mixing with 1 L of tap water to produce the required turbidity of 46 ± 0.3 NTU in each test.

To assess floc re-growth, flocs were broken by re-introducing a rapid shear mix at 200 rpm for 1.5 min using the jar tester (Phipps and Bird, 7790-900B, USA). The rotor speed was then reduced to 30 rpm for further 25 min to determine the floc re-growth ability of the various coagulants. To compare the flocculating capacity of the seed extracts and the purified proteins as coagulant aids, a predetermined seed extract dose was added 45s to the end of the coagulation test with AS, as a primary coagulant.

Figure 4.5 Mastersizer2000 instrument connected to a Jar test.

The Mastersizer was connected to a jar test apparatus and the liquid suspension was monitored by continuously drawing water through the optical unit of the Mastersizer as shown in Figure 4.5 and returning to the jar tester. Pumping was via a peristaltic pump (Watson-Marlow, 323S, USA) positioned on the return tube with 4.8 mm internal diameter peristaltic pump tubing. The inflow and the outflow were located 10 mm above the blade of the jar tester and opposite each other. Measurements were taken every 35s for the duration of the experiment, and the automated results automatically logged onto a computer. The flow rate was optimised to avoid floc breakage by conducting several experiment runs. It was observed during the preliminary tests that when the flow rate was high, floc size was smaller indicating the breaking of flocs while at slow flow rate, flocs settles in the tube, thus, affecting floc measurement results. The coagulant dosage was the dose obtained from the preliminary jar test results. 5 mg/l of AS was used as primary coagulant with 50 mg/l of the extracts used as either primary

coagulant or as coagulant aids. Similarly, the purified protein dosage adopted in the study was between 0.1 and 0.6 ml/l as primary coagulant and as coagulant aids.

4.15 Floc strength and floc recovery factors

To understand the properties of the coagulated flocs, it is important to consider the floc strength and floc recovery ability to its original size after exposure to high shear force. Floc strength, however, reveals the resistance of the floc against stress and can be assigned a strength factor. A high strength factor value is an indication that the floc is resistant to breakage. Similarly, the recovery factor reveals the ability of the floc to re-grow to its original size after breakage. Here, a high floc recovery factor is regarded as good in terms of ability of the floc to re-grow to its original size after breakage due to the introduction of high shear rate. Floc strength and recovery factors were calculated using Equations 4.4 and 4.5 following previous studies by (Sun et al., 2011, Xiao et al., 2011, Yu et al., 2014, Zhao et al., 2015a):

$$S_f = \frac{d2}{d1} \times 100 \qquad \text{Eq. 4.5}$$

$$R_f = \frac{d3 - d2}{d1 - d2} \times 100 \qquad \text{Eq. 4.6}$$

Where d_1 is the average median floc size established at the steady phase before breakage, d_2 is the median floc size achieved after it was subjected to high shear rate. The average median size, d_3 is the average median floc size recovered at the final steady phase after floc breakage.

Growth rate was measured using the equation 4.5 below following (Zhao et al., 2013b):

$$G.R = \frac{\Delta size}{\Delta time} \qquad \text{Eq. 4.7}$$

4.16 Zeta potential measurement

Zeta potential measurements were performed to determine the surface charge potential of the seed extracts samples and that of the colloidal particles. Zeta potential is one of the crucial parameters that affect and determine the stability of a suspension and can be used as an indicator of coagulation performance.

In this study, the zeta potential was measured using Zetasizer Nano ZSP (Malvern instrument, UK). Instrument temperature was kept constant at 25°C throughout the test runs. The instrument was allowed to equilibrate for 120s before optimising the cell optics and the driver voltage. An average of 10 measurements was made for each sample. The measured data was automatically logged onto a computer and the zeta potential measurement recorded. A mean value of 7 measurements was calculated using the Zetasizer software. The sample cuvette was rinsed ten times with DI water and then finally rinsed with the next sample to be measured. The zeta potential measurement was undertaken on the raw water sample, and also on the treated water samples after 1hr sedimentation in a series of jar test experiments to determine the coagulation mechanism and performance of the samples. Measurements were conducted with ±2% accuracy over the entire measurement cycle.

CHAPTER 5 COAGULATION POTENTIAL OF HIBISCUS EXTRACTS

5.1 Background

Access to clean water has been a major challenge to many developing countries around the world. This enormous problem has brought about several innovative works to screen out naturally-occurring extracts of either plant or animal origin in water treatment. Nowadays, there has been increased interest in the study of natural extracts as a coagulant. Nevertheless, only a few of such plants have so far been screened for their coagulation activity, to date. MO seed, fava beans, *Cactus Opuntia* pad, Mustard seed and several other natural products have been studied as potential water treatment alternatives to the synthetic coagulants (Jahn, 1986, Zhang et al., 2006, Kuki et al., 2015). Chitosan, a product obtained from chitin (of animal origin) has also been tested in water treatment in this regard (Kawamura, 1991). Preliminary investigation of some of these natural extracts has so far provided encouraging results for people in low-income countries. However, most of these studies were concerned with the removal of turbidity in water because of its importance in assessing the overall water quality.

Turbidity is a parameter that affects the physical appearance of water. Its presence in water is capable of providing cover for bacteria against disinfection chemicals. Some bacteria in water can react and consume the disinfectant chemical such as chlorine thereby reducing the available residual chlorine in the distribution system. Thus, it is a requirement in drinking water treatment that turbidity should be removed to prevent the growth and re-growth of pathogens in the distribution networks. Earlier work has reported that one of the main causes for many waterborne disease outbreaks is related to

turbidity problem (Mac Kenzie et al., 1994), as in the case of 1993 Milwaukee cryptosporidium infection, which remains the biggest epidemic in the United States.

Thus, the work reported in this chapter focuses on objectives (1) and (2):

- **To investigate the potential of using Hibiscus plant seed species as coagulants.**
- **To develop a simple method of improving the efficiency of the extracts and to compare the results obtained after purification using IEX chromatography.**

  The work reported in this chapter has been published in Journal of water and health and Vatten Journal of water management and research (see Chapter 1).

5.2 Application of Hibiscus extracts as coagulants

Jar test experiment was conducted to assess the quality of water treated with OCE, SCE and KCE in terms of turbidity removal. Figures 5.1, 5.2 and 5.3 show the performance of the three extracts in treating water with initial turbidity of 200, 100 and 50 NTU respectively. The dosage of each of the three extracts was added as coagulant to synthetic kaolin water with initial turbidity of 200 NTU, as shown in Figure 5. 1. The reason for choosing these levels of turbidity is that the average turbidity in Nigerian river after rainfall event could be as high as 600 NTU, but after settlement, it can go down to between 50 and 200 NTU. The results show that, at the end of the coagulation process, the lowest residual turbidities were achieved with coagulant doses of 60 mg/l of OCE and SCE, and 80 mg/l of KCE, yielding turbidity removal efficiencies of 98, 94 and 90% respectively. Therefore, while higher coagulant dose was required to achieve the minimum residual turbidity with the KCE sample; its turbidity removal efficiency was lower compared with that of OCE and SCE. However, the concentration of protein

in the extracts was 1.018 mg/ml in OCE, 0.918 mg/ml in SCE and 0.631 mg/ml in KCE obtained from protein concentration analysis following (Bradford, 1976).

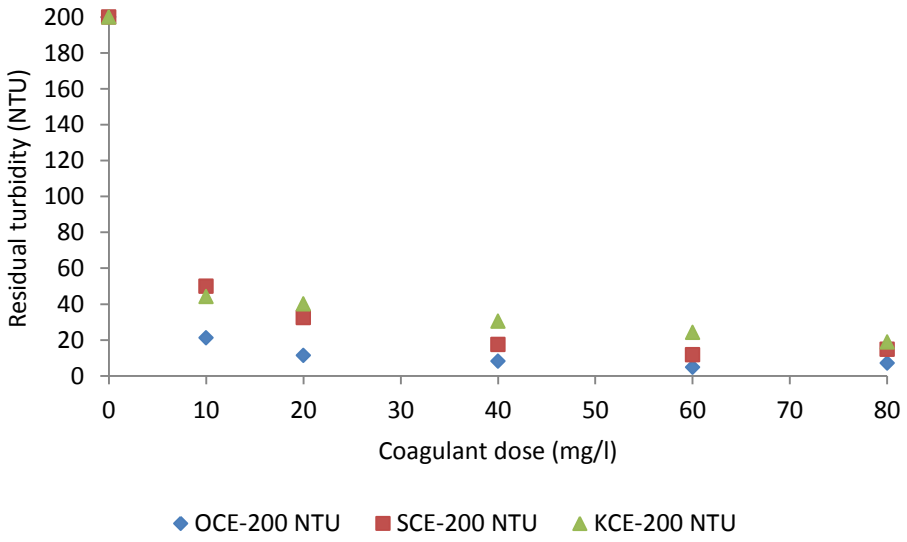

Figure 5.1 Performance of natural coagulants in treating 200 NTU turbidity water.

With these concentrations, the amount of protein used for coagulation to achieve these performances was 6.108 mg with OCE, 5.508 mg with SCE and 5.048 mg with KCE respectively. Additionally, it was observed that at coagulant dose higher than the optimum value, residual turbidity began to increase mainly due to re-stabilisation of the suspended particles in the water.

The results of the coagulation experiments using OK, SB and KE seeds extracted with 1.0 M NaCl concentration are presented in Figure 5.2. Water with initial turbidity of 100 NTU was used in this experiment. The results show that maximum turbidity removal performance of approximately 91% was achieved with 40 mg/l of OCE while 60 mg/l dose was the optimum coagulant dose required for SCE and KCE to achieve the maximum turbidity removal of approximately 88 and 79% respectively. However, there was a notable decrease of protein concentrations used for coagulation due to a reduction

in turbidity of the water. The amount of proteins used in the test was 4.072 mg in OCE, and 3.786 mg in KCE, which correlates with the reduction in water turbidity. The decrease in particle concentration, however, did not yield any change in protein concentration requirement for effective coagulation with SCE, which used the same, 5.508 mg of proteins. The results also show a decline in coagulation performance across all the extracts due to reduced turbidity.

An interesting relationship was found between coagulant dose demand and particle concentration, where a decrease in turbidity from 200 to 100 NTU, causes a reduction in coagulant dose demand. Katayon et al. (2004) compared the performance of MO seed extract in treating low and high turbidity water and reported poor turbidity removal in the low turbid water.

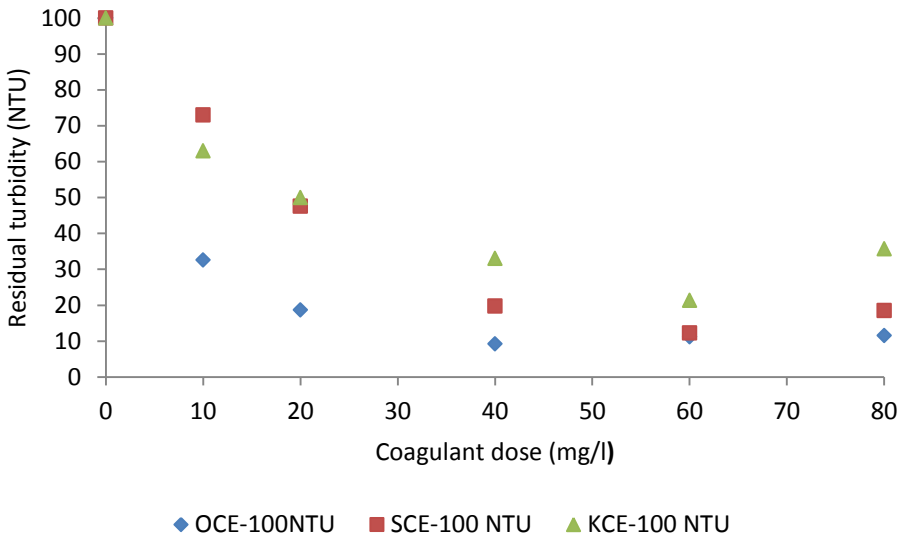

Figure 5.2 Performance of NATURAL coagulants in treating 100 NTU turbidity water.

Figure 5.3 shows jar test results for investigating the performance of OCE, SCE and KCE in water with initial turbidity of 50 NTU. When the turbidity of the water was decreased further from 200 to 50 NTU, optimum coagulant dose demand also

decreased, indicating the dependence of coagulant dosing on particle concentration for efficient turbidity removal. Earlier studies have reported that, for effective flocculation activity to occur, amount of polymer dosed should be proportionate to the particle concentration in order to make the collision efficiency α = 1 (Birkner and Morgan, 1968, Gregory and Barany, 2011, Gregory, 2005).

In the work reported here, a 40 mg/l dose of both OCE and SCE was used to achieve a final residual turbidity of less than 7 NTU, while a 60 mg/l dose of KCE caused a reduction in turbidity from 50 to 13.75 NTU. Turbidity removal performance was observed to follow the order $OCE\ (86\%) > SCE\ (85\%) > KCE(73\%)$. To achieve this performance by the extracts, the amount of protein used for coagulation was 4.072 mg in OCE and 3.786 mg in KCE while it declined to 3.672 mg in SCE compared with 5.508 mg used in the medium turbidity water.

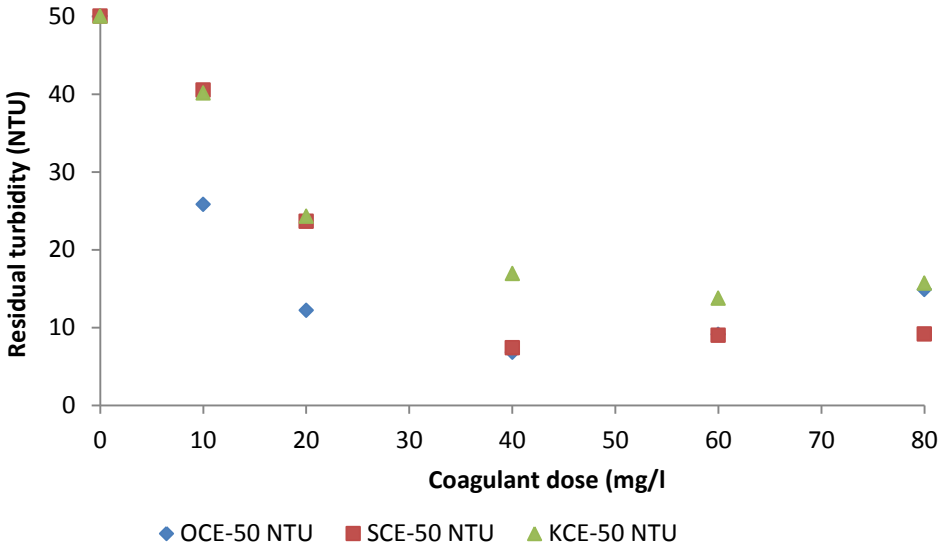

Figure 5.3 The performance of OCE, SCE and KCE as coagulants in 50 NTU turbidity water.

Similarly, the poor performance by the extracts was likely caused by the reduction in turbidity of the water to 50 NTU. An attempt to increase the coagulant dose beyond the

optimum limit did not enhance turbidity removal efficiency, rather the turbidity of the water increased considerably. Naturally-occurring extracts such as MO have been shown to exhibit poor coagulation performance in low turbidity water (Ndabigengesere and Narasiah, 1998a).

The statistical significance of coagulant dose demand due to increased water turbidity reveals that the optimum dose that achieved the lowest residual turbidity were significantly ($p<0.05$) dependent on the initial turbidity. An increase in water turbidity from 50 to 200 NTU caused an increase in coagulant dose, with $r^2 = 0.8929$ in OCE and KCE, and $r^2 = 0.9643$ in SCE. The optimum coagulant doses required to achieve successful turbidity removal with OCE and SCE were 40 and 60 mg/l for (50 and 100), and 200 NTU, corresponding to 4.072 and 6.108 mg, and 3.672 and 5.508 mg in SCE protein while KCE required between 60 and 80 mg/l doses corresponding to 3.786 and 5.084 mg respectively. Katayon et al. (2006) found the dependence of MO coagulant dose on the level of initial turbidity to be significant with an r^2 value equal to 0.985. As seen in this study, any further addition of coagulant dose above the optimum dosages did not result in improved efficiency, rather, the final residual turbidity significantly ($p<0.05$) increased mainly due to overdosing.

5.2.1 Effect of coagulant dose on pH and optimum coagulation pH

For decades, traditional inorganic coagulants have been used in water treatment to remove turbidity in raw water. Chemical coagulants such as AS [$Al_2(SO_4)_3.14H_2O$] and ferric sulphate [$Fe_2(SO_4)_3$] are employed within certain pH range for optimum performance, but their addition to colloids in water can affect the treated water pH (Kawamura, 1991). Several studies have reported that the application of natural extracts

in water treatment does not affect the pH of the water (Ndabigengesere et al., 1995, Ndabigengesere and Narasiah, 1998a, Diaz et al., 1999, Zhang et al., 2006) and that there is no standard operational optimum pH (Ndabigengesere and Narasiah, 1996), known to date.

Figure 5.4 presents the effect of coagulant addition on treated water pH during the jar tests experiments using AS, MO and OCE, SCE and KCE by incrementally dosing 10−100 mg/l of each natural coagulant and AS to water with an initial turbidity of 200 NTU. Figure 5.4 shows that the treated water pH remained largely unaffected when the natural coagulants of OCE, SCE and KCE were used. Similarly, MO extract did not alter or suppress the pH of the water as a coagulant. However, AS dosing to 40 mg/l produced an approximately linear reduction in pH from an initial value of pH 7.36 to pH 6 with 10 mg/l dose, then to pH 4.7 with 40 mg/l. It was followed by a reduced rate of pH change (pH 5 to less than pH 4.3 when coagulant dosed from 40 to 100 mg/l). Furthermore, the result shows that after the treatment process, the raw water pH was significantly ($p < 0.05$) altered with alum as a coagulant.

The study observed a buffering effect which was brought about by the reaction between the amino groups (NH_2) and the carboxylic group – COOH – in the seed proteins. The buffer capacity (BC) was estimated to be 0.017 with MO, 0.016 with OCE, 0.029 in SCE while it was 0.011 in KCE sample. The small change in final pH was also likely caused by the balancing of hydrogen ion in the seeds and the raw water hydroxide ion (Katayon et al., 2004). While some previous studies have shown the most efficient coagulation pH using natural extracts to be above the neutral pH (Okuda et al., 2001b, Zhang et al., 2006), the study reported here investigated the performance of OCE, SCE and KCE on different pH ranges.

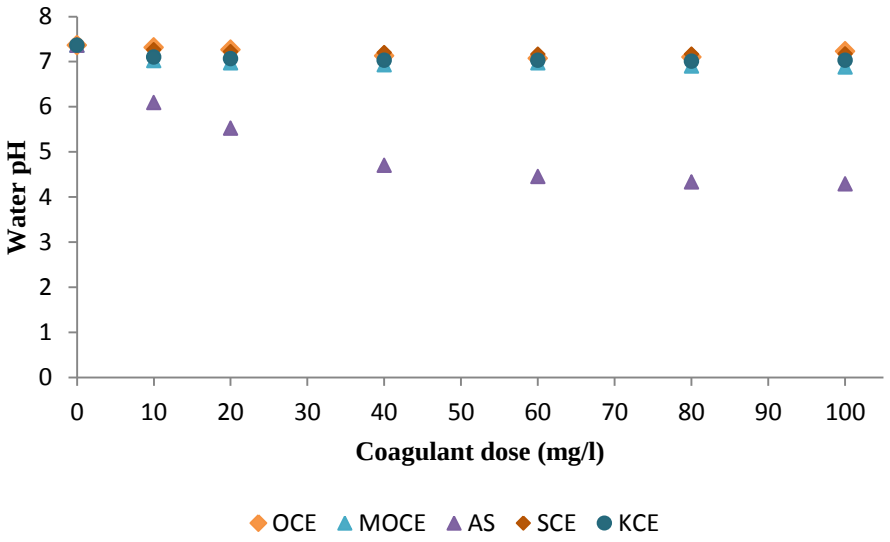

Figure 5.4 Effect of different natural coagulants addition and AS on water pH using 200 NTU.

Different pH values were employed in order to determine the optimum coagulation pH because of its importance in water treatment and also as it affects the stability of protein. Figure 5.5 shows the evaluation of optimum coagulant dosages that achieved the maximum turbidity removal in the coagulation process using the various extracts on 200 NTU water. Specifically, a coagulant of 60 mg/l of (OCE and SCE) and 80 mg/l of KCE were used on different pH range (4−9) in 200 NTU water and their efficiencies evaluated. All the extracts achieved minimum residual turbidity at pH 4 (acidic level) and the maximum residual turbidity was found at pH 9 (alkaline level) for all the samples. The effect of pH adjustment in removing turbidity in water using extracts was seen to be significant ($p < 0.05$) at lower pH than alkaline pH levels. Turbidity removal efficiencies were 99% for OCE and 98% for (SCE and KCE respectively) at pH 4. However, it is not practically possible to treat water at such a low pH value because drinking water guideline stipulates pH of between 6.5 and 7.5 otherwise an additive must be introduced to bring the pH to acceptable level. All the extracts significantly (p

< 0.05) achieved turbidity removal above 94% at pH 6. Furthermore, OCE was found to achieve up to 95% efficiency at neutral pH. Hence, this pH range (6.5 to 7) is within the acceptable limit for drinking water treatment. It is noteworthy that the treatment efficiency was reduced to 80, 65 and 56% in OCE, SCE and KCE respectively at pH 9. The percentage reduction in performance was also noted to be significant ($p < 0.05$), with high reduced rate observed in KCE, followed by SCE and then OCE was found to be the least affected in this regard. The lowest reduced rate was recorded in OCE, which declined from 99 to 80% while the deterioration rate was most pronounced in SCE and KCE samples, from 98% in both cases to 65 and 56% respectively.

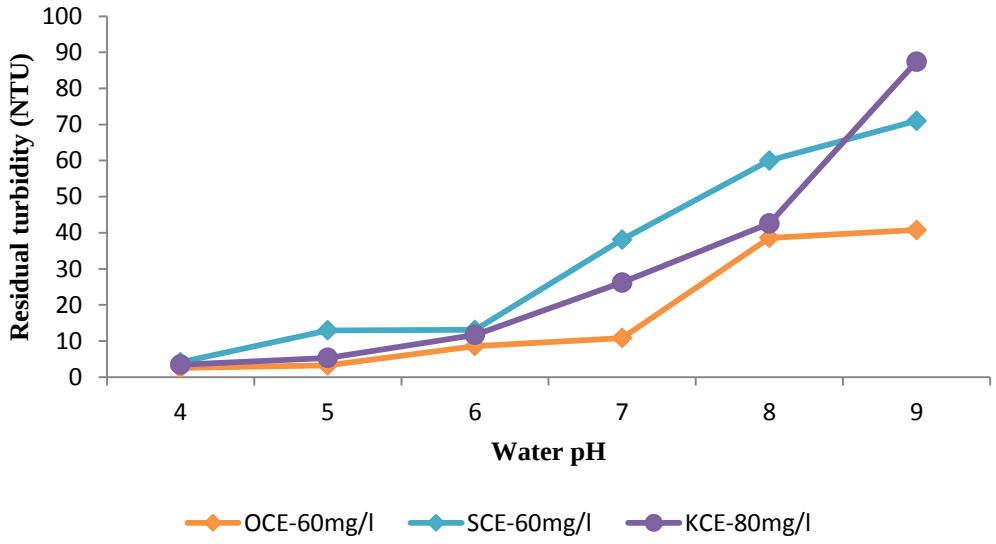

Figure 5.5 Performance of natural coagulants on different pH values on 200 NTU water.

5.2.2 Effect of temperature variation on Hibiscus extracts stock solution

Protein is said to become denatured when its folding structure is altered as a result of exposure to certain elements of physical factors (e.g. heat), causing the protein to become biologically inactive or its overall primary activity affected. Proteins can also degrade and denature upon storage, with such denaturation leading to visible

aggregation and turbidity formation (Sharma and Luthra-Guptasarma, 2009). In some instances, proteins can be re-natured but in most cases the denaturation is irreversible. The work reported in this thesis evaluated the effect of denatured OCE, SCE and KCE against the native state as alternative coagulants. Their performances were assessed after heat treatment to some specified temperature ranges.

Tables 5.1, 5.2 and 5.3 show the performance evaluation of the heated extracts to 60, 97 and 140 °C temperatures for 6, 4 and 2 hours respectively. The wide temperature ranges and time was done in order to understand the relationship between the two parameters because in many developing countries, the main source of energy is firewood which is difficult to control. The observation is based on high temperature with short heating duration and low temperature with longer duration because Physical factors such as extreme temperature condition can denature proteins. Therefore, a wide temperature range was selected with regards to people in developing countries. Table 5.1 shows the effect of heat treatment on a stock solution of OCE used in treating synthetic water with 200 NTU. The results showed an improvement in treatment efficiency by the heated extract compared with the non-treated sample. Highest turbidity removal efficiency of greater than 97% was achieved with 80 mg/l dose while the non-treated extract only achieved 93% performance. It is noteworthy that the optimum coagulant dose used was 80 mg/l in all cases, with both treated and non-treated extracts. At 10 mg/l coagulant dose, the performance of non-treated OCE was approximately 80% exceeding the maximum performance of 75% recorded by the 60 °C heated sample and 67% was achieved in both stock solutions heated to 97 and 140 °C respectively. Additionally, a modest improvement in treatment efficiency was observed across all the treated samples when coagulant doses were increased. However, the performance of the non-treated

sample deteriorated at 20 mg/l but improved upon the addition of more coagulant dose into the treatment process. The observed trend in performance by the non-treated sample at 20 mg/l was not clear, although a better understanding of the process would have been achieved if protein sequence analysis was conducted. However, this is one of the difficulties arising from polymer study, requiring thorough protein analysis which is beyond the scope of this study. Regardless of the nature of the sample used in the coagulation process, at 100 mg/l dose and above, the deterioration in water quality was witnessed across OCE samples but was most pronounced at 100 mg/l dose due to re-stabilisation caused by overdosing. Furthermore, the 60 °C heated extract outperformed those heated to 97 and 140 °C temperatures respectively. On the other hand, OCE heated to 140 °C for 2 hours was found to be the most effective coagulant at 80 mg/l, and this was the optimum dose in all the samples.

Table 5.1 Effect of heat treatment on the performance of OCE on 200 NTU turbidity removal.

Coagulant Dose (mg/l)	ΔT=0 % Reduction	ΔT=60^{o}C (6 hrs) % Reduction	ΔT=97^{o}C (4 hrs) % Reduction	ΔT=140^{o}C (2 hrs) % Reduction
10	79.8	74.5	67.0	66.5
20	74.5	91.1	82.5	84.1
40	88.8	96.4	94.0	94.2
60	90.8	96.5	96.0	96.5
80	93.3	97.8	97.8	97.9
100	82.8	96.8	96.7	96.8

Table 5.2 presents the performance of SCE, which was heated to a range of temperatures. As in OCE, SCE samples were heated to 60, 97 and 140 °C for 6, 4 and 2

hours respectively. The effect of temperature variation on SCE performance was evaluated in treating synthetic water with a turbidity of 200 NTU. The results show that with 10 mg/l dose, a notable reduction in turbidity was found across all the extracts. The non-treated sample achieved approximately 82% turbidity removal while the 60 and 97° C heated samples achieved 90% coagulation efficiency. Similarly, with 10 mg/l dose, the performance of the 140 °C heat-treated sample attained a turbidity removal efficiency of 70%. Further addition of coagulant resulted in increased performance until the optimum dose. The optimum coagulant dose to achieve the lowest residual turbidity was 80 mg/l; the native sample recorded 93% while the samples heated to 97 and 140 °C achieved 96 and 92% turbidity removal respectively. On the other hand, the performance of SCE heated to 60 ºC was 94% with 60 mg/l dose.

Table 5.2 Effect of heat treatment on the performance of SCE on 200 NTU turbidity removals in water.

Coagulant Dose (mg/l)	ΔT=0°C % Reduction	ΔT=60°C (6 hrs) % Reduction	ΔT=97°C (4 hrs) % Reduction	ΔT=140°C (2 hrs) % Reduction
10	81.7	89.5	89.9	69.5
20	83.8	89.9	93.3	78.9
40	91.3	90.9	94.0	84.5
60	91.5	93.7	94.3	88.6
80	92.5	93.0	96.4	92.0
100	90.2	91.0	82.4	88.9

The maximum coagulant dose used in the study was 100 mg/l. With 100 mg/l dose, the performance of all the extracts deteriorated except that of the native sample which

attained 90% compared with 82 and 89% attained with 97 and 140 ºC stock sample respectively.

Laboratory measurements were conducted on the coagulation performance of KCE in a water sample with a turbidity of 200 NTU. Table 5.3 presents the effects of temperature on a stock solution of KCE which was heated to 60, 97 and 140 °C for 6, 4 and 2 hours respectively. Different doses (10 – 100 mg/l) of the extract were applied in the coagulation test. The results revealed that, water sample which had been treated with the heated KCE at 140ºC for 2 hours achieved the highest turbidity reduction of approximately 99% with 60 mg/l dose while the 60 and 97°C treated extracts achieved 98 and 97% performance with a reduced dose of 40 mg/l respectively. At a higher coagulant dose of 80 mg/l, the native sample achieved only 91% performance. The behaviour of KCE was distinctly different from that of OCE and SCE. The performance of KCE after heat treatment was significantly higher because, at 10 mg/l dose, all the samples recorded significant efficiencies of > 91% while the 60 °C treated sample achieved > 95% performance. An increase in dose resulted in increased performance throughout the coagulation process, although, there was reduced performance beyond 60 mg/l across all the heated extracts. However, the overall coagulation performance of the sample was impressive even at starting dose of 10 mg/l.

One notable feature of KCE was that heating to 60 °C improved its performance across all doses. Similarly, KCE heated to 140 °C showed the same trend but its performance deteriorated slightly at 100 mg/l dose, from 98.4 to 98.5%. However, the general performance of denatured KCE deteriorated after the optimum dose of 80 mg/l except for the native sample which only deteriorated at 100 mg/l to achieve 85% efficiency.

Table 5.3 Effect of heat treatment on the performance of KCE on 200 NTU turbidity removals.

Coagulant Dose (mg/l)	ΔT=0°C %Reduction	ΔT=60°C %Reduction	ΔT=97°C %Reduction	ΔT=140°C %Reduction
10	77.7	95.1	91.1	93.5
20	80.0	97.3	95.0	96.2
40	84.8	98.2	96.5	98.1
60	87.9	97.5	95.7	98.7
80	90.5	97.3	95.0	98.4
100	85.1	95.6	93.8	98.5

5.2.3 Effect of storage duration on Hibiscus seed stock solution

One crucial physical factor that can cause protein denaturation is storage time. Several proteins can be denatured and lose their stability within minutes upon storage. In order to understand the impact of protein storage on coagulation activity of seed extract for people in low-income countries, the extracts were stored and their subsequent coagulation potential evaluated.

The effect of storage time on the performance of OCE, SCE and KCE stock solution was investigated as shown in Figures 5.6, 5.7 and 5.8. A stock solution of the extracts was prepared and stored at room temperature of 19 $\pm$ 2 °C. Fresh OCE was prepared and then stored in a 200 ml open beaker at room temperature between 1 and 14 days interval to observe its denaturation process. Performance evaluation was then conducted using 1, 3, 7, 10 and 14 days stored extracts. This is vital in developing countries, where electricity supply is a major challenge, and the cost of obtaining modern, temperature-controlled storage facilities is prohibitive. Figure 5.6 shows the effect of storage time on

the performance of OCE using a varying dosage of the coagulant on water with an initial turbidity of 130 NTU. The results indicate that the performance of OCE as coagulant increased with storage time from day 1(fresh sample) to day 10, after which its effectiveness in respect to turbidity removal, deteriorated.

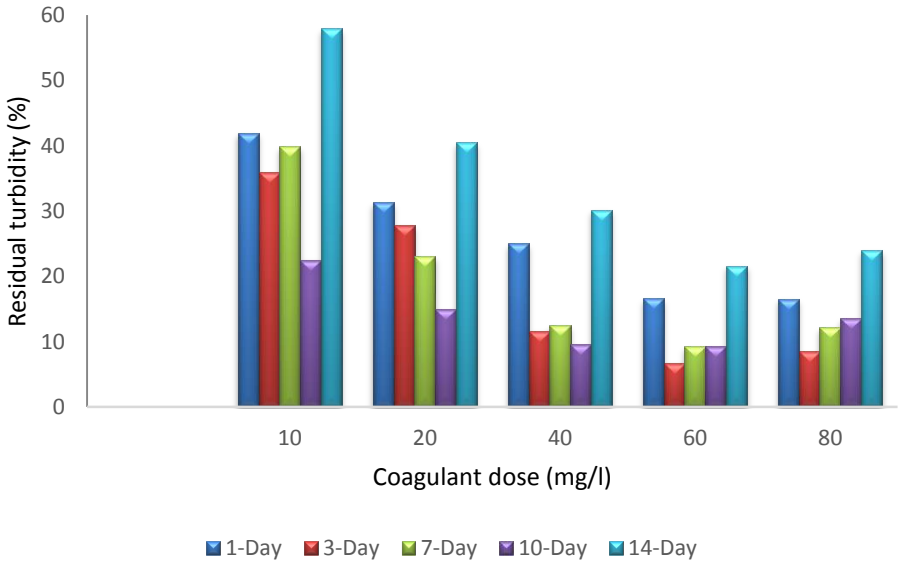

Figure 5.6 Effect of storage time on the performance of OCE in 130 NTU turbidity removal.

Optimium performance was observed with the 3−day stored sample when OCE dosed at 60 mg/l, yielded a reduction of 95% in turbidity. OCE stored for 7 and 10 days achieved a maximum reduction of 93% with the same coagulant dose of 60 mg/l, respectively. However, at lower doses of between 10 and 40 mg/l, the 10 days stored sample yielded performance of between 83 and 93% compared to 72 and 91% of the 3 day sample. Under the same dosage condition, the 7 day sample achieved a performance of 69 and 90% respectively. While fresh extract required 80 mg/l dose, its performance was lower than that of the stored samples, yielding a maximum reduction of 87% from 130 NTU against the 94% recorded with stored extract. It is clear that the act of storing OCE for a period not exceeding 10 days is beneficial in improving its water purification potential,

and does not incur an additional cost which offers a tremendous advantage over the use of the fresh coagulant. A further test was investigated on the turbidity removal potential of SCE, following storage for 1, 3, 7, 10 and 14 days respectively. Figure 5.8 shows that the performance of SCE as coagulant increased with storage duration to day 10, beyond which its efficiency deteriorated. In a comparable fashion to that of OCE, optimum performance was observed with 80 mg/l dose of SCE stored for 3 days. Turbidity reduction was approximately 97%, from 130 NTU to less than 4 NTU. At the end of the 7 and 10 day storage time in both cases, maximum turbidity removal was 89% using a coagulant dose of 60 mg/l from 130 NTU to approximately 14 NTU. However, with the 14-day storage, residual turbidity was 34 NTU at a coagulant dose of 20 mg/l, yielding only 74% removal efficiency.

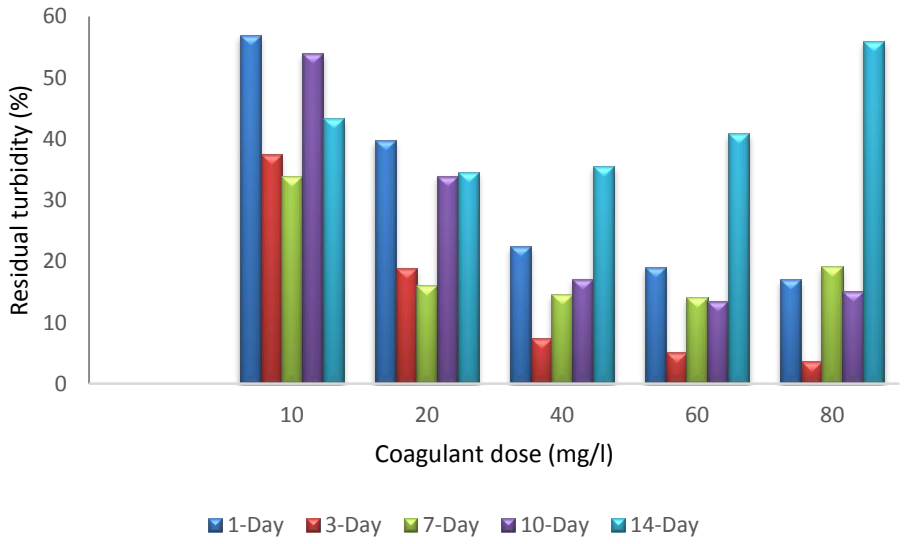

Figure 5.7 Effect of storage time on the performance of SCE in 130 NTU turbidity removal.

Interestingly, at 10 mg/l dose, the 14-day sample outperformed the samples which were stored for 1 and 10 days. Similarly, at 20 mg/l dose, optimum performance of the samples stored for 10 and 14 days was somewhat similar, producing a reduction of

approximately 74%. Furthermore, at 10 and 20 mg/l coagulant doses, the overall performance was better achieved by sample which was stored for 7 days. Fresh samples appear to have exhibited poor performance across the doses adopted in the study against any other sample investigated.

A further investigation was conducted on the performance of KCE in order to identify the most appropriate time for stock solution before any deterioration in quality as a coagulant. Figure 5.8 shows the efficiency of KCE as a coagulant after storing the stock solution for 1, 3, 7, 10 and 14 days. The results revealed that by storing KCE samples for these days did not yield as significant performance as witnessed in both OCE and SCE extracts.

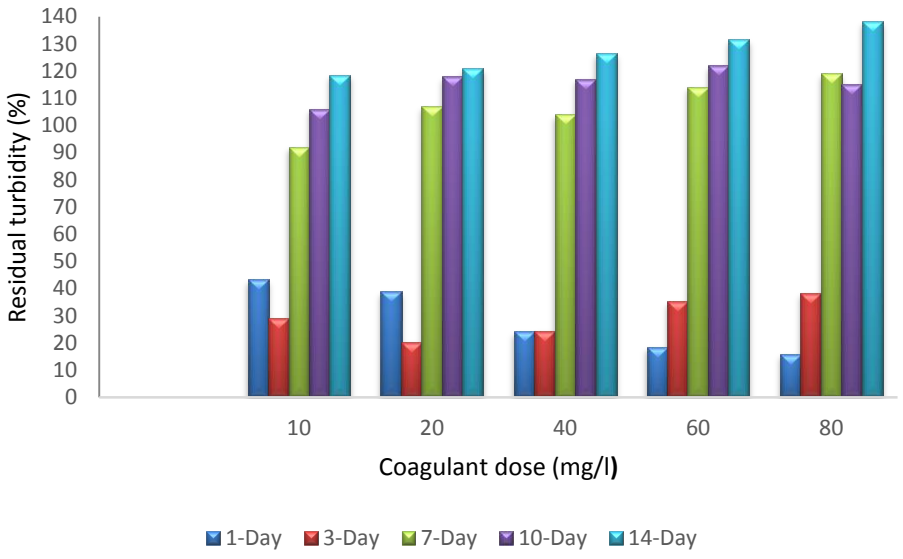

Figure 5.8 Effect of storage duration on the performance of KCE in 130 NTU turbidity removal.

The performance of fresh KCE at 80 mg/l was approximately 88% turbidity removal while KCE, which was stored for 3 days, yielded turbidity reduction of 84.4% with 20 mg/l dose. Additionally, KCE which had been stored for 7, 10 and 14 days yielded no meaningful coagulation performance. With higher doses, residual turbidity in treated

water was found to be more than the initial turbidity, especially with the 14 days stored extract.

However, at 40 mg/l dose, fresh and the 3 day stored extract achieved 81% turbidity removal performance, residual turbidity was less than 25 NTU, from 130 NTU in the water. Similarly, maximum turbidity reduction was observed with a dose of 80 mg/l in water treated with the 10−day than in the 7-day extract. It was clear that storing KCE for a lengthy time did not in any way improve its performance, rather its water treatment potential deteriorated. Although the efficiency of the 3-day stored sample was lower than the fresh extract, it required only half of the fresh sample dose to achieve the 84% removal efficiency. The result further shows that the 3−day sample demonstrated good performance compared with the other extracts across the doses used in the treatment process.

5.3 Coagulation mechanism of extracts

An assessment of the coagulation mechanism of the extracts was conducted on kaolin and river water. Zeta potential measurement was used to evaluate the coagulation mechanisms which depend on the electrostatic forces between charges carried by the colloids in the system. Synthetic kaolin water with initial turbidity of 55 NTU and Bourn brook river water with an initial turbidity of 19 NTU were evaluated in a series of jar test experiments. The low river turbidity was the measured level available during the period of the test, hence the reason for adopting low synthetic water turbidity was because all the extracts showed poor performance on low turbid water and this will give a better understanding of the performance of the extracts.

5.3.1 Coagulation mechanism of the extracts on synthetic water

The results on the effect of surface charge potential and turbidity reduction by OCE, SCE and KCE on a synthetic water with a turbidity of 55 NTU are presented in Figure 5.9. It is noteworthy that all the raw seeds used in the study were stored in a watertight polyethene bags unprocessed at room temperature of 19±2 °C for 24 months to evaluate the effect of aging of the seeds. The results show that the kaolin water has an initial zeta potential of −15.5 mV, indicating that kaolin particles are negatively charged (Katayon et al., 2004, Miller et al., 2008). During the coagulation process, a 20 mg/l dose of OCE was used to achieve a residual turbidity of less than 3.5 NTU, corresponding to 94% removal efficiency while the zeta potential of OCE (O_{zp}) treated water increased from −15.5 to −4.39 mV likely due to adsorption. The significant increase in zeta potential of the water after treatment could be due to the effect of some tap water constituents. In addition, the surface charge potential of OCE was −8.3 mV as shown in Table 5.4. Incidentally, the zeta potential of OCE also increased substantially from −8.3 to −4.39 mV in the clarified water. Earlier work by Ndabigengesere et al. (1995) reported the zeta potential of MO stock solution to be +6 mV and suggested that MO consists of cationic proteins. The amount of protein used for coagulation was 2.036 mg from the 20 mg/l dose, indicating that 17.964 mg of the extract are contaminants compounds in the seed that may affect its coagulation ability. Further coagulant dose addition was also investigated in order to understand the effect of increasing the anionic charge in the treatment process. The addition of more dose from 40 to 100 mg/l resulted in increased turbidity due to re-stabilisation and at the same time, the zeta potential decreased further from −4.39 mV at an optimum dose to −6.6 mV at 40 mg/l, followed by a linear reduction to −8.06 mV at 100 mg/l dose.

The coagulation mechanism of SCE in treating synthetic water was investigated. Optimum performance was achieved with 60 mg/l dose which yielded a reduction of 88% from 55 NTU to less than 7 NTU. At the optimum dose, the zeta potential of SCE (S_{zp}) treated water increased from −15.5 to −6.31 mV. Table 5.4 also shows the surface charge potential of SCE as a coagulant to be −6.4 mV, higher than that of OCE, which was −8.3 mV. Coagulant dose above 60 mg/l resulted in an increase in residual turbidity. For example, at a 100 mg/l dose, the residual turbidity was 10.82 NTU, representing 80% reduction and the zeta potential also decrease from −6.31 mV with 60 mg/l dose to −9.44 mV at 100 mg/l due to charge reversal. The coagulation mechanism presented by SCE was typical of charge neutralisation. The amount of protein used for coagulation was 5.058 mg while the remaining 54.492 mg used in the 60 mg/l dose extract are contaminants.

The application of KCE as a coagulant was also evaluated and its coagulation mechanism determined after treating 55 NTU water. The surface charge potential of KCE was found to be −8.3 mV similar to that of OCE (Table 5.4). Various doses of KCE sample was added to the synthetic water and the residual turbidity decreases continually until the maximum dose of 100 mg/l. Turbidity removal performance was 85%, with 100 mg/l as the optimum dose. However, the work presented here observed that KCE behaved differently to OCE or SCE, and the zeta potential response was quite the opposite of coagulation mechanism of OK and SB seeds. In KCE, residual turbidity decreased continually as coagulant dose increased which resulted in an increase in zeta potential until the maximum dose of 100 mg/l was reached. The zeta potential of KCE (K_{zp}) final water increased from −15.5 to −6.67 mV at the end of the treatment.

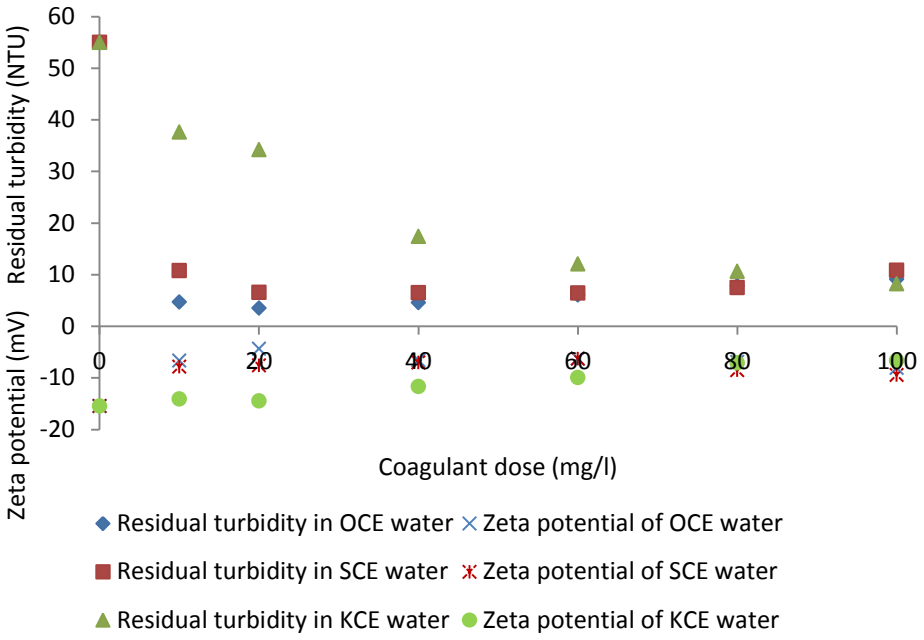

Figure 5.9 Coagulation mechanism of OCE, SCE and KCE in 55 NTU synthetic water.

The degree of charge neutralisation action was different requiring more dose to destabilise the kaolin particles. The amount of protein used for coagulation in the KCE treated water was 6.31 mg from the 100 mg/l dose whereas the remaining 93.69 mg in the extract was contaminants added into the treatment process.

Table 5.4 Surface charge potential of crude, purified and contaminants in Hibiscus seeds.

Samples	OK	SB	KE
Zeta potential of Purified seeds	−10.1	−11.4	−8.1
Zeta potential of Crude seed extracts	−8.3	−6.4	−8.3
Zeta potential of Contaminants	−10.3	−14.1	−5.1

While OCE and SCE required doses of 20 and 60 mg/l respectively, the KCE sample used the maximum dose of 100 mg/l to destabilise the kaolin particles in the raw water to achieve turbidity reduction.

5.3.2 Coagulation mechanism of the extracts in river water

To understand further the coagulation mechanisms of the extracts in water treatment. OCE, SCE and KCE were applied to treat river water with similar range of doses used in treating the synthetic water. Figure 5.10 shows the performance of varying doses of OCE, SCE and KCE in river water with a turbidity of 19 NTU and zeta potential of −12.3 mV.

The results show a maximum turbidity removal of 98% was achieved with 40 mg/l dose of OCE, and the zeta potential remained largely unaffected at −12.6 mV. The addition of more coagulant dose eventually led to overdosing leading to re-stabilisation. The zeta potential in the water marginally increased from −12.3 to −11.7 mV, with 100 mg/l dose. Overall, there was a relative change in zeta potential and the optimum dose did not correspond to the highest zeta potential value in the water due to background effect (zeta potential) from NOM in river water. Under this condition, the coagulation mechanism was by adsorption and bridging action.

Meanwhile, when a 40 mg/l dose of either SCE or KCE was used, turbidity reductions were 96 and 83%, from 19 NTU to less than 0.68 and 3.25 NTU respectively. At the optimum coagulant dose, the zeta potentials value increased marginally in SCE to −11.4 mV while −10.6 mV was found in KCE treated water. Again, the coagulation mechanism is unlikely to be by charge neutralisation, but due to adsorption and bridging action. However, river water treated with these extracts presents two common features; firstly, the optimum coagulant dose that achieved the minimum residual turbidity was 40 mg/l, and secondly, at 20 mg/l dose, the zeta potential in the treated water remained unaltered at −12.4 mV from −12.3 mV across all the samples. This is clearly due to the

background impact of zeta potential from other river water constituents (NOMs) in the system.

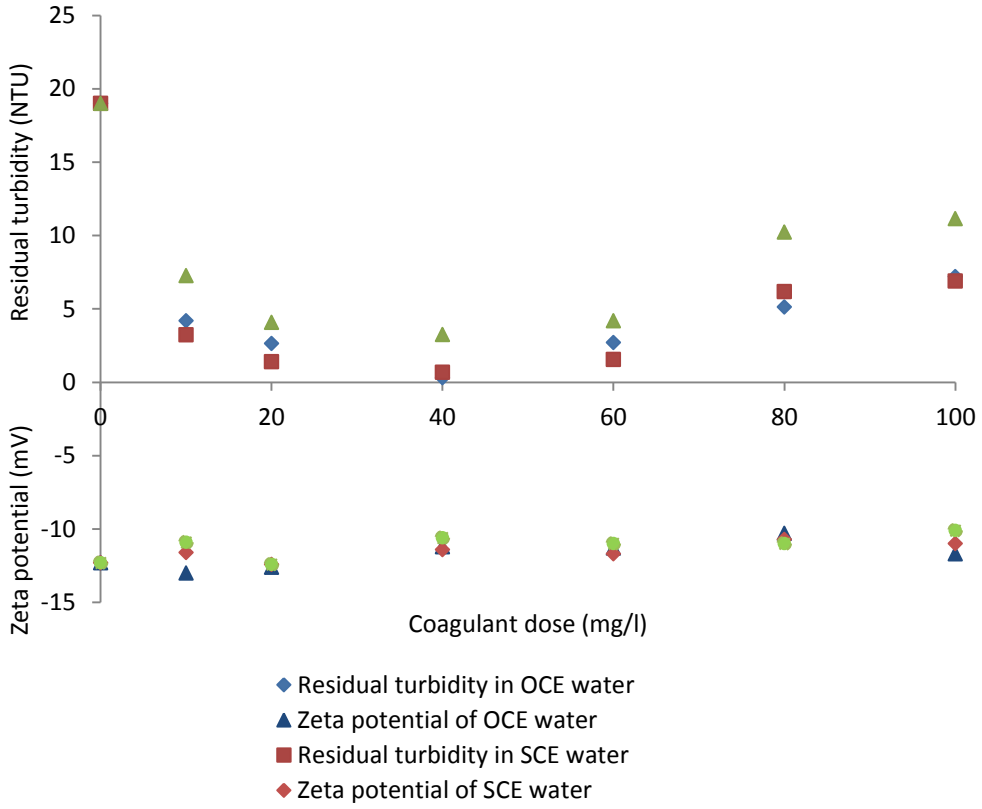

Figure 5.10 Coagulation mechanism of OCE, SCE and KCE in 19 NTU river water.

In addition, re-stabilisation was also observed after the addition of more coagulant dose in the water and the zeta potential increased accordingly. For instance, at 80 mg/l dose, the zeta potential of the final water was between −10.3 mV and −11.0 mV for all the extracts. At 100 mg/l dose, the final zeta potential value was reduced from −10.3 mV to −11.7 mV in OCE treated water. The water sample which had been treated with 100 mg/l of SCE had an overall zeta potential reduction from −10.7 mV to −11.0 mV while that of KCE increases from −11.0 mV to −10.1 mV.

Again, measurement of the zeta potential of the treated water with 60 and 80 mg/l doses reveals a zeta potential of −11.0 mV with KCE as a coagulant. There was no consistency in the coagulation mechanism by the extracts in river water treatment. While the coagulation mechanism by the extracts was not consistent in river water, the overall removal of turbidity was considerably higher in river water than in the synthetic water. However, there were situations where the zeta potential of the treated water increased, but such an increase was not substantial enough to adjudge the coagulation mechanism to be due charge neutralisation action.

5.4 Effect of storage time on raw seeds and coagulation performance

The effect of storage duration of raw seeds on coagulation performance was evaluated in river water with a low turbidity of 19 NTU. The seeds were stored at room temperature of 19 ± 2 °C for 24 months. Moreover, because natural coagulants are poor in coagulating low turbidity water (Muyibi and Okuofu, 1995), it is important that the study investigated their coagulation performance on low turbidity water. Katayon et al. (2006) investigated the effect of different storage conditions of raw MO seed and observed better performance with the fresh extract than in seed which had been stored for one month (Katayon et al., 2006). In this study, coagulants extracted from all the seeds were used in treating synthetic and river water with turbidities of 55 (Figure 5.9) and river water of 19 NTU (Figure 5.10) respectively. Waters with initial turbidity of 50 NTU (Figure 5.3), and 55 NTU (Figure 5.9) with the stored raw seeds were used to assess the performance of the coagulants. The results showed that maximum turbidity reduction of 94% was achieved with the stored raw seed at 20 mg/l dose compared with 86% achieved with the fresh extract. Similarly, a maximum of 88% turbidity reduction was obtained with stored SB seed extract against the 85% turbidity reduction recorded

when the fresh extract was applied. Stored KE seed outperformed the fresh extract as well; it achieved 85% performance against 73% recorded by the fresh sample.

River water with a very low turbidity of 19 NTU was investigated as shown in Figure 5.10. OCE and SCE were found to have caused a substantial reduction in turbidity in the river water. Both extracts yielded turbidity reduction of $>$ 98% from 19 NTU to less than 1 NTU. Similarly, KE extract achieves up to 94% performance using the same dose of 40 mg/l. The overall performance by the extracts was achieved with the stored seed extracts compared with the fresh extracts after harvesting. This results, however, is at variance with that of (Katayon et al., 2006) who observed a better performance with fresh MO seed. In each case, turbidity removal was significantly ($p < 0.05$) higher with stored samples than with the fresh seed extracts. For example, the performance of the extracts in treating river water with very low turbidity of 19 NTU was better than kaolin possibly due to background surface charge on residual DOC, despite the assertions that natural extracts are poor in coagulating low turbidity water.

5.5 SDS-PAGE analysis of the crude and purified proteins

SDS-PAGE analysis was conducted on the extracts in order to obtain information on their compositions as well as to determine the molecular weight (MW) of the different protein fractions. The analysis is the most widely used method of separating protein by electrophoresis to denature the protein using polyacrylamide gel as a support medium. The analysis will give a better understanding of the molecular size of the coagulant protein (s) in the seeds. The various bands and sizes of proteins are depicted in Figures 5.11 and 5.12. Figure 5.11 shows some similar distinct protein bands across all the extracts. Each extract, however, consists of multiple heterogeneous bands, having

varied protein composition and physical characteristics. The various protein bands in OCE lane 1, SCE lane 5 and KCE lane 6 in Figure 5.11 were compared in order to provide information regarding their MW. The results show that the band between 40 and 46 kDa is similar for SCE and KCE. However, the concentration of protein band with MW of 46 kDa was found in all the extracts, including OCE.

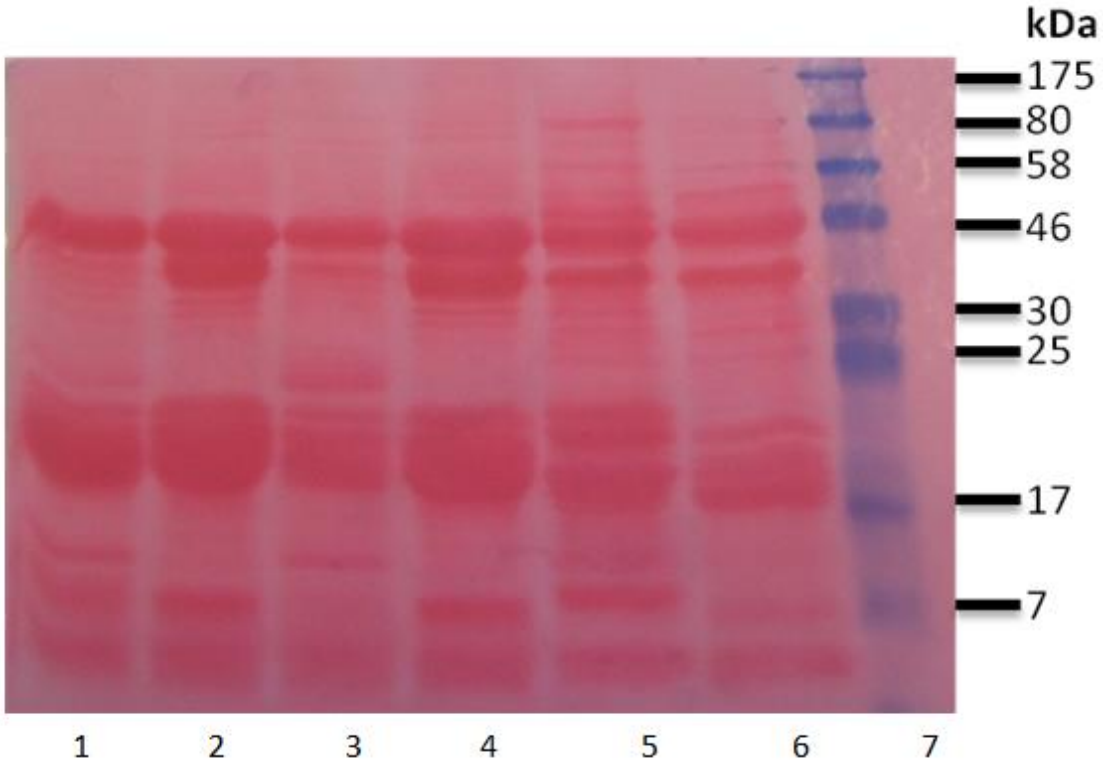

Figure 5.11 SDS-PAGE analysis of OK, SB and KE. lane 1, 2 and 3 OCE @ 120 °C/ 3 hrs, stored 3 days, @ 120 °C/3 hrs and 3 days, lane 5, 6 and 7 are SCE, KCE protein marker (7-175 kDa).

There is also clear evidence of similarity in protein band between 17 and 21 kDa in SCE and KCE, but the densest protein band above 17 kDa was observed in SCE. Interestingly, however, a faint protein band can be discerned even above the 50 kDa distinct band in SCE and KCE, which was not visible in OCE. Most notably, the protein concentration of the band with MW of 17 kDa is more discernible in OCE extract than in the SCE and KCE samples. Similarly, the protein bands above the 7 kDa are faint but

visible in OCE compared to the SCE and KCE samples. Overall, there are clear similarities in protein bands between SCE and KCE compared to the OCE sample. In order understand the MW of the coagulant protein, the purification process is another option adopted that would deepen our understanding of their potential after conducting activity assay on each of the coagulant eluted proteins.

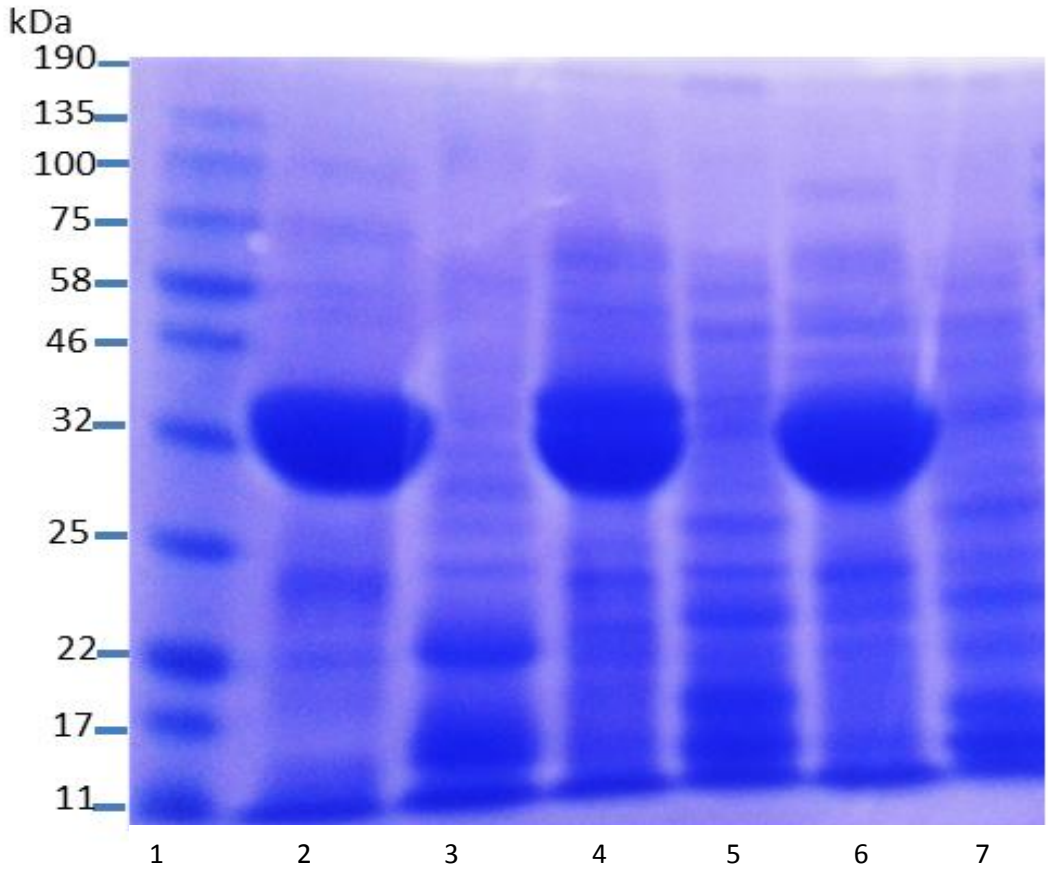

Figure 5.12 SDS–PAGE analysis of Hibiscus eluted with 0.3M NaCl. Lane 1 Marker; Lane 2, 3 are POP, unabsorbed OK, Lane 4, 5 are PSP, unabsorbed SB, Lane 6, 7 are PKP, unabsorbed KE.

Figure 5.12 presents the SDS-PAGE analysis of purified protein fractions eluted with 0.3 M NaCl. The SDS-PAGE results show a single protein band across all the samples. The fraction eluted with the 0.3 M was considered in this work because of its coagulation activity. It is logical to believe that most, if not all, of the bands that were visible in the crude extracts were eliminated after the purification, except the protein

bands with MW of 39 kDa. The single densest protein band with MW of between 30 and 39 kDa was adjudged to be responsible for the coagulation activity.

The concentration of the proteins across this band was similar except that the band was moderately dense in PSP but slightly wider in POP. It is also likely that there exists a small unit of coagulant protein with MW of 11 kDa as seen in the SDS-PAGE analysis. However, its presence may not have any significant influence on the coagulation activity because it is observed in a similar band even in the contaminant ladder in lane 3, 5 and 7.

5.6 Coagulation potential of purified Hibiscus seed proteins

Hibiscus plant seeds obtained from OK, SB and KE seeds were purified in an IEX column using varying concentrations of NaCl solutions. For example, the eluent was prepared by dissolving 0.3, 0.5 and 1.0 M NaCl in phosphate buffer solution. An activity assay performed to screen out fractions with potential coagulation showed that, only fractions eluted with 0.3 M NaCl were found to have coagulation activity and so this was used in the subsequent coagulation investigations. Figure 5.13 presents the performance of POP, PSP and PKP in removing turbidity in water using purified proteins obtained from the IEX column. At the optimum coagulant dose, the purified protein dose employed in the study was reduced significantly ($p<0.05$). For instance, a maximum turbidity removal of approximately 95% was achieved with 0.495 mg/l dose of POP while a 0.74 mg/l dose was used to achieve 94 and 92% using PSP and PKP respectively. It can be seen here that there was no significant difference ($p>0.05$) in performance between POP and PSP. However, purified protein was found to perform

optimally at a reduced dosage compared with the crude extracts to achieve minimal residual turbidity.

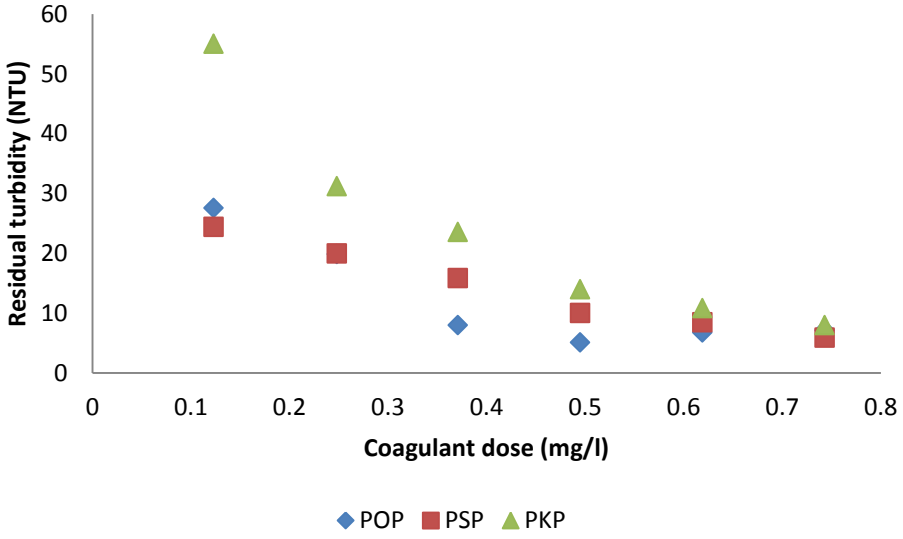

Figure 5.13 Performance of POP, PSP and PSP as coagulants in 100 NTU turbid water.

5.7 Sludge volume production with crude, purified proteins and AS

A jar test was used to assess the performance of all the coagulant proteins and AS based on the volume of sludge produced after 1-hour sedimentation, using water with an initial turbidity of 100 NTU. Using a 0.495 mg/l dose of POP, the amount of sludge produced was 0.5 ml Similarly, at 0.74 mg/l dose of PSP and PKP and 5 mg/l of AS, sludge volume production was approximately 0.54 and 0.4 ml in PSP and PKP while AS produced 2.1 ml. The ratio of sludge volume generated by AS to POP, PSP and PKP was 4:1, 4:1.1 and 5:1. Additionally, when the crude sample was tested, sludge production was higher compared with the purified proteins, 1.1ml in water which had been treated with OCE and 1.3 ml in SCE and KCE respectively. The sludge volumes obtained with the CSEs were also lower than the sludge volume generated by AS during the treatment. Although, the small sludge volume produced with either purified or crude

proteins are smaller, their sludge is biodegradable and non-toxic which offer further advantage of using natural coagulants as an alternative to synthetic coagulants. Ndabigengesere et al. (1995) reported that volume of sludge produced by AS was five times higher than that of MO seed extracts.

5.8 Summary

Coagulation experiments were conducted on three Hibiscus species in water treatment. All the three samples show significant turbidity removal in high than in low turbidity water similar to observations by (Ndabigengesere and Narasiah, 1998a, Katayon et al., 2006). The effect of pH adjustment, temperature and storage of the extract due to denaturation show improved performance against the non-treated samples. However, all the extracts tested did not affect the pH of the treated water with low sludge volume compared to AS. Similarly, the SDS-PAGE analysis of crude and purified samples shows interesting results. While crude extract show multiple MW proteins in the sample, purified protein show a single protein band with MW of 39 kDa. Furthermore, the zeta potential measurement indicated the main mechanism of coagulation was by adsorption and bridging action because all the extracts are anionic.

CHAPTER 6 - CHARACTERISTICS OF COAGULATED HIBISCUS FLOCS

6.1 Background

In water treatment, accelerated sedimentation is achieved by aggregating the flocs via slow mixing process (flocculation) to form larger macro flocs facilitating removal in a sedimentation tank. Therefore, in order to achieve satisfactory treatment, coagulated flocs must demonstrate sufficient strength so as not to be broken by the turbulent flow field found in the flocculator and clarifier. Thus, the merit of each coagulant is judged based on certain parameters such as floc strength, growth, size and density. Previous work has observed that smaller flocs are more likely to resist rupture than larger flocs but may pose some challenges during removal compared to bigger flocs (Boller and Blaser, 1998, Jarvis et al., 2005c), as the mechanism and general mode of floc transportation is hampered if the flocs are small in size and so cannot settle effectively. Thus, it is difficult under normal plant condition to prevent floc breakage, particularly in highly turbulent areas where flocs are prone to breakage, and consequently, the regrowth potential of flocs post-rupture is of interest.

Following previous work which monitored floc growth, breakage and re-growth phases using different inorganic coagulants by (Jarvis et al., 2005b, Yu et al., 2012, Xu et al., 2014), This study investigates the applicability of organic materials as coagulants in water treatment. Recently, preliminary investigation of some of these natural extracts such as *MO, Cactus latifaria* and Mustard seeds, have shown coagulation potential in those plants (Jahn Samia, 1998, Diaz et al., 1999, Bodlund et al., 2014). However, most of these studies only assessed the coagulation potential of the extracts; whereas

problems related to floc strength and recovery have not been investigated, despite their importance in the treatment process.

The work presented in this thesis considers the use of Hibiscus seed extracts as an alternative material to the synthetic coagulants. Therefore, this chapter focuses on research objective (3). This work has been published in Water Research journal (see Chapter 1):

- **To investigate floc growth, breakage, re-growth and strength of water coagulated using hibiscus seed extracts and purified proteins.**

Laboratory experiments to determine floc properties were conducted with crude seed extracts and purified protein samples as in Chapter 4. Crude samples were extracted by dissolving the seed powder in 1M NaCl solution while the purification was conducted using a HiTrap 1 ml anionic IEX column. To measure floc properties, a laser diffraction instrument, Mastersizer 2000, Malvern, UK, was connected to a jar test apparatus where the suspension was continuously monitored through the optical unit of the Mastersizer via a peristaltic pump. Turbid water was rapidly mixed at 200 rpm for 1.5 min during which coagulant was added with a flocculation period of 25 min at 30 rpm for floc growth. Flocs were then broken at 200 rpm for 1.5 min and the speed for flocs recovery was observed at low mixing rate, at 30 rpm for another 25 min. The 200 rpm was adopted for the floc breakage because this is the highest shear rate anticipated throughout the entire process (in the flocculator). Additionally, since jar test is used to simulate the condition in the water treatment plant, 200 rpm was used during the coagulation to disperse the coagulants effectively. The flocculation process adopted 30 rpm for floc growth similar to the condition used in the preliminary jar test while the 1.5 min was chosen, 30 second longer than the initial coagulation process to observe the

resistance of the flocs against the blade shear force. The entire process is shown in chapter 4.

6.2 Results

6.2.1 Floc strength and floc recovery factors

In order to obtain a better understanding of the properties of coagulated flocs, it is important to consider the floc strength and floc recovery after exposure to high shear force. Floc strength reveals the resistance of flocs to stress and can be described using a strength factor. Similarly, the recovery factor reveals the ability of a floc to re-grow after breakage.

6.2.2 Floc size, growth rate of Hibiscus seeds as primary coagulants

Figure 6.1 shows the effects of floc formation and breakage experiments using crude seed extracts of OK, SB and KE and AS as primary coagulants. The results revealed that floc growth rates achieved by the crude extracts as primary coagulants were very slow during the growth phase. However, the floc growth rate was faster in OK than AS, demonstrating shorter growth time to achieve the maximum floc size. OK also produced an equivalent median floc size similar to that of AS (approximately 300µm). The amount of protein used for coagulation was determined following Bradford (1976), with 5.09 mg found in OK, 4.59 mg in SB and 3.16 mg in KE extracts respectively, in the 50 mg/l dose. The 300µm diameter AS floc size reported in this study is the same as that obtained by Zhao et al. (2013b), who used *Enteromorpha* extract as a coagulant. At steady state, the median floc size for SB and KE were approximately 176µm and 142µm respectively, lower than the 300 µm flocs generated by AS before breakage.

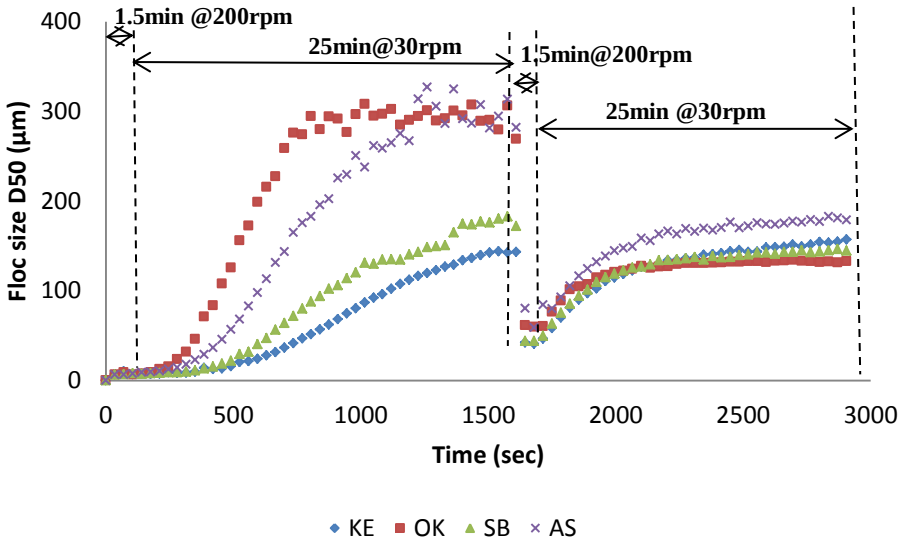

Figure 6.1 Floc growth, breakage and re-growth of KE, OK and SB and AS as coagulants.

While OK and AS achieved the largest floc size after reaching the steady growth state, it is, however, likely that SB and KE flocs did not reach the steady growth phase when the high shear rate was re-introduced. Thus, the size of the flocs generated by SB and KE could have grown larger than the floc sizes witnessed before the reintroduction of the higher shear, if the flocculation process using all the extracts were allowed to continued to the steady growth state. This will provide a clearer comparison between all the coagulated flocs in Figure 6.1.

6.2.3 Floc growth rate of OK, SB and KE extracts as primary coagulants

In the work reported here, other floc characteristics such as floc growth time and floc growth rate were also evaluated. Essentially, this evaluation was performed in order to determine the time required for the coagulation and flocculation processes to be completed using Hibiscus extracts as primary or as coagulant aids. Table 6.1 shows the floc growth time and floc growth rate recorded by all the extracts. A shorter floc growth time of 13.42 min was achieved by flocs formed with OK extract to reach the steady

state while flocs generated with AS, achieved a longer floc growth time of 20.42 min. Under the same flocculation condition, it took much longer time, approximately 25.5 min for SB and 22.16 min for KE flocs, yet they did not reach the steady phase (Figure 6.1). Additionally, flocs generated by OK assumed a faster growth rate of 21.95 µm/min than AS flocs which achieved a floc growth rate of 15.36 µm/min. When the floc growth rate of OK was compared with those of SB and KE flocs, OK recorded a floc growth rate that was approximately three times faster than that of SB and KE flocs. The floc growth rate was 7.22µm/min for SB and 6.79µm/min for KE respectively. Flocs formed with OK extract are larger in size, achieved faster growth rate and took the shortest time to reach the steady growth phase than those of AS, SB and KE samples under the same flocculation condition. OK was shown to have a higher protein concentration of 25% as reported by (Oyelade et al., 2003), compared to SB and KE seed with a lower protein content of 18.8% (Rao, 1996) and 13.04% (Mariod et al., 2010) respectively. The protein concentration in the extracts was 1.018 mg/ml in OK, 0.918 mg/ml in SB and 0.631 mg/ml in KE, and used in the study.

Several studies have indicated that the main agent of coagulation in natural extracts is the presence of a dimeric cationic protein with molecular mass of 6.5 and 14kDa (Ndabigengesere et al., 1995, Ghebremichael et al., 2005, Bodlund et al., 2014). However, the work reported here found that the main agent of coagulation in the Hibiscus extracts is an anionic compound with MW of 39 kDa and a surface charge potential of − 8.3, − 6.4 and – 8.3 mV in OK, SB and KE respectively, as shown in Chapter 5. La Mer (1966) observed greater adsorption of anionic potato starch due to the COOH groups. In such case, the adsorption effects of an anion would outweigh the electrostatic repulsive force between the colloids and the polymer like charges.

Table 6.1Floc characteristics of OK, SB and KE, and AS as primary or coagulant aids.

Parameter		Extract + AS			Extract			AS
		AS+OK	AS+SB	AS+KE	OK	SB	KE	
Floc d_{50}	(µm)	665	672.46	693	294.5	176.99	150.56	313.68
Growth T	(min)	10.5	7.58	10.5	13.42	24.5	22.16	20.42
Growth R	(µm/min)	63.3	88.72	66	21.95	7.22	6.79	15.36

6.2.4 Floc size and growth rate of OK, SB and KE extracts as coagulant aids

Figure 6.2 shows the floc growth, breakage and regrowth characteristics of AS and AS+extract flocs in water treatment. 50 mg/l of each of the extracts was combined with 5 mg/l of a predetermined dose of AS. Additionally, the amount of protein in the 50 mg/l extracts used for coagulation was found to be 5.09, 4.59 and 3.16 mg in OK, SB and KE respectively. The floc growth patterns were found to be similar for all the extracts for the duration of the experiments. At steady state, when used as coagulant aids, AS+SB, AS+KE and AS+OK produced floc sizes of 696µm, 701µm and 722µm respectively, but did not re-grow to their original sizes after breakage. This result is in agreement with the work reported by Yu et al. (2009), who observed that flocs formed by charge neutralisation and bridging action are larger than flocs generated by simple charge neutralisation. Figure 6.2 revealed that the d_{50} values for the AS+extract combinations were more than twice the floc size of AS used alone as a primary coagulant (approximately 300µm).

Floc growth of AS+OK, AS+KE and AS+SB assume a rapid growth within a few minutes of the coagulation process, although the growth was faster in AS+SB extract than in AS+KE and AS+OK samples. Table 6.1 shows that floc growth time was

shortest in AS+SB flocs, which achieved an average growth time of 7.58 min to reach the steady state compared to 10.5 min growth time recorded by both AS+OK and AS+KE flocs to reach the steady floc growth phase. Similarly, AS+ SB extract achieved the fastest floc growth rate of more than 887 µm/min than in AS+OK and AS+KE flocs. Although AS+OK and AS+KE have the same growth time, the growth rate was slightly faster in KE, which achieved a floc growth rate of approximately 66 µm/min compared with 63.3 µm/min growth rate recorded by AS+OK flocs.

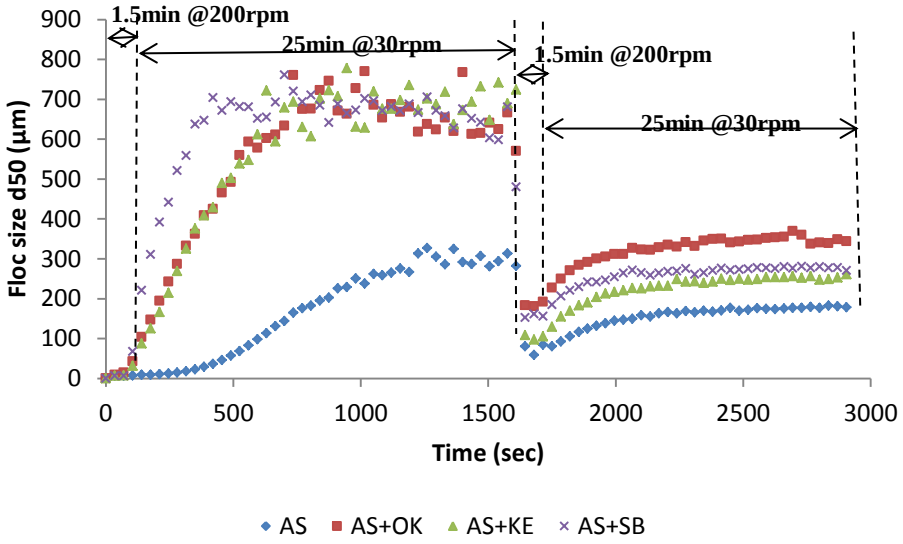

Figure 6.2 Floc growth, breakage and re-growth of AS and AS+extracts as coagulant aids.

The results presented here also noted the importance of particle concentration in promoting floc growth and size for adsorption to be effective. For instance, Muyibi and Evison (1995b) and Ndabigengesere et al. (1995) reported in separate studies that MO extract was found to be ineffective in coagulating low turbidity water. Similarly, Lee et al. (2001) used a low molecular weight polymer (10 and 50 kDa) as a primary coagulant and observed that the polymer was more efficient in the treatment of water with high turbidity. The low MW of the coagulant protein reported in Chapter 5 revealed that all

the seed samples have a small MW protein of 39 kDa. Thus, the size of the floc in the work reported here may have increased beyond that size if higher turbidity water had been used. However, water samples with higher turbidity than the one used here were found to affect the measurement due to light obscuration resulting low and small floc formation. Furthermore, the presence of many macromolecules in the extracts as shown in the SDS-PAGE in the preceding (Chapter 5) showed that the extracts have many low MW proteins and polysaccharides with long carbon chains.

6.2.5 The sizes of the regrown flocs of the *Hibiscus* seeds

The study further proceeded to investigate the post-rupture floc sizes produced by all the samples. The d_{50} values for all samples were found to decrease rapidly with the re-introduction of the high shear rate at 200 rpm. After breakage, the flocs began to re-grow when slow mixing was re-introduced. Examination of Figure 6.1 shows that when the extracts were used as primary coagulants, while the size of the re-grown flocs was almost the same, (approximately 146µm), the breakage was most severe in OK, due to its high lipid content, because flocs formed under such conditions are more fragile (Jarvis et al., 2005b). Furthermore, flocs generated by KE possess a higher strength factor than flocs produced by OK and SB because they are smaller in size. After re-growth, the floc sizes were similar across all the extracts; 146µm and 173µm for AS flocs.

Figure 6.2 shows that as coagulant aids, AS+OK produced the largest regrown floc size of 350µm at steady state, compared to 280µm and 274µm in AS+SB and AS+KE respectively. Again, the results show no significant difference between AS+SB and AS+KE flocs at steady state before and after breakage. The behaviour and response of

the samples to the breaking force was similar but more extensive in AS+KE followed by AS+SB whereas the amount of breakage recorded in AS+OK was found to be lower. The performance of AS+OK was superior to that of AS+SB and AS+KE after floc breakage, due to the high protein concentration in OK extract.

6.2.6 Influence of low pH on the strength of the coagulated flocs

To examine the impact of low pH coagulation, the growth, breakage and re-growth of flocs formed by OK, SB and KE seed extracts at pH 4 are presented in Figure 6.3. The test was conducted on low pH coagulation because it was observed in Chapter 5 that the coagulation performances of all the extract were higher at pH 4. At lower pH, the average floc sizes of SB and KE were observed to be approximately 210µm and 174µm respectively; i.e. larger than their corresponding floc sizes of 176µm and 142µm when used as primary coagulants at neutral pH. However, during the same growth period, the d_{50} floc size of OK decreased from 300µm to 240µm at pH 4. The impact of low coagulation pH was also investigated regarding floc growth rate and floc growth time. Table 6.3 presents floc growth rate and floc growth time achieved by each of the extracts at low pH 4. The results revealed that the shortest floc growth time of 7.58 min was achieved by OK, followed by 8.16 min in KE and the longest time was recorded by flocs coagulated with SB extract, which attained the steady floc growth phase in 9.3 min. On the other hand, the floc growth rate at low pH also indicates a similar pattern of behaviour to that of floc growth time achieved by OK at neutral pH. OK achieved the fastest floc growth rate of 26.45 µm/min while KE attained a growth rate of 20.02 µm/min. However, the slowest flocs to grow were those formed with SB extracts, which achieved a low floc growth rate of 18.45 µm/min. Furthermore, OK whose floc sizes

deteriorated at low pH were the fastest flocs to grow and shortest floc growth time to reach the steady growth phase than SB and KE flocs.

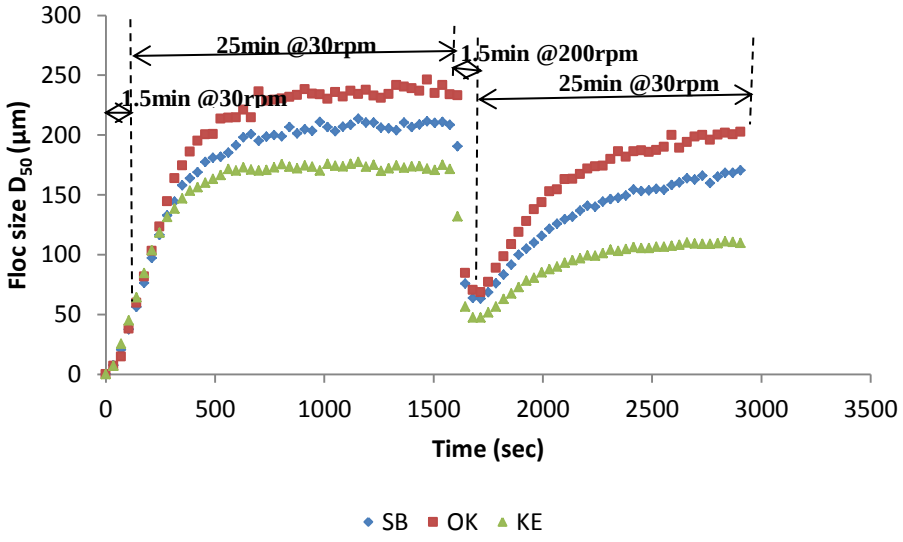

Figure 6.3 Floc growth, breakage and re-growth using OK, SB and KE crude extracts at pH 4.

6.2.7 Floc strength and recovery factor of the *Hibiscus* extracts

Table 6.2 summarises floc strength and floc recovery ability of coagulants extracted from *Hibiscus* plant seeds. The results show that as coagulant aids, OK exhibited the highest floc strength of approximately 25.5% exceeding KE and SB with 21.8% and 15.0% respectively. As primary coagulants, while the floc strength factor increased from 21.8 to 33.8% in KE and 15.0% to 25.0% in SB, the strength of the OK-derived flocs deteriorated from when used as a coagulant aid to when used as a primary coagulant. Conversely, the slight decrease in floc strength from 25.0% to 23.3% in OK is thought to be due to the coating of floc surfaces by the lipid in OK seed. After floc breakage, a notable floc recovery ability was seen in both SB and KE as primary coagulants (100% and 76.5% respectively). Following its low floc strength

performance, OK again showed a corresponding poor floc re-growth by recovering only 32.6% of its original floc size.

Further evaluation of the flocs after breakage when all the extracts were used as coagulant aids shows that OK has the highest floc recovery factor of 38.6% compared to 26.6% and 23.5% recovery ability achieved by SB and KE respectively. The work reported here observed a direct relationship between floc strength factor and floc recovery factor in both OK and KE extracts. The results show that poor floc strength led to limited floc recovery, whereas stronger flocs exhibited a level of significant floc re-growth.

Table 6.1 shows that there was a small difference between the floc strength in OK and KE extracts at low pH (29.3% in OK, 28.7% in KE and 33.3% in SB). In this case, the acidic pH value played a significant role in improving the strength of OK and SB. However, the floc strength of KE at pH 4 when used as a primary coagulant is reduced compared to its strength at neutral pH (33.8% to 28.7%). Table 6.2 further shows that the re-growth ability of flocs formed by the three extracts at low pH after floc breakage, indicating a floc recovery of 75.7% for OK, 65.7% for SB and 46.8% for KE. In separate studies, Cao et al. (2010) and Sun et al. (2011) reported that flocs formed in acidic pH region were stronger and more recoverable than flocs generated in alkaline conditions. However, despite the high floc strength of 33.3% recorded at pH 4 by SB, floc re-growth was 65.7%; i.e. lower than the 100% floc recovery ability recorded when its floc strength was 25% as primary coagulant. The cause of this is likely due to a change in protein confirmation of SB at low pH which affected its binding activity during floc re-growth.

Table 6.2 Floc strength and recovery of OK, SB and KE extracts as primary or as coagulant aids.

Parameters	Crude coagulant		
	OK	SB	KE
Strength factor (%)			
• CE+AS @ neutral pH	25.5	15.0	21.8
• CE @ neutral pH	23.3	25.0	33.8
• CE @ pH4	29.3	33.3	28.7
Recovery factor (%)			
• CE+AS @ neutral pH	38.6	26.6	23.5
• CE @ neutral pH	32.6	100	76.5
• CE @ pH4	75.7	65.7	46.8

6.3 Characteristics of the purified protein flocs

The performance of purified seed proteins on floc growth and size as primary coagulants or as coagulant aids is presented in Figures 6.4, 6.5 and 6.6. In the work reported here, coagulant protein doses used in the experiments were 0.123, 0.37 and 0.74 mg/L with a pre-determined AS dosage of 5 mg/L obtained from preliminary jar test results. The concentration of the proteins obtained from the purification process was 1.238 in POP, 1.211 in PSP and 1.092 in PKP mg/ml.

6.3.1 Floc growth and size of POP, PSP and PKP

Figure 6.4 shows that the largest floc size of approximately 741μm was recorded with 0.123 mg/l dose of POP, was added to 5 mg/l dose of AS, as a coagulant aid. Further increase in coagulant aid dose from 0.123 mg/l to (0.37 and 0.74 mg/L), led to a decrease in floc size, producing median d_{50} floc sizes of 490μm and 502μm respectively. These conditions of increasing coagulant aid dose to 0.37 and 0.74 mg/l resulted in the formation of smaller floc sizes.

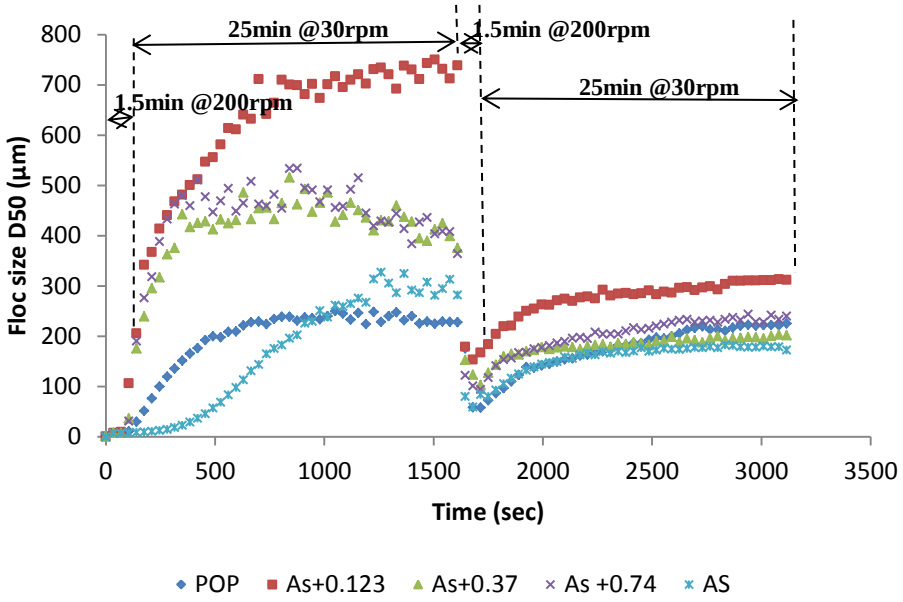

Figure 6.4 Growth, breakage and re-growth of POP flocs as primary or as coagulant aids.

The combination of coagulant protein+AS reduces the available particle surfaces for charge neutralisation, resulting in insufficient adsorption sites for interparticle bridging. Figure 6.4 also shows that, with POP as primary coagulant, floc size was smaller, 200µm in diameter due to steric inhibition.

Meanwhile, the study evaluated the character of flocs generated with the purified proteins in terms of floc growth rate and floc growth time as shown in Table 6.4. The result revealed that, despites the larger floc size produced with 0.123 mg/L of POP as coagulant aid, the growth time was longer, approximately 10.5 min to reach the steady phase, whereas the 0.37 mg/L and the 0.74 mg/L doses achieved the shortest floc growth time of 4.6 and 6.42 min to attain the steady state respectively.

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phase, whereas the 0.37 mg/L and the 0.74 mg/L doses achieved the shortest floc growth time of 4.6 and 6.42 min to attain the steady state respectively. Similarly, the addition of 0.37 mg/L dose produced the fastest floc growth rate of 94.04 µm/min whereas the 0.74 mg/L dose achieved a floc growth rate of 71.53 µm/min.

Table 6.3 Floc growth properties of Hibiscus extract at pH4.

Parameters	OK @ pH4	SB @ pH4	KE @ pH4
d_{50} (µm)	200.5	171.6	163.4
Growth time (min)	7.6	9.3	8.2
Growth rate (µm/min)	26.5	18.5	20.0

It is noteworthy that the 0.123 mg/L coagulant aid dose which had the longest floc growth time produced the slowest floc growth rate of 60.96 µm/min. Furthermore, the time required for flocs generated by 0.123 mg/l dose of POP as a primary coagulant, was 8.16 min to reach the steady phase which was shorter than the floc growth time recorded with the same dose as a coagulant aid. In addition, the 0.123 mg/L of PPP achieved a floc growth rate of 24.51 µm/min as a primary coagulant, indicating a slow floc growth rate when compared with its performance as a coagulant aid. When the performance of POP was compared with AS as a primary coagulant, floc formed with POP produced a shorter floc growth time and the fastest floc growth rate than AS, which recorded a floc growth time of 20.42 min and floc growth rate of 15.32 µm/min.

Figure 6.5 shows the growth, breakage, and re-growth of aggregated floc formed with PSP. A faster initial floc growth rate was exhibited by the PSP sample with coagulant aid doses of 0.123, 0.37 and 0.74 mg/L in combination with AS than when used as the

primary coagulant. At steady state, maximum floc sizes d_{50} of 580µm and 519µm were achieved with 0.123 mg/L and 0.37 mg/L coagulant aid doses respectively.

The strength of flocs generated with 0.74 mg/L dose of PSP and POP in conjunction with AS are weaker compared with their strength as primary coagulant as well as when used at lower coagulant aid doses. At steady state, PSP assumed a different pattern of floc growth, where the absolute deviation of the median floc size about the mean value was found to be greater than in flocs generated by POP. However, the re-growth pattern taken by the flocs was similar regardless of coagulant aid dosage and also irrespective of pre-breakage floc size.

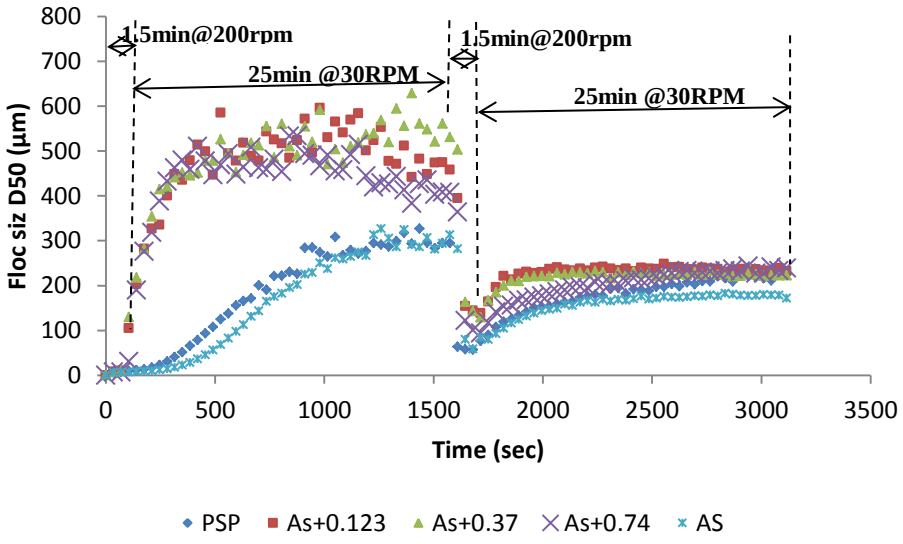

Figure 6.5 Growth, breakage, and re-growth of PSP flocs as primary or as coagulant aids.

During flocculation, thread-like flocs, visible to the naked eye under lamination, grow in length and circumference. At the end of the measurement period, the regrown flocs reached a steady phase d_{50} floc size of 243µm in all the samples, including flocs formed by PSP as primary coagulant. It is noteworthy that, when used as a primary coagulant, PSP produced an initial median floc size, which ranges between 295 and 300µm similar

to the floc generated by AS as coagulant, (approximately 300µm) in diameter. However, the ionic strength of PSP species was not sufficient to compress the double layer during coagulation as shown by its surface charge potential.

Figure 6.6 shows that the addition of 0.37 mg/L and 0.74 mg/L doses achieved the shortest floc growth time of 5.25 and 5.83 min respectively whereas, with 0.123 mg/l dose, floc growth time was 6.58 min as coagulant aids. However, when employed as primary coagulant, the 0.123 mg/l dose achieved a floc growth time of 13.41 min. Similarly, the floc growth rate was notably faster with the 0.37 mg/L coagulant aid dose, achieving floc growth rate of 88 µm/min, followed by the 0.74 mg/L dose which recorded a floc growth rate of 82.25 µm/min against the 69.97 µm/min attained with 0.123 mg/L.

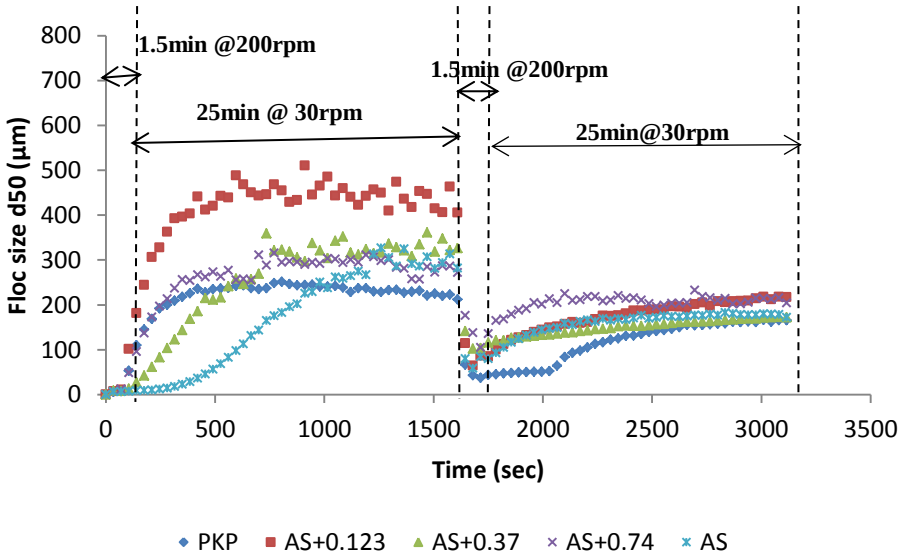

Figure 6.6 Growth, breakage, and re-growth of PKP flocs as primary or as coagulant aid.

After having the longest floc growth time, the 0.123 mg/l dose used as primary coagulant was found to have the slowest floc growth rate of 16.54 µm/min.

Table 6.4 Floc properties of POP, PSP and PKP as coagulant aids and as primary coagulants.

	AS+POP	(mg/l)		AS+PSP	(mg/l)		AS+PKP	(mg/l)		Coagulant	0.123(mg/l)	
Parameter	0.123	0.37	0.74	0.123	0.37	0.74	0.123	0.37	0.74	POP	PSP	PKP
d_{50} (µm)	640.1	432.6	459.3	460	462	479.5	441.3	211.3	256.9	200.0	221.8	236.9
Growth T (min)	10.5	4.6	6.4	6.6	5.3	5.8	7.0	8.2	5.3	8.2	13.4	5.3
Growth R (µm/min)	61.0	94.0	71.5	69.8	88.0	82.3	63.0	25.9	48.9	24.5	16.5	45.1

The relationship between floc growth rate and time was also clear as in POP. While floc growth time achieved with 0.123, 0.37 and 0.74 mg/L doses of PSP as coagulant aids were approximately shorter than time recorded by 0.123 mg/L as primary coagulant, the growth rates was 4 times faster by the coagulants aids than that of PSP as primary coagulant.

Figure 6.7 shows floc growth, breakage and re-growth performance when PKP was used as primary coagulant and as a coagulant aid. A maximum median floc size of 480μm was recorded when 0.123 mg/L dose of PKP as coagulant aid in conjunction with AS. The results show a decrease in floc size as coagulant aid dosage increases from 0.123 mg/L to 0.74 mg/L doses, similar to the trend of floc growth shown by POP and PSP. A maximum floc size of 335μm was achieved with 0.37 mg/L of PKP, and 310μm diameter floc size was generated with 0.74 mg/L dose. The decrease in floc size due to increased coagulant aid doses can be overcome by improving bridging condition due to insufficient sorption sites. La Mer (1966) postulated that optimum dosage corresponds to half of the particle surface coverage.

Table 6.4 shows the analysis of floc growth time and floc growth rate of PKP as coagulant or as coagulant aid in conjunction with AS. The results show that PKP exhibited a different pattern of behaviour compared with either POP or PSP flocs. The 0.74 mg/l dose of PKP achieved the shortest growth time of 5.25 min as primary coagulant, followed by 0.123 mg/l dose which attains a floc growth time of 7 min. The longest floc growth time of 8.16 min was recorded when 0.37 mg/L dose was used as primary coagulant. Furthermore, the fastest floc growth rate of 63.04 μm/min was achieved with a coagulant dose of 0.123 mg/L while at coagulant aid dose of 0.74 mg/L, floc growth rate was 48.94 μm/min compared with the slowest growth rate of 25.89

μm/min attained by 0.37 mg/L dose. At the end of the flocculation process, a 45.13 μm/min floc growth rate was recorded with 0.123 mg/L dose of PKP as primary coagulant.

6.3.2 The sizes of the regrown flocs of purified seed proteins

The re-growth potential of flocs formed from coagulated water with 0.123, 0.37 and 0.74 mg/L doses of POP as coagulant aid was investigated. These conditions of increasing coagulant aid dose to 0.37 and 0.74 mg/l resulted in the formation of smaller floc sizes. Interestingly, the regrown ability of flocs formed from water coagulated with 0.37 and 0.74 mg/l doses of POP as coagulant aid, and when 0.123 mg/l dose of POP as primary coagulant, was much smaller. Overall, the floc sizes attained were approximately 300μm with 0.123 mg/L, 196μm with 0.37 mg/L and 232μm in both 0.74 mg/L and POP as primary coagulant, despite their small pre-breakage floc sizes.

Similarly, at the end of the measurement period, with PSP as coagulants, the re-grown flocs reached a steady state d_{50} floc size of 243μm in all the samples, including flocs formed by PSP as primary coagulant. It is noteworthy that, when used as primary coagulant, PSP produced an initial median floc size, ranging between 295 and 300μm similar to the floc generated by AS as coagulant, (approximately 300μm).

Again, the recovery ability of flocs generated by 0.37 and 0.74 mg/l of PKP as coagulant aid and PKP as primary coagulant was found to be higher than floc recovered by 0.123 mg/l of PKP as coagulant aid. While the d_{50} size of the re-grown floc was 201μm with 0.123 mg/L dose of PKP, the 0.37 and 0.74 mg/l doses achieved a post-breakage steady d_{50} size of 164μm and 211μm respectively. Thus, as coagulant aid, PKP exhibited the greatest floc strength and re-growth capability at a higher dose even

though the initial floc size was smaller. There was a modest, yet noticeable, amount of thread-like flocs using PKP, but this was less than that observed in PSP flocs.

6.3.3 Floc strength and recovery factor of purified Hibiscus seed proteins

Table 6.5 presents floc strength and recovery ability of three purified hibiscus proteins used as primary coagulants and as coagulant aids. When the purified seed proteins were used as primary coagulants, the results show that POP achieved the highest strength factor of 24.3% and 21.7% in PSP while 18.2 % was achieved with PKP. Flocs formed by AS achieved a strength factor of approximately 20%. In addition, the results show that flocs formed with POP and PSP dosed as coagulant aids can resist marginally higher shear with a 0.123 mg/L dose compared to AS at 5mg/l dosage under the same coagulation and flocculation conditions. Furthermore, the addition of the 0.123 mg/L dose as coagulant aid with AS produced larger floc size with corresponding decrease in floc strength of POP and PKP, whereas floc strength formed by PSP remain largely unchanged. This result agrees with Jarvis et al. (2005a) who observed that the resistance of smaller flocs in turbulent flow regions is higher than that of larger flocs. Interestingly, when the coagulant dose was increased from 0.123 mg/L to 0.37 mg/L, and maintaining the same shear rate, the strength factor was broadly the same for POP (20.8% to 20.7%) but increased slightly for PSP from 21.4% to 22.2%. In the case of PKP, the increase was significantly higher, from 14.0% to 31.3%.

Further increase in coagulant aids dose to 0.74 mg/L caused further floc strength decline in POP and PSP. Under the same coagulation conditions, floc strength improved further in PKP from (31.3% to 35.6%). PKP flocs were found to behave in a similar fashion to AS in work reported by (Yu et al., 2014), who showed that an increase in alum dose during coagulation resulted in increased floc strength.

Table 6.5 Floc strength and recovery factor of purified proteins as primary and as coagulant aids.

Parameters	POP	PSP	PKP	AS
Strength factor (%)				
• Primary coagulant	24.3	21.7	18.2	20
• AS + 0.123mg/L	20.8	21.4	14.0	_
• AS + 0.370 mg/L	20.7	22.2	31.3	_
• AS + 0.740 mg/L	19.2	19.5	35.6	_
Recovery factor (%)				
• Primary coagulant	70.7	71.4	64.3	50
• AS + 0.123 mg/L	27.3	25.7	38.0	_
• AS + 0.370mg/L	28.4	25.9	25.1	_
• As + 0.740mg/L	36.9	31.4	59.0	_

The work reported here noted that an increase in PKP dosage from 0.123 to 0.74 mg/L resulted in a further increase in floc strength factor, from 18.2 to 36.0%. Although PKP has low protein content as reported earlier, flocs generated by 0.37 and 0.74 mg/L doses of PKP were stronger than flocs formed by POP and PSP under the same dosage condition. The result demonstrated that if PKP is used as coagulant aid with AS as the primary coagulant, at a higher dose of 0.74 mg/L, the improvement in floc strength was significantly higher compared with 0.123 and 0.37 mg/L doses. Further investigation revealed that floc reversibility of the purified proteins was better when the samples were used as primary coagulants, with PSP, POP, and PKP re-growing to 71.4%, 70.7% and 64.3% of their original size respectively, whereas AS flocs recovered only 50% of their original size. At higher coagulant aid dose of 0.74 mg/L, the recovery factor improved across all the samples compared to 0.123 mg/L dose. While floc recovery ability was 59.0% in PKP and 36.9% in POP, it was lowest in PSP, achieving 31.4%. Again, at the 0.123 mg/L dose, floc recovery by the PKP was much higher than the maximum floc re-growth achieved by PSP and POP at 0.74 mg/L as a coagulant aid. It is noteworthy,

however, that the re-growth of PSP and PKP flocs was the same, approximately 25% at 0.37 mg/L dose, whereas under the same shear condition, POP superseding this floc recovery to achieve a 28.4% floc re-growth ability.

6.4 Summary

Floc properties of Hibiscus plant species were investigated as shown above. The results showed smaller floc growth when the extracts were used as primary coagulant, but a doubling of floc size was achieved as coagulant aid in combination with AS. The increase in size was observed in both crude samples and with much lower dosage of the purified proteins as coagulant aids. However, an increase in coagulant aid doses resulted in reduced floc size due to overdosing. Furthermore, a notable impact of low pH coagulation shows some relative increase in floc size in both SB and KE treated water compared with deteriorated size in OK. However, there was improvement in floc strength as witnessed in SB and OK flocs while a reduced strength was seen in KE flocs compared to their observed strength as primary coagulants. The post-breakage floc recovery was seen to be higher as primary coagulant than when the extracts were used as coagulant aids. Conversely, an increased in coagulant aid dose of the purified proteins in combination with AS resulted in greater increase in floc recovery.

CHAPTER 7 - EFFECT OF NOM IN HIBISCUS-TREATED WATER

7.1 Background

One of the greatest challenges of using natural extracts in drinking water treatment is the continuous increase of organic loads, either as DOC or TOC in the clarified water (Ndabigengesere and Narasiah, 1998a). DOC contribution from natural plants and animals in water can react with chlorine during disinfection to form DBPs such as THMs and HAAs, which can adversely affect human health (Liu et al., 2014, Peng et al., 2016). Additionally, there is another issue of change in taste, odour and colour emanating from the treated water after lengthy storage caused by decomposed NOM (Ndabigengesere and Narasiah, 1998a). Jahn (1986) recommended that water treated with natural extract be consumed within 24 hours. As such, natural extracts can only be used as a POU at household level in drinking water clarification. Hence, there is a need to investigate this issue further and if possible, identify an alternative method of processing the extracts to improve the quality of water treated with natural products.

Given these challenges, the work reported in this chapter focuses on objective (4) of this thesis:

- **To apply fluorescence excitation-emission matrices to monitor, evaluate and characterise the composition of NOM in treated water using crude extract, and purified seed proteins as coagulants.**

7.2 Organic nutrients addition with crude extracts

The impact of NOM addition in treated water using OCE, SCE and KCE are shown in Figure 7.1. Varying coagulant dose of the extracts ranges from (0 – 100 mg/l) were

applied to assess the effect of DOC addition in the clarified water. Similar works have been reported elsewhere by Ndabigengesere and Narasiah (1998a) and Okuda et al. (2001b) who observed that the main setback in using MO extract as a water treatment coagulant was the release of organic and nutrient compounds in water. Crude extracts may contain compounds other than proteins, such as lipids, ashes, carbohydrates, and many other molecules. However, it is not clear whether inorganic substances may also be added from the crude extracts which can also affect the overall water quality. Figure 7.1 shows clearly that the use of Hibiscus seed extracts in water treatment can significantly increase final water DOC. The highest increase was found in OCE, followed by SCE and KCE treated water. At a lower coagulant dose of 20 mg/l, residual DOC increases to approximately 8 mg/l in SCE and KCE while in OCE treated water, DOC concentration was 10 mg/l. It appears that the increase in DOC was caused by every stepwise increase in coagulant dose, and the concentration rose to a maximum value using coagulant dose of 100 mg/l. With 100 mg/l dose, DOC in the water increased from 6.7 mg/l to approximately 19 mg/l in OCE, 17 and 15 mg/l in KCE and SCE respectively.

In assessing the concentration of organic loads in the treated water, it was observed that the amount of protein used for coagulation varied depending on the coagulant dosage. For instance, at 10 mg/l dose, the amount of protein used for coagulation was 1.018 mg in OCE, 0.918 mg in SCE and 0.631 mg in KCE respectively. The maximum coagulant dose used throughout the experiment was 100 mg/l. At such a high dosage, the amount of protein used for coagulation was increased to 10.18, 9.18 and 6.31 mg in OCE, SCE and KCE treated water respectively.

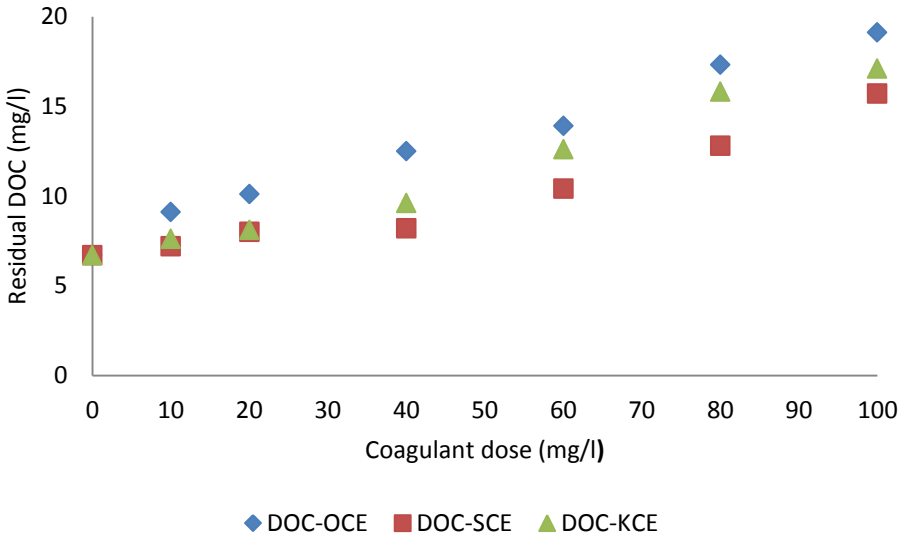

Figure 7.1 Impact of DOC in treated water with OCE, SCE and KCE.

Figure 7.1 shows the trend of organic matter loading by the extracts. The results confirm the earlier findings (Chapter 5) that Kenaf seed contain high organic matter with low protein contents. In KCE treated water, DOC concentrations were found to be higher than in SCE water with the highest overall effect of OM noticed in OCE. The nutrients loading were observed to be independent of protein content in the seeds. Because despite the low protein content in Kenaf seed, DOC concentration in KCE-treated water was higher than in water which had been treated with SCE.

7.3 Impact of organic loads in water treated with purified proteins

Figure 7.2 shows the improvement in water quality in terms of DOC concentration using purified seed proteins. The purification of the coagulant proteins was conducted in an IEX column, and fractions eluted with 0.3, 0.5 and 1.0 M NaCl solutions. The protein fraction eluted with 0.3 M NaCl solution was observed to have a significant coagulation potential in the activity assay. The results show that the coagulation process achieved a reduction in NOM in the treated water with POP, PSP and PKP. For

example, a residual DOC concentration in treated water decreases with coagulant doses of 0.123 and 0.495 mg/l. Also, it was observed that the 0.123 mg/l dose achieved a significant reduction in DOC concentration across all the samples. The performance of POP was 25.4% while PSP and PKP samples recorded 24 and 18% DOC removal in treated water respectively. An increase in coagulant dose resulted in an increase in DOC concentration, indicating charge reversal scenario. For instance, the 0.249 mg/l dose achieved a decrease in percentage DOC removal from 25.4 to 22.4% in POP, 10.5% in PSP and 3.0% in PKP respectively. Further increase in dose to 0.495 mg/l recorded a marginal decline in DOC concentration with 5.6 and 6.5 mg/l representing 16.4% and 3.0% DOC removal in POP and PSP. Under the same coagulation condition, DOC concentration remained fairly unaffected using PKP, 6.7 mg/l in both raw and treated water.

Again, the performance of the purified proteins was assessed using a very high dose of 2.476 mg/l. In this case, residual DOC concentration was found to increase by approximately 11.0% in POP, 10.5% in PSP and 19.4% in PKP respectively. Similar observation was made in Chapter 6 where the 0.123 mg/l dose was found to improve floc growth, size, and strength but a decline in these characteristics was witnessed at higher doses. It is noteworthy that the use of purified proteins at lower doses did not add organic nutrients in the treatment process; rather it aided a slight reduction in DOC concentration in treated water. Furthermore, at the maximum coagulant dose of 2.476 mg/l, only 1.0 mg/l of DOC was added to the final water with POP and PSP, whereas the increase was relatively higher, about 1.3 mg/l in PKP treated water. It is likely that a better DOC removal result would have been achieved if a lower dose other than 0.123 mg/l was used. The protein purification process, in this case, revealed that all the

samples were obtained in their pure state, free from any organic compounds, which resulted in a higher coagulation activity than the crude extracts.

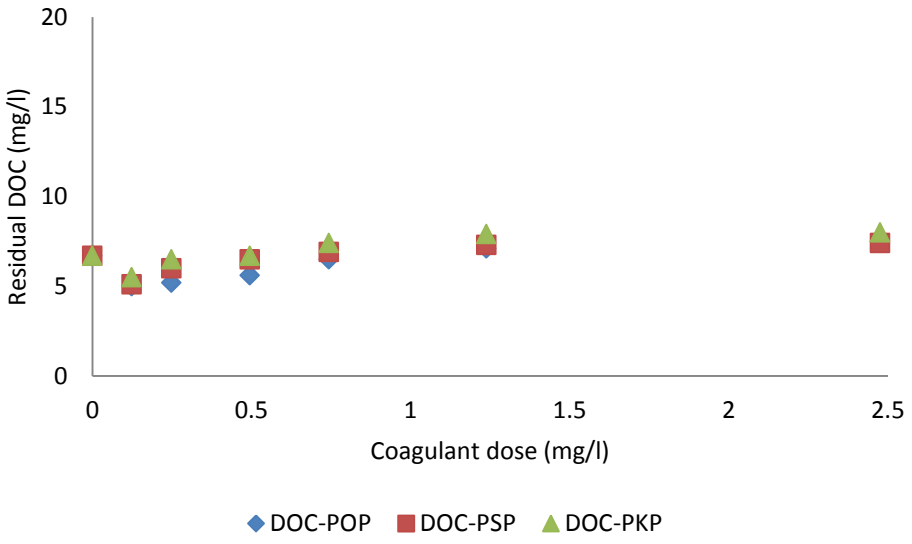

Figure 7.2 Impact of DOC in water treated with POP, PSP and PKP.

7.4 Characterisation of NOM in water using fluorescence-EEMs

Again, in order to obtain a broader understanding of the character and impact of NOM in treated water, the relationship between DOC removal and fluorescence EEMs data, was analysed in water after jar test experiments. The fluorescence-EEM technique was employed for the assessment of DOC removal in treated water using either crude or purified coagulant proteins obtained from the seeds. EEM data present a unique overlap of fluorescence intensities over different excitation and emission wavelengths (Bridgeman et al., 2011).

Within the fluorescence EEM, the presence of organic matter can be visualised as peaks, and these peak were classified by Coble (1996) as; peaks A and C (humic and fulvic-like substances) while peak T and B (tryptophan and tyrosine-like proteins) obtain at a shorter emission wavelengths. In all cases, the relationship between

fluorescence intensity observed at excitation wavelength 220 – 230 nm, and emission wavelength 340 – 360 nm was compared with residual DOC concentration in treated water. Previously, Gone et al. (2009) and Bieroza et al. (2009) showed in separate studies using river and freshwater samples that the combination of peak C emission wavelength and peak T fluorescence intensity may be used as an indicator of TOC removal. Conversely, Gone et al. (2009) and Markechová et al. (2013) have also reported that peak T was least well-removed and can be used to assess residual DOC post-coagulation.

Table 7.1Fluorescence EEMs peaks intensities (Bridgeman et al., 2011)

Peaks description		Excitation wavelength (nm)	Emission wavelength (nm)
Humic substances	A	237-260	400-500
Humic substances	C	300-370	400-500
(Highly coloured)	C_1	320-340	410-430
	C_2	370-390	460-480
Tyrosine-like protein	B_1	225-237	309-321
	B_2	275	310
Tryptophan-like protein	T_1	275	340
	T_2	225-237	340-381
Humic (marine)	M	290-310	370-410

However, Henderson et al. (2009) observed that peak T and/or peak C can be used effectively to monitor contamination in recycled water. Furthermore, recently, several studies have extensively investigated fluorescence fingerprints of OM obtained from EEMs data to locate fluorescence peaks and their intensities in raw and treated waters (Baker, 2005, Bieroza et al., 2009, Zhu et al., 2014, Carstea et al., 2014). Fluorescence major peaks as revealed are presented together with their intensities in Table 7.1. The

fluorescence peaks nomenclature reported here have been adopted from other studies (Bridgeman et al., 2011, Markechová et al., 2013).

7.4.1 Fluorescence EEMs of OM in water treated using Hibiscus extracts

The results showing fluorescence peaks and their intensities are presented in Table 7.2, and fluorescence EEMs are shown in Figures 7.3 and 7.4. 50 mg/l of each of the extract was used in the coagulation unit based on other results (Chapter 5) to identify the optimum dose for coagulation. A visual observation of the EEMs of Bourn Brook raw water in Figure 7.3a reveals its OM composition. Three fluorescence peaks, (T, B and A) are visible in the water sample. The three fluorescence peaks observed in this study are the most commonly identified fluorophores in a water sample (Baker et al., 2008, Gone et al., 2010, Markechová et al., 2013). Peak A fluorescence is attributed to humic substances while both peak B and peak T fluorescence are protein-like substances, mainly related to algal and microbial activity derived OM.

The fluorescence signatures of the treated water with OCE, SCE and KCE (Figures 7.3b, 7.3c and 7.3d) show significant fluorophore presence compared to raw water post-coagulation. Most notably, the shape and location of peaks were similar for OCE and SCE-treated waters. Peak T_1 and T_2 fluorescence were more dominant in clarified water than in raw water with evidence also of peak B_2 fluorophore presence. The opposite result was observed in OCE and KCE treated water, where higher fluorescence intensities were noted as shown in Table 7.2. Figures 7.3b and 7.3c show high values of peaks T_1 and T_2, and peak C fall below detection limit after the treatment. Many studies have often linked peak T to sewage pollution and regarded it as an indication of microbial presence (Baker, 2002, Baker et al., 2008). This study observed an increased

fluorescence signal in the region of protein-like peaks (T and B) from the seeds as anticipated because these seeds are sources of proteins. While peak T has been related to microbial presence (Baker et al., 2008) and can be used to monitor contamination in water (Henderson et al., 2009), in this case, peak T was as a result of secondary contribution from the seed proteins (1.018 mg/ml in OCE and 0.918 mg/ml in SCE). The amount of protein used in the coagulation process was 5.09 mg in OCE and 4.59 mg in SCE respectively out of the 50 mg/l dose used in the study.

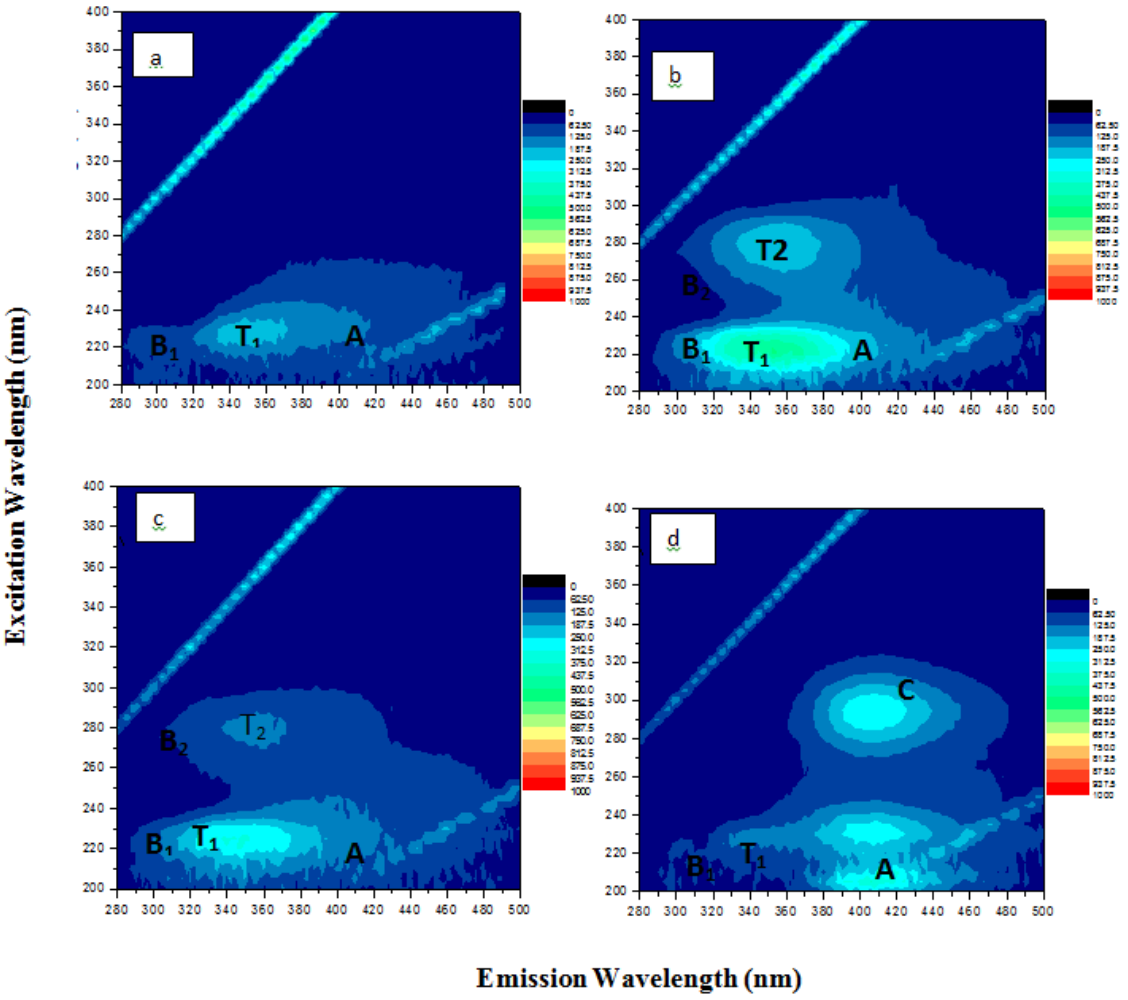

Figure 7.3 Fluorescence EEMs of (a) raw water (b), OCE treated-water (c), SCE treated-water (d) KCE-treated water.

Figure 7.3d, showing water treated using KCE clearly identifies peak C, a humic-like substance with high fluorescence intensity visible in clarified water but absent from the raw water. Increases in peaks T_1 and B_1 were also note in KCE-treated water, and T_2 and B_2 were not detected. One possible reason for this could be KCE has low protein concentration (0.631 mg/l, from protein concentration measurement). Here, the amount of protein used in the coagulation process was 3.155 mg. Earlier work has reported that the entire kenaf plant (seed inclusive) has high fibre content which is use as animal feeds (Webber III et al., 2002), and this might have resulted in the presence of peaks (A and C) humic-like substances with higher fluorescence intensities and decreased emission wavelength.

7.4.2 Fluorescence EEMs of OM in treated water with purified proteins

Typical fluorescence EEMs (Figures 7.4a, 7.4b, 7.4c and 7.4d) indicate the OM composition in raw and clarified water before and after treatment with POP, PSP and PKP. In this study, the protein fraction eluted with 0.3M NaCl solution was used because of its coagulation potential as observed in a coagulation activity assay and from preliminary jar test experiments (Chapter 5 and 6).

The amount of protein in each sample was quantified to be 1.238 mg/ml in POP, 1.210 mg/ml in PSP and 1.092 mg/ml in PKP respectively. Figure 7.2 shows that the 0.123 mg/l dose provided greater performance regarding DOC removal. Therefore, fluorescence fingerprints of all treated water using 0.123 mg/l coagulant dose were assessed (Figure 7.5). Furthermore, the impact of two coagulant doses, 0.248 and 0.495 mg/l were considered on residual DOC concentration and data regarding their fluorescence intensities are presented in Table 7.3. The percentage removal of DOC and

percentage decrease in fluorescence intensity was compared at the end of the treatment. After using the 0.495 mg/l dose of PKP in the coagulation process, the result indicated no single observed effect on treated water DOC; the concentration remained largely unchanged with no adverse impact on DOC concentration.

Gone et al. (2009) reported that the decrease in peaks T, A and C fluorescence intensities and fluorescence-inferred DOC removal in raw and treated water can be used as a useful tool to predict DOC removal and (Hudson et al., 2008, Gone et al., 2010) suggested that fluorescence intensity could be a relative measure of fluorophores concentration though it depends on the fluorophore. The results show that peaks T and A became indistinct after the treatment in all samples, while peak B, a tyrosine-like substance, was the least eliminated, and its presence was still visible post-coagulation. Additionally, while the crude extracts have shown high fluorophores in the region of tryptophan-like peaks, the purified proteins revealed its potential to eliminate both the tryptophan and humic substances, peak T and peak A respectively.

The raw water sample peaks were detected at these centres with the following excitation-emission wavelength and fluorescence intensity: peak T (230/348 nm and 238 au.), Peak B (220/302 nm and 122 au) and peak A (230 /411 nm and 147 au.). However, one important contrasting feature associated with the clarified water sample is that it is characterised by an increase in emission wavelength with reduced fluorescence intensity. Figures 7.4b and 7.4c show that the peaks were observed at the following excitation-emission wavelength and intensities.

Table 7.2 Major fluorescence peaks wavelength and intensities using crude and purified proteins.

Samples	Peak T_1			Peak T_2			Peak B_1			Peak B_2			Peak A			Peak C		
	ex (nm)	em (nm)	int (au)	ex (nm)	em (nm)	int (au)	ex (nm)	em (nm)	int (au)	ex (nm)	em (nm)	int (au)	ex (nm)	em (nm)	int (au)	ex (nm)	em (nm)	int (au)
Raw water	230	348	238	285	360	60	220	302	122	275	275	34	230	411	147	335	413	52
Treated CE																		
▪ OCE	220	350	671	280	352	156	265	310	217	280	310	80	220	410	295	320	428	73
▪ SCE	225	348	333	280	352	145	225	306	164	280	310	73	220	411	165	320	411	57
▪ KCE	225	342	516	280	352	250	225	310	211	280	310	87	220	411	191	320	410	60
Treated PP																		
▪ POP	230	354	129	285	360	40	220	304	87	275	302	32	220	421	125	320	418	45
▪ PSP	230	354	133	285	360	44	220	304	74	275	304	33	220	418	146	320	426	50
▪ PKP	225	356	141	285	360	43	220	302	91	275	304	37	220	410	130	320	421	44

Table 7.3 Percentage DOC and fluorescence intensity removal in POP, PSP and PKP treated-waters.

	DOC (%) removal			FI (%) removal	
Dose (mg/l)	POP	PSP	PKP	POP	PSP	PKP
0.123	25	24	18	46	44	41
0.248	22	10	3	42	43	35
0.495	3	3	0	43	42	36

They have similar peak T (230/354 nm and 129−133 au.), peak B (220/304−308 nm, and 87−91 au.) and peak A (220−225/421 nm and 125 au.). Figure 7.4d shows the fluorescence fingerprints in PKP treated water. The various peaks were found at excitation-emission wavelength and intensity as follows; peak T (220/356 nm and 141 au.), peak B (225/302 nm and 91 au.) and peak A (235/410 nm and 130 au.) accordingly.

Once more, to offer a better understanding of the relationship between DOC removal and peak T fluorescence intensity in the clarified water, the percentage removals of these two parameters were calculated. For both cases, the percentage removals of DOC concentration and fluorescence intensity were observed to be appreciably higher in 0.123mg/l treated waters. It achieved 25%, 22% and 3% DOC removal while the decrease in OM fluorescence intensity was 46%, 42% and 43% using 0.123, 0.248 and 0.495 mg/l doses respectively. Similarly, the percentage DOC removal with PSP was observed to be 24%, 10% and 3% which correspond to 44%, 43% and 42% decrease in fluorescence intensity after the coagulation process. Additionally, equal percentage removal of DOC was observed with the 0.495 mg/l dose in both POP and PSP treated water and their performance on fluorescence reduction equivalent. As expected, however, the lowest percentage DOC removal was observed in PKP treated water. The results show that the 0.123 mg/l dose achieved 18% and

3% with 0.248 mg/l whereas 0% DOC removal was recorded with the 0.495 mg/l dose. Under the same condition, the corresponding percentage decrease in OM fluorescence intensity was 41%, 35%, and 36% respectively.

The overall performance shows that the highest proportion of DOC removal was recorded with the lowest coagulant dose of 0.123 mg/l. In comparison, the maximum decrease in OM fluorescence intensity between the raw and clarified water also occurred with 0.123 mg/l, even though the performance margin was small across the different doses. Furthermore, there was no clear correlation observed between the two parameters (Figure 7.5). For instance, when the percentage DOC removal was 0 in PKP clarified water, the decrease in OM fluorescence intensity was 36% while with 3% DOC removal the reduction in fluorescence intensity was 35% under the same experimental condition. A similar scenario was also noted when the percentage removal in DOC was 22 and 3% in POP, the corresponding decrease in fluorescence intensity was 42 and 43%, giving little or no clear relationship. The results show that while, the decrease in fluorescence intensity was a measurement all the OM composition in the water, DOC is a fraction of TOC in water. Hence, the correlation between the two parameters, if any, was unclear even though a decrease in DOC concentration showed a corresponding decrease in fluorescence intensity.

Furthermore, an increase in DOC concentration resulted in increased fluorescence intensity as seen with the crude samples. Overall, any relationships are not linear, and are based on some degree of interdependency. Similarly, the application of PSP as a coagulant showed similar behaviour between percentage reduction in fluorescence intensity and percentage residual DOC concentration in clarified water.

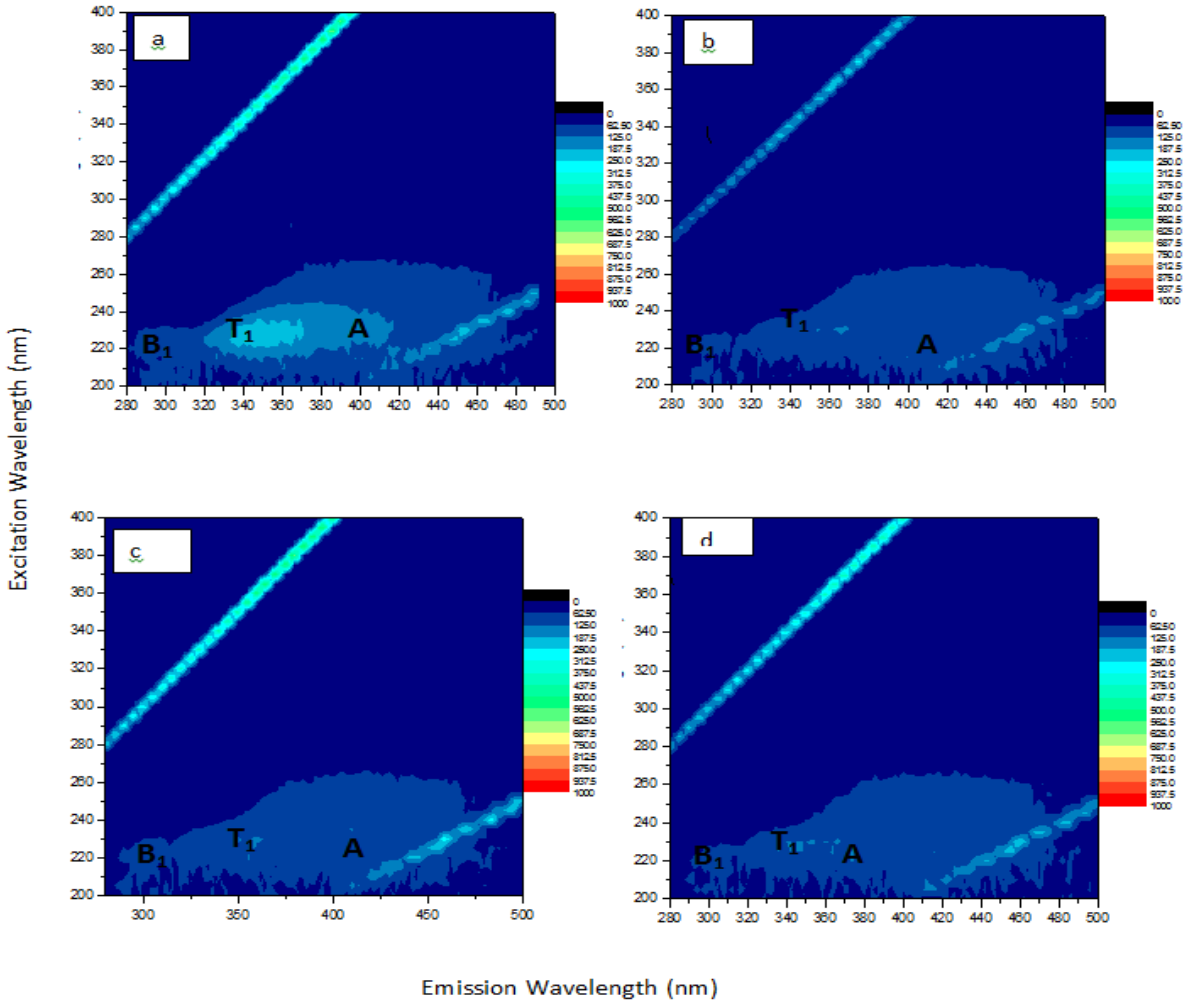

Figure 7.4 Fluorescence EEMS of (a) raw water peaks (b), POP treated-water (c), PSP treated-water (d) PKP-treated water.

Figure 7.5 shows the relationship between a decrease in percentage fluorescence intensity and percentage DOC removal in water treated using purified Hibiscus seeds. Although, the relationship was not linear, it is clear that a high removal of DOC in water resulted in significant decrease in fluorescence intensity. As expected, the performance of POP as coagulant in terms of forgoing relationship was higher which closely followed by PSP and PKP the least effective. However, there was significant difference in percentage fluorescence reduction in PKP treated water with increased DOC removal. In all these cases, fluorescence intensity seems to be a useful tool in assessing the level of organic contaminants than DOC removal as other organic compounds may be present which can only be detected by their

fluorescence measurement. However, the combined effect of the two relationships can also be a useful indicator of pollution of organic origin.

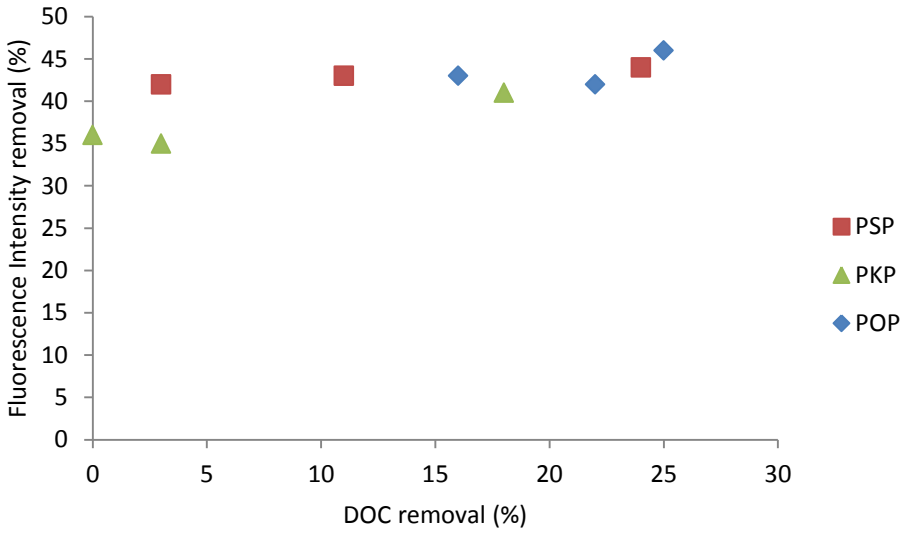

Figure 7.5 Percentage fluorescence and DOC removal in water using POP, PSP and PKP.

7.5 Coagulant protein spectra in purified Hibiscus protein suspensions

Figure 7.6 presents the fluorescence peaks of both the contaminants (i.e. weakly bound protein to the matrix) and purified proteins suspension obtain from Hibiscus seeds. The fluorescence EEMs of the coagulant protein suspension present the likely spectra of the coagulant protein in the different Hibiscus seed species. Figures (7.6a, d, and g) present the various peaks in the contaminants as observed in the weakly bound POP, PSP and PKP respectively. The locations of the peaks are similar to each other indicating that all the seeds belong to the same plant species. The dominance of peaks T and B in all the contaminants revealed that they contain high protein contents.

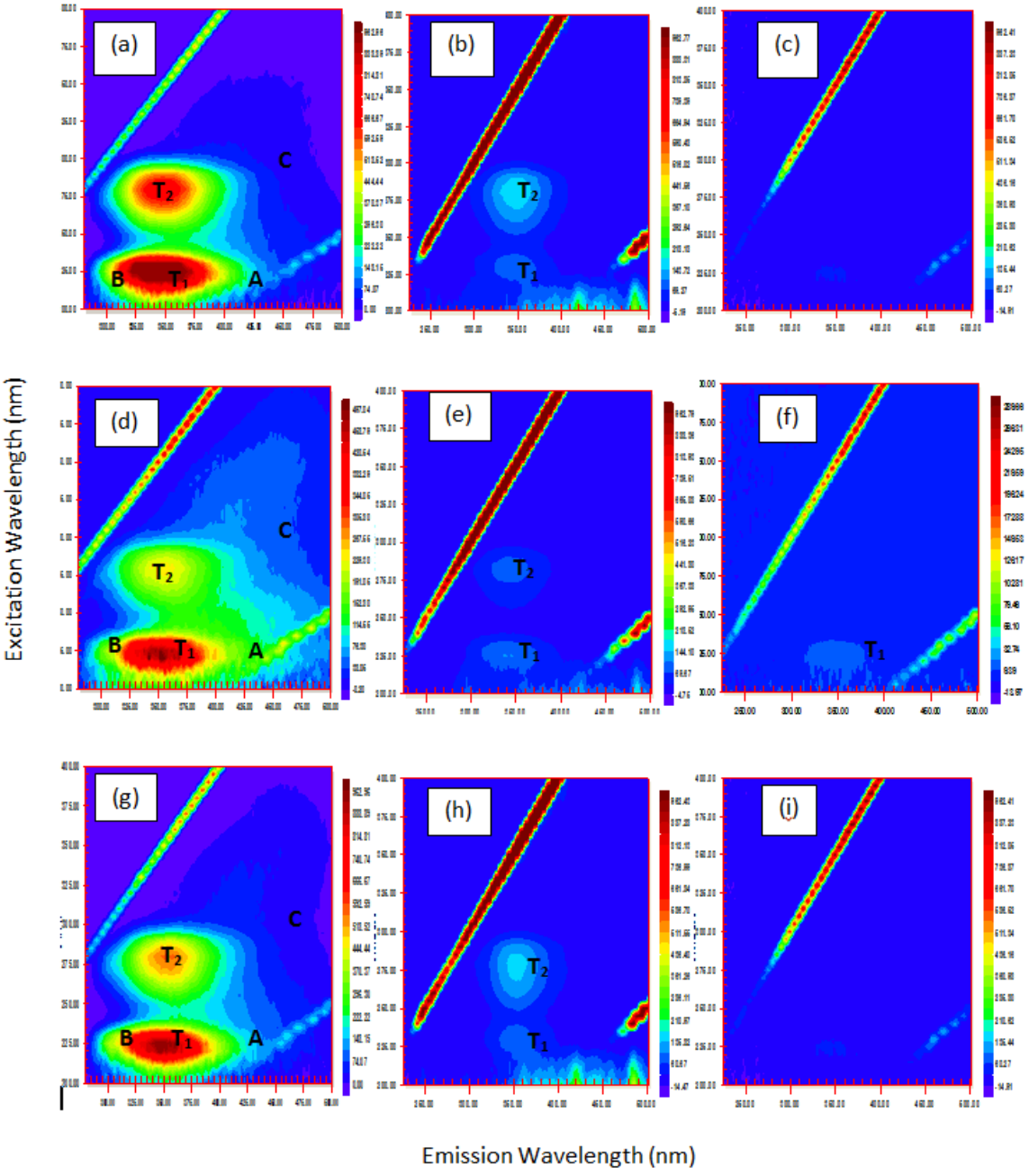

Figure 7.6 ***EEMs spectra of purified proteins (a) POP unabsorbed (b) POP - 0.3M (c) POP-0.5M (d) PSP unabsorbed (e) PSP - 0.3M (f) PSP - 0.5M (g) PKP unabsorbed (h) PKP - 0.3M (i) PKP - 0.5M NaCl.***

Figures (7.6b and c, e and f, then h and i) are the matrices of eluted fractions of *okra*, *sabdariffa* and *kenaf* proteins with 0.3 and 0.5 M NaCl solutions. Fractions eluted with the 0.3 M NaCl concentration contain coagulant protein compounds as revealed from preliminary jar test results. Peaks T_1 and T_2 are visible in all the samples after protein purification.

Additionally, all the samples eluted with 0.5 M NaCl solution showed no visible fluorophore signal and did not coagulate particles in water when tested. Under-coagulation condition with the 0.3 M suspensions, the Peak disappeared after the process, indicating its binding and adsorption ability with the NOM as seen in Figure 2b, c and d.

7.6 Summary

The impact of NOM in water coagulated with natural Hibiscus species were assessed in both crude and purified forms. Similarly, the coagulant proteins in the seeds were evaluated using fluorescence EEM peaks. At the end of the experiment, it was observed that water treated using the crude extract samples contributes significant organic loads into the treated water while the purified protein slightly reduced the DOC concentration in the final water both at optimum dosage. Peak T intensity was seen as a useful tool in evaluating the presence of DOC in water. However, in the absence of protein sequence analysis, it was observed that fluorescence spectroscopy could be used base on fluorophores signature to identify or characterised protein in a sample and their observed excitation and emission wavelengths used to know the particular protein in the sample base on the peak region.

CHAPTER 8 ANTIMICROBIAL POTENTIAL OF HIBISCUS SEED PROTEINS

8.1 Background

Traditional water treatment processes employ a disinfection unit to eliminate bacteria and viruses in water, using chlorine-based compounds (Farré et al., 2012) and the health benefits are well-documented and understood (Orme et al., 1990). However, this important unit process is too expensive to be utilised fully in the developing world because of cost issues associated with the import of and procurement of disinfecting chemicals. Consequently, the absence of such a critical unit process has increased the vulnerability of many rural communities to waterborne disease outbreaks and death. However, in many developing countries, natural extracts have successfully been used in treating ailments such as diarrheal disease (Dubreuil, 2013). Also, Ingale and Gandhi (2016) authenticated the claim of using MO aqueous extract in treating epilepsy and anxiety patients. The presence of antimicrobial agents in natural plant extracts are well-established and have been investigated against some isolated microbes (Ebrahimzadeh et al., 2009, Gothandam, 2010, Bindhu and Umadevi, 2013), e.g. *Cryptosporidium parvum oocysts* as reported by Petersen et al. (2016). However, despite its widely acclaimed applications in folk medicine (Matthews et al., 2009), there is a little information regarding the use of natural extracts as a disinfectant in water treatment. Therefore, it is important to understand the potential of natural extracts in water treatment especially their antimicrobial properties.

Three important locally available materials that are worthy of further investigation are OK, SB and KE seeds as presented here. The work reported considered both their crude and purified proteins as alternative disinfectant to the traditional chemicals in water treatment.

This chapter presents the results base on objective (v) of this thesis.

- **To study the antimicrobial potential of Hibiscus seeds using crude and purified coagulant protein samples.**

8.2 Bacterial inactivation potential of Hibiscus seed extracts

The inactivation of *total coliforms*, *faecal coliform* and *E-coli* was investigated with extract doses range from 50 – 200 mg/l. Figures 8.1 and 8.2 show the inactivation of *faecal coliform* and *E-coli* in raw water using OCE, SCE, and KCE.

8.2.1 Inactivation of faecal coliform using Hibiscus seed extracts

Figure 8.1 presents the disinfection potential of OCE, SCE and KCE against *faecal coliform* in raw water. The results revealed that the inactivation of *faecal coliform* increases with increase in dosage from 50 to 200 mg/l, after one-hour sedimentation. At 50 mg/l dose, OCE achieved 55% inactivation compared with SCE which recorded 53% and KCE 43% inactivation of the *faecal* bacteria respectively. With a 100 mg/l dose, the inactivation of OCE increased from 55 to 60%, while that of SCE increased from 53 to 62% while under the same conditions, KCE achieved an improved performance, from 42 to 53%. Similarly, at 200 mg/l dose, the inactivation of *faecal coliform* rose to 70% in OCE, 67% in SCE and 58% in KCE respectively. These results are quantitatively similar to those of Shaheed et al. (2009) who achieved increased inactivation of *faecal coliform* with a high dosage of Luffa cylindrical seed extracts. The work reported here shows that the inactivation of *faecal coliform* in water with Hibiscus seed extracts was dose dependent.

Furthermore, the study considered the standard LRV and compared it with the calculated percentage inactivation of *faecal coliform*. The LRV and percentage inactivation of *faecal coliform* were <1-Log (90%) across all samples doses. Additionally, the maximum overall inactivation of *faecal coliform* was 70% in OCE, 67% in SCE and 58% in KCE, using 200 mg/l dose. These results show that although all the extract might possess antimicrobial

agents, the inactivation of *faecal* bacteria was partial, considering WHO recommendation which stipulates a zero presence of *faecal coliform* in 100 ml water sample (WHO, 1998). Thus, it is unlikely for such a standard to be met with a current dosage. However, at higher doses other than the one use in the study the inactivation of faecal coliform would have been closer towards achieving the recommended WHO standard.

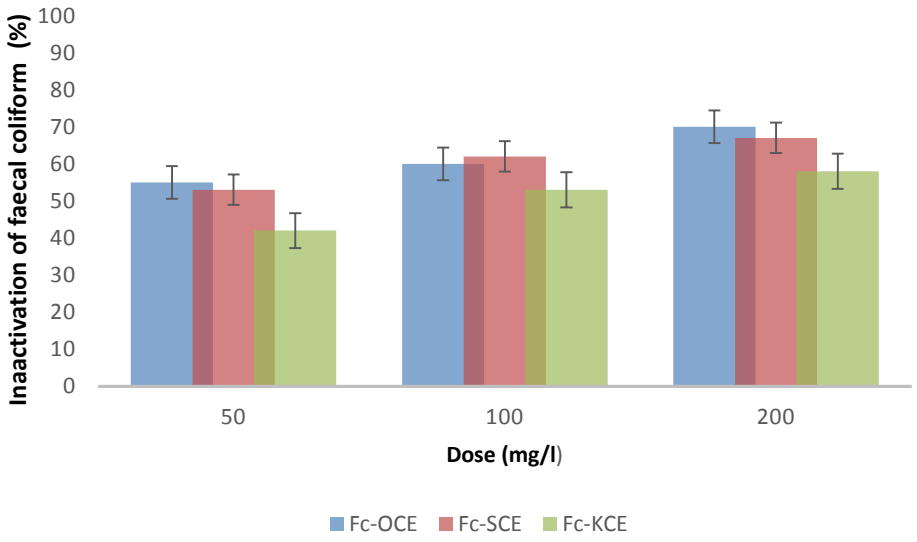

Figure 8.1 Inactivation of *faecal coliform* in River using OCE, SCE and KCE.

8.2.2 Inactivation of *E-coli* using *Hibiscus* seed extracts

The antimicrobial impact of the seed extracts against *E-coli* bacteria was also investigated and Figure 8.2 presents the inactivation of OCE, SCE and KCE against *E-coli* bacteria in the raw water. At 50 mg/l dose, 66% and 54% inactivation of *E-coli* were recorded in OCE and SCE while KCE achieved 49% performance respectively. At a dose of 100 mg/l, the inactivation of OCE deteriorated to 57% while minimal increased inactivation was witnessed in SCE, achieving 56%. However, a marked improvement in inactivation of *E-coli* was found in in KCE treated water with 100 mg/l dosage, achieving 58% from an initial 49% using 50 mg/l. Furthermore, a high concentration of Lysine and tryptophan compounds has been

reported in OK and SB seeds (Ismail et al., 2008) while other antimicrobial compounds such as tannin and alkaloids is also reported elsewhere (Hossen et al., 2013). While tryptophan can support the growth of E-coli bacteria (WHO, 1970), tannin was found to inactivate an isolated *E-coli* bacteria (Min et al., 2007).

With 200 mg/l dose, the inactivation of *E-coli* in OCE was also noted to be lower compared with 50 mg/l dose, achieving only 61% from an initial inactivation of 66%. Under the same condition, SCE achieved a small increase in performance to 60% from an initial 54% while as expected, KCE achieved the greatest inactivation of 65% from 49%. It is noteworthy that between 50 to 200 mg/l dose, the inactivation of both *faecal coliform* and *E-coli* bacteria in water using KCE achieved approximately 16% improvement. Similarly, the standard LRV of all the extracts against *E-coli* was <1-Log (90%), similar to the impact of the extracts against *faecal coliform*, where the results were not WHO compliant.

8.2.3 1.1.1 Inactivation of total coliform bacteria with *Hibiscus* seed extracts

Following 60 min sedimentation time, all the extracts recorded significant inactivation of faecal coliform and E-coli bacteria using varying doses of 50, 100 and 200 mg/l respectively. However, the most widely used indicator of pollution in water is the general coliform groups and E-coli bacteria. Although, the presence of E-coli in a water source is undoubtedly an indication that pollution of faecal origin has occurred from either man or animal. All members of the coliform groups may be of faecal origin and so any pollution should be treated as such except otherwise proved. Therefore, it will be of interest to test the sample against total coliform count in the raw water.

The inactivation of bacteria was investigated using OCE, SCE and KCE in raw water sample. The results showed that all large and small wells tested positive for *total coliform* count, (reads 2419.6 MPN/100ml).

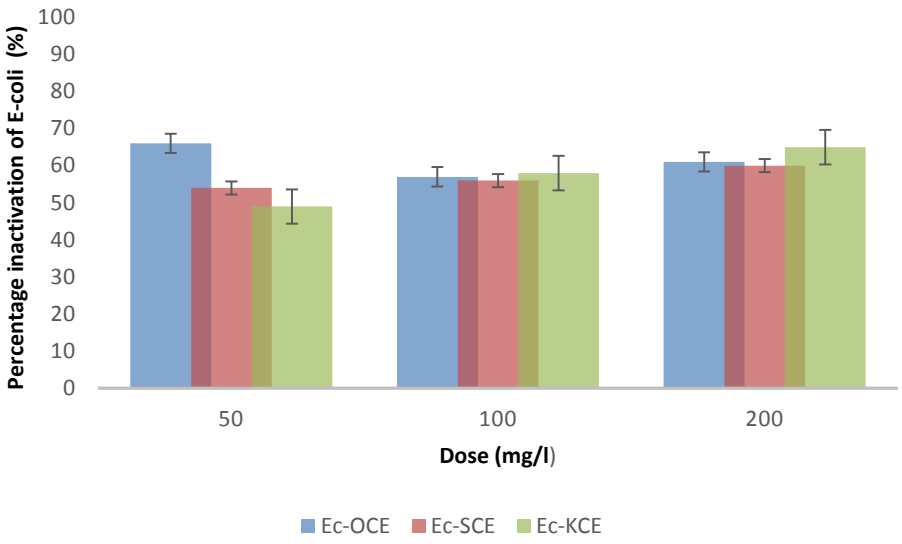

Figure 8.2 Inactivation of *E-coli* bacteria in water using OCE, SCE and KCE.

Treated samples were analysed for *total coliforms, faecal coliform* and *E-coli* after 72 hours treatment following Ndabigengesere and Narasiah (1998b). A significant increase in bacterial counts was observed, indicating the regrowth of *faecal* and *E-coli* count after storage. A maximum count of 2419.6/100ml was observed for *total coliform* and *faecal counts*, with a similar result for *E-coli*. It is clear therefore that either all the extracts possess other organic compounds that can support the growth and regrowth of bacteria in the treated water or that the microorganisms were largely not inactivated post treatment.

8.2.4 Inactivation of bacteria with purified *Hibiscus* proteins and AS

Tables 8.1 and 8.2 show the results for initial bacterial inactivation with POP, PSP, and PKP, and then AS in raw water and their results after lengthy storage before and after treatment. The calculated percentage reduction in total coliform count, faecal coliform and *E-coli* was also compared with the standard LRV. The raw water contained a bacterial colony of 517.2/100 ml for the total coliform count, 90.8/100 ml for E-coli and 114.5/100 ml for the faecal count.

The results show a significant decrease in bacteria count using 0.74 and 1.48 mg/l doses of POP, PSP, PKP and 15 mg/l of AS as a control obtain from preliminary jar test results. The percentage inactivation of *total coliform* count was approximately between 95 and 100% with either dose of the purified proteins while AS produced a maximum percentage inactivation in *coliform* count of 93%. Most notably, the 1.48 mg/l dose recorded the best removal efficiency in the coliform count of approximately 100% was achieved with POP, PKP and PSP respectively. In all cases, the standard LRV of *total coliform* was 2.7−log (99.9%). However, at 0.74 mg/l dose, the LRV of total coliform was found to be similar, 1.7−log (99%) for all purified proteins while the calculated percentage inactivation of *coliform* count was 99 and 98% respectively. With 15 mg/l dose of AS, the LRV was <1−log (90%). It is observed that most of the bacteria in the raw water are sensitive to the purified proteins at a higher dose.

The inactivation of *E-coli* and faecal species recorded almost 100% performance across all the samples using both 0.74 mg/l and 1.48 mg/l doses while AS achieved only 78 and 76% removal of *E-coli* and *faecal coliforms* respectively.

The LRV was approximately >2−log (99%) with the proteins whereas AS recorded a LRV of <1−log (90%) in *E-coli* and *faecal* inactivation in raw water.

8.2.5 The regrowth of bacteria after 72-hour treatment

Further experiment was performed on the regrowth of bacteria after 72-hour treatment in the re-suspended sludge in order to compare the results with that of regrowth of *total coliform* in Table 8.2. The results revealed a complete regrowth of *total coliform* in all the extract-treated water (2419.6/100ml, not in table), thus indicating of the presence of other microbes in the water that are largely unaffected by the extracts during the coagulation process. A complete bacterial kill of *E-coli* and *faecal coliform* after the lapse time of 72 hours was seen in treated water with the purified proteins. The results show strong antibacterial agents in the seed with

a high potential *E-coli* and *faecal coliform* inactivation that could achieve the WHO standard of zero presence of the two indicator organisms.

8.3 Summary

The inactivation potential of three *Hibiscus* seed species were assessed in Bourn brook river water. Both crude and purified protein was used in the water treatment process and their effects against *E-coli*, *faecal* and *total coliforms* evaluated. It was observed that, while the crude samples achieved only partial inactivation of bacteria with a LRV of <1(90%), the purified samples recorded approximately 100% bacterial inactivation with a LRV of > 2.7 (99.9%) which achieved the WHO zero presence of *E-coli* and *faecal coliform* per 100 ml of water. Similarly, after 72-hr post treatment, complete bacterial regrowth were noted in water treated using crude extract samples whereas with the purified proteins there was no observed regrowth in bacterial colonies after 72-hr post treatment. It is noteworthy that the use of purified *Hibiscus* protein offer clear advantage over the crude sample in water treatment, performing the double function as a coagulant and disinfectant simultaneously.

Table 8.1 Inactivation of *Coliform, E-coli* and *faecal* count in water using POP, PSP, PKP and AS

		POP (mg/l)		PSP (mg/l)		PKP (mg/l)		AS (mg/l)
Parameters	Raw water	0.74	1.48	0.74	1.48	0.74	1.48	15
Initial turbidity (NTU)	11.9							0.92
Number of positive wells for coliform:								
▪ Large wells	49	17	8	2	1	9	–	23
▪ Small wells	27	3	1	2	0	1	–	5
Total coliform count (MPN/100ml)	517.2	24.1	9.7	4.1	1	10.9	0	36.8
Percentage inactivation of coliform count (%)		**95.34**	**98.13**	**99.21**	**99.81**	**97.89**	**100**	**93**
LRV (%)		**90**	**99**	**99**	**99.9**	**99**	**99.9**	**90**
Number of positive wells for E-coli:								
▪ Large wells	40	–	1	–	–	–	–	15
▪ Small wells	7	–	1	–	–	–	–	3
E-coli count (MPN/100ml)	90.8	0	2.0	0	0	0	0	21.1
Percentage inactivation in *E-coli* count (%)		**100**	**99.61**	**100**	**100**	**100**	**100**	**78**
LRV (%)		**99**	**99**	**99**	**99**	**99**	**99**	**90**
Number of positive wells for faecal coliform:								
▪ Large wells	43	–	–	–	–	–	–	18
▪ Small wells	9	–	–	–	–	–	–	4
Total faecal count (MPN/100ml)	114.5	0	0	0	0	0	0	26.9
Percentage inactivation in *faecal* count (%)		**100**	**100**	**100**	**100**	**100**	**100**	**76**
LRV (%)		**99**	**99**	**99**	**99**	**99**	**99**	**90**

Table 8.2 Regrowth of *Coliform, E-coli* and *faecal* count after 72 hours using POP, PSP, PKP and AS.

		POP (mg/l)		PSP (mg/l)		PKP (mg/l)		AS(mg/l)
Parameters	Raw water	0.74	1.48	0.74	1.48	0.74	1.48	15
Initial turbidity (NTU)	11.9							0.92
Number of positive wells for coliform:								
▪ Large wells	49	49	49	49	49	49	49	29
▪ Small wells	27	48	48	32	48	48	48	15
Total coliform count (MPN/100ml)	517.2	2419.6	2419.6	686.7	2419.6	2419.6	2419.6	68.0
Percentage regrowth in *coliform* count (%)		**400**	**400**	**130**	**400**	**400**	**400**	**46**
Number of positive wells for E-coli:								
▪ Large wells	40	–	–	–	–	–	–	15
▪ Small wells	7	–	–	–	–	–	–	9
E-coli count (MPN/100ml)	90.8	0	0	0	0	0	0	28.4
Percentage regrowth in *E-coli* count (%)		**0**	**0**	**0**	**0**	**0**	**0**	**25**
Number of positive wells for faecal coliform:								
▪ Large wells	43	–	–	–	–	–	–	18
▪ Small wells	9	–	–	–	–	–	–	11
Total faecal count (MPN/100ml)	114.5	0	0	0	0	0	0	35.9
Percentage regrowth in *faecal* count (%)		**0**	**0**	**0**	**0**	**0**	**0**	**26**

CHAPTER 9 - DISCUSSION

9.1 Background

This chapter discusses the results presented in chapters 5, 6, 7 and 8 as they relate to each of the main objectives of this research work, using the three Hibiscus plant species in drinking water treatment.

- **Coagulation potential of Hibiscus seeds in household water treatment**

The first in-depth investigations on the use of OCE, SCE and KCE as coagulants in water treatment using turbidity values of 50, 100 and 200 NTU are presented in Chapter 5. All the findings evaluated show similarity with previous works on coagulation activity of natural extracts where the addition of coagulant resulted in minimal residual turbidity. At higher coagulant dosage, particle re-stabilisation occurred. The re-stabilisation of the particles was due to steric repulsion between the coagulant and the particles.

The presence of complex low MW proteins in the extracts was believed to be responsible for the enhanced coagulation process in treating high turbidity water (Lee et al., 2001). Earlier work by Kilduff et al. (1996) and Antov et al. (2010) had shown higher adsorption ability of smaller macromolecules derived from natural materials per unit weight of the adsorbent. Performance declined with turbidity (200-100-50 NTU) as reducing particle concentration resulted in fewer and weaker adsorption sites to provide effective bridging. Consequently, one of the essential requirements for effective flocculation performance as suggested by Bolto and Gregory (2007), is that there should be enough surfaces on the particles for adjoining attachment by other particles to encourage bridging. To minimise this effect, La Mer (1966) postulated that the optimum coagulant dose should cover approximately 50% of particle surface.

OCE achieved final residual turbidity of 5 NTU, which is compliant with the WHO and Nigerian drinking water standards (SON 2007). It is noteworthy that this action is associated with a higher amount of extractable protein concentration useful for coagulation in OCE, but lower in KCE and SCE samples. The work presented here agree with the results reported by Katayon et al. (2006) and Ndabigengesere and Narasiah (1998b) who observed significant coagulation activity by MO extract in high turbidity water. Turbidity removal was effective in high turbidity water because destabilisation is influenced by a high rate of particle collision efficiency with the coagulants for bridging. OCE also outperformed both SCE and KCE in the medium and low turbidity water, with reduced coagulant dose due to reduced particle concentration. Most importantly, the demand for a higher coagulant dose in high turbidity water than in low turbidity water was due to the increased particle concentration, although the relationship was non-linear, which was in agreement with the relationship observed by Pernitsky and Eng (2004).

Results on the impact of coagulant addition on treated water pH and turbidity removal agree with earlier work which employed different natural coagulants. The effect of pH on the extract in this study and MO in water treatment show similar behaviour. The treated water pH largely remains unaffected as a result of the buffering effect of (NH_2) and (COOH) groups in the extract which present an amphoteric behaviour. The incremental dosing of all the extracts including MO extracts yielded a plateau curve whereas alum was found to depress the pH of the water. In aqueous solutions, amino acids contain weak α-amino groups (basic) and week α-carboxylic groups (acidic) which buffers the water. Furthermore, each of the basic and acidic amino groups contains in its side chain an ionizable group, and so the combined affect act as effective buffers during coagulation, resisting any change in pH of the water. Thus, the use of OCE, SCE and KCE as coagulants offer an advantage over synthetic coagulants since

no chemical addition is needed to control the pH of the treated water and can be adopted as POU water treatment.

Furthermore, the relationship between coagulant doses at different pH was investigated as it affects protein stability, using the optimum coagulant doses. At pH4, turbidity removal was significantly ($p<0.05$) higher in OCE compared with SCE and KCE. Although all the extracts are anionic, the overall results for all the extracts at low pH met the WHO standard for residual turbidity in treated water. Thus, the improved coagulation efficiency between the colloids and the coagulants was primarily accomplished at low pH where kaolin particles are observed to be less negative than at neutral pH which encourages double layer compression (Gregory, 2005, Yin, 2010). While Okuda et al. (2001b) and Zhang et al. (2006) demonstrated that the most effective pH for coagulation using MO and *Cactus Opuntia* mucilage to be in the alkaline region, the study reported here observed that the acidic region is the most effective pH for coagulation. This demonstrates that the amino acid composition of proteins in different plants may have different coagulation activities. Therefore, the difference in coagulating property may be attributed to the type of proteins in Hibiscus seed, although this requires further investigation.

The performance of denatured protein extract was assessed based on different storage duration. Ageing of Hibiscus seed extracts was shown to have a significant impact on its coagulation performance. A lower dosage of denatured suspensions was required to obtain measurable turbidity removal efficiency by OCE and SCE with storage time of up to ten days but the efficiency of the suspensions degraded after that. In both cases, the best performance was observed using the 3-day old extract followed by 7 days, thus, suggesting that denaturation by storage can eliminate certain-coagulation hindering proteins in the extracts. In particular, there was discrete release of coagulant protein with considerable activity

following storage, resulting in increased coagulation efficiencies of the extracts. Conversely, protein storage presented an exceedingly complex scenario. A denatured KCE sample by storage did not yield any improvement in performance due to its low protein concentration. Another possible reason for low performance in KCE is that the remaining coagulant proteins after denaturation are low compared with the particle concentration resulting in low collision efficiency. However, the results are not in agreement with the findings of Katayon et al. (2004), who noted a decrease in turbidity removal efficiency of MO extracts stored longer than a day. The performance trend in KE extract is not surprising because the denaturation may have removed some proteins with limited coagulation potential in the KE seed, thus disrupting its random configuration, having little protein left for coagulation. Consequently, the unique unfolding behaviour between OK and SB and MO extracts may be attributed to the difference in their protein compounds, ionic strength and surface charges.

While some studies have shown that MO extract is a cationic electrolyte (Ndabigengesere et al., 1995, Kwaambwa and Maikokera, 2007), OK, SB and KE extract were found to be anionic in this work. Certainly, proteins can be denatured within few hours of storage, and such differences in behaviour can lead to different structural conformation. A wide range of characteristics can be exhibited by denatured proteins, from reduced solubility to communal aggregation. After the tenth day, the degradation in performance was severe, extensively caused by the aggregation, precipitation and repugnant odour emission from the protein samples. Additionally, there was another issue of physical protein agglomeration and adhesion on the container which could have added to the degraded performance after the tenth day.

It has been acknowledged that there are many other physical factors or processes which can cause protein denaturation. Heating is one such process, and so samples were denatured at different temperatures and their coagulation efficiencies evaluated after the heat treatment.

The comparative performance of OCE and SCE under extreme temperature conditions showed some similarities in their coagulation behaviours. The Jar test results revealed a definitive improvement in performance after heat treatment due to a change in protein conformation structure. The performance of the denatured OCE and SCE was impressive, exceeding that of native samples at the same optimum dosage. It is believed that the heating process changed the protonation pattern of the proteins, possibly by destroying the secondary and tertiary structure, with a renewed enhancement in their coagulation activity. At low dosage, there was limited coagulating compound, and after the elimination of proteins with partial activity, the amount of dispersed coagulant available for coagulation was limited in the denatured extract than in the non-treated samples.

A clearer understanding of the impact of protein denaturation by heating was seen in KCE. Surprisingly, after heat treatment, KCE achieved a significant improvement in coagulation efficiency than in OCE and SCE. The denaturation of the proteins was seen to be more advantageous in KCE where its Non-treated sample presented the worst performance due its low protein concentration which was further subdued by the activity of other contaminants. Furthermore, the degree of improvement was varied across the denatured samples at doses of 20-60mg/l, but improved performance was observed at higher doses across all the extracts. Thus, heating can improve the coagulation potential of Hibiscus extract for people in developing countries, where access to clean water is a major challenge. It was evident that even after the deterioration, the denatured samples still outperformed the maximum efficiency recorded by the non-treated extract. The heat treatment yielded a considerable amount of coagulant protein occasioned by the removal of coagulant-hindering proteins. The extracts are thermo-stable after heat treatment, which is advantageous for people in rural areas where the main source of energy is firewood. Furthermore, the heating process improved the effectiveness of the filtration process which could be done within minutes due

cracking and melting of the lipids. In the non-treated extracts, the lipids have the potential to clog the filter pores.

This study shows that the denaturation of proteins that are partially sensitive to storage time and temperature in water treatment are beneficial because the elimination of such proteins further improves the quality of the coagulant protein. The two processes are simple and straightforward to adopt as protein purification technology in developing countries, requiring no technical know-how.

The SDS-PAGE analysis of the crude extracts shows multiple protein bands in each of the extract as a clear manifestation that there are various heterogeneous protein compounds in the seeds. The MW of these proteins varies according to size and bands, each having different physical characteristics. Although observations of the protein profile show the physical appearance of the various bands to be somewhat similar, they have varied coagulation activity. The concentration of the bands indicates much overlapping across the protein compounds that may or may not have coagulation activity. Determination of the particular protein band, size or MW responsible for the coagulation activity in the extracts is challenging. Some of the proteins are contaminants with no potential activity while others may contain partial activity. Additionally, the presence of such proteins may render the coagulation process ineffective and could contribute to the overall NOM in the treated water. The presence of NOM in water poses challenges for water treatment, creating odour, taste, colour and encouraging bacterial growth (Bolto, 1995, Broin et al., 2002, Ali et al., 2010). Thus, NOM removal is necessary to minimise health concern related to DBP formation in the treated water, especially if chlorine is used as disinfectant (Singer, 1999, Bridgeman et al., 2011, Liu et al., 2014).

To facilitate the removal of the overlapping proteins in the band with limited coagulating activity and other compounds, protein purification by IEC was adopted. The purification of the proteins presented an identical single protein band with low MW of 39 kDa in the SDS-PAGE analysis. The work reported found that only the protein fraction eluted with the low ionic strength of 0.3 M NaCl solution has coagulation activity, thus, indicating the purity of POP, PSP and PKP in the SDS-PAGE analysis. Apart from the coagulation potential observed with the 0.3 M fraction, water treated with this sample also showed no sign of organic nutrient loads. Contaminants that were not bound to the matrix were eluted as unabsorbed. It was evident that all the other protein bands seen in the crude extracts were contaminants or proteins with weak or no coagulation activity which could add more NOM in the treated water. It is noteworthy that the unabsorbed and weakly bound proteins to the matrix, and the strongly bound proteins to the matrix that were either eluted with the starting buffer or with the high ionic strength of 0.5 and 1.0 M NaCl were non-coagulant proteins.

Comparative analysis of the crude and the purified protein has shown the presence of many macromolecules in the extracts while the purified proteins consist of a single small size protein. This observation was seen in MO protein having two distinct protein bands when it was isolated and characterised by Gassenschmidt et al. (1995), Ghebremichael et al. (2005) and Ghebremichael et al. (2006) with increased adsorption properties. It is thought that the elimination of other non-coagulant protein, are the reasons for the increased coagulation activity, initially retarded due to protein-protein complexes (Bodlund et al., 2014). Assessment of the coagulation activity of POP, PSP and PKP showed increased water treatability of the proteins compared to the crude extracts at a reduced dosage of the purified proteins. The low dose requirement of the purified coagulant protein samples was caused by increased adsorption action of the proteins (Gassenschmidt et al., 1995).

Furthermore, after one hour sedimentation time, sludge generated by natural extracts and AS were measured and compared. The sludge volume formed with the purified proteins was smaller compared with AS sludge. The explanation for this is that the sludge produced when using the extracts resulted from agglomeration of the coagulated particles that settled as macro flocs with no form of precipitate. In the case of AS, sludge production was due to the formation of acidic hydroxide precipitate. Furthermore, sludge produced from natural coagulants is biodegradable and can be used as fertiliser on farms because it in non-toxic. The low sludge volume generated by Hibiscus seeds facilitates a reduction in the cost of sludge handling in water treatment compared with AS flocs which are difficult to dewater (Letterman, 1999, Ndabigengesere and Narasiah, 1998a).

As the work reported here investigates the coagulation mechanism of the extracts using both kaolin and river water. Measurements of zeta potential before and after treatment in kaolin water using the three extracts show a significant increase in zeta potential in the treated water. Even though the coagulants are anionic, and kaolin particles are negatively charged as demonstrated by Katayon et al. (2004), the coagulation mechanism requires further investigation, because it unlikely by charge neutralisation. Most notably, during adsorption, kaolin particle can formed hydroxylated surfaces in water (Sharp, 2005). For optimum flocculation performance, zeta potential should be near zero (Kleimann et al., 2005, Bolto and Gregory, 2007, Hunter, 2013, Morfesis et al., 2009). In all these cases, the zeta potential operating window of the extracts was $-6.67 < \zeta < -4.39$ mV, with minimal turbidity in the treated water. The difference in the zeta potential operating window was due to the coagulation mechanism by the natural extract, which was by adsorption and bridging While in the UK river water with multi-charged ions, metal coagulants are used, the effect of surface charge on the particles may be quite different.

The zeta potential of the river water presented in this work is within the range reported by (Sharp, 2005). The coagulation mechanism in river water was different due to background effect of NOM in the raw water. A similar observation was reported by Mpofu et al. (2003) who investigated anionic polyacrylamide acrylate copolymer in kaolin water. These findings suggest that the mechanism exhibited by Hibiscus extracts is unlikely to be via charge neutralisation, but rather by adsorption due to the positioning of the surface shear plane. River water may consist of variably charged ions and various NOM from different sources which may affect the coagulation mechanism. Many studies have shown that the presence of bivalent ions such as magnesium and calcium can also enhance flocculation activity (Tripathi et al., 1976, Choy et al., 2015).

- **The growth, breakage and regrowth of coagulated Hibiscus flocs**

For the first time floc formation and breakage experiments were conducted to assess the strength of flocs generated by Hibiscus extracts in water treatment. The study provided an improved understanding of the potential of the seeds in water treatment. Both crude and purified proteins were considered as primary coagulants or as coagulant aids with AS in the treatment process.

The results show that the median d_{50} floc size formed by the coagulants to be smaller in SCE and KCE, compared to the traditional metal salt coagulant, AS, before breakage because of their low protein concentration and secondly due their high NOM compounds. NOM can negate floc growth through steric repulsion and modification of the thickness of the hydrodynamic layer (Sharp et al., 2006). While high lipid contents in seed can affect floc growth potential, the presence of high protein concentration, especially in OK seed was likely responsible to have been responsible for its larger flocs, equivalent to that of AS-flocs. However, both OCE and AS achieved the largest floc size after reaching the steady growth

state, but from the growth plot, it would appear to be unlikely that either SCE and KCE derived-flocs had reached the steady growth phase before the re-introduction of the high shear rate. Hence, the size of the flocs generated by SCE and KCE could have grown larger had additional flocculation time been applied before reintroducing the breakage shear rate.

Assessment of the most cost-effective water treatment process could be based on the performance of the coagulant that produces larger flocs within a shorter coagulation time (and hence faster floc growth rate). The floc growth properties presented in this work clearly show that OCE produced larger flocs with a shorter growth time compared to AS, although flocs formed by AS performed better in this regard than SCE and KCE flocs. Most importantly, the high performance in OCE is linked to higher protein contents in OK seed as reported by (Oyelade et al., 2003), compared to SB and KE seed with a lower protein content also reported by (Rao, 1996) and (Mariod et al., 2010) respectively.

The post-rupture floc sizes produced by all the samples as primary coagulants show a significant initial decrease in d_{50} values, followed by a gradual decline as the high shear rate continued. The operational significance of the high shear rate was to introduce an extension of protein carbon chain and subsequent straining of the middle bond, causing rupture and production of smaller MW protein (Scott et al., 1996). However, with low MW, after the re-introduction of the slow stirring, floc collisions were encouraged and re-growth ensued. Furthermore, despite the severe effect of the breakage shear on OCE flocs, all samples produced almost the same size of re-grown flocs. This evidence confirms the reports that NOM/DOC including lipids can affect the strength of the bond because flocs formed under such conditions are more fragile (Jarvis et al., 2005b, Sharp et al., 2006). Furthermore, flocs generated by KCE possess a higher strength factor than flocs produced by OCE and SCE because KE seed is characterised by low lipid contents. Hence, its protein may have a higher

affinity to bind to other molecules in aqueous solution. The observation that the re-grown flocs were similar across all the extracts is considered to be a result of the effect of surface charge re-distribution. In the solid-liquid interface, several conditions can affect protein conformational structure and can play a critical role during the coagulation process (Yu and Somasundaran, 1996).

The combination of AS with Hibiscus extracts as coagulant aids improved the coagulation performance of the extracts. Improvements were influenced greatly by the adsorption and bridging action of the extracts after AS had provided the needed charge neutralisation effect. The subsequent addition of the extracts provided effective adsorption as a polishing step to bridging the flocs, so as to increase the effective particle collision radius to give greater contact opportunity to form larger flocs. The AS produced the double layer compression through charge neutralisation of surface charges on the colloids which encourages bridging bonding by the extracts. The SDS-PAGE results present many varieties of macromolecules in the extracts which controlled the rate of polymer bridging to obtain a compact structural bond. This result agrees with the work reported by Yu et al. (2009), who observed that flocs formed by charge neutralisation and bridging action are larger than flocs generated by simple charge neutralisation. The production of larger flocs that can settle faster greatly improved the coagulation and flocculation processes.

The fastest floc growth rate was seen in the AS+SB combination compared to AS+KE and AS+OK because its protein has higher affinity for bonding with other molecules under this condition. This phenomenon may be explained by the distinct feature of its side chain and again its binding affinity which witnessed higher interaction activity within the AS aqueous media. Overall, proteins may have similar structures but could exhibit different behaviour, as such, the two basic controlling factors that govern coagulation by natural extract are protein conformation and charge properties (Yu and Somasundaran, 1996). As coagulant aid, it is

likely that the high lipid in OK seed has effectively suppressed the charge neutralisation potential of AS through surface coating of the particle, as reported elsewhere (Ali et al., 2010).

When used as a coagulant aid, the largest regrown size was found in AS+OK compared to that of AS+SB and AS+KE. The high performance was due to the charge neutralisation action of AS, which reduces the separation distance created by the OM and establishes more contact opportunity. OK, having the highest protein concentration, took advantage of this for re-adsorption and bridging of the broken flocs. Interestingly, there was no significant difference between flocs formed by AS+SB and AS+KE at steady state before and after breakage. These results further show the dependence of flocculation rate by the extracts on the amount of protein in the seed and the effect of the overall NOM in the water.

As expected, the characteristics of OK-derived flocs resulted in a higher strength factor because stronger flocs can lead to larger floc growth (Muehle, 1993). However, Sharp et al. (2006) indicated that larger flocs are fragile but other than floc size, the study shows that the strength of the flocs may also depend on other factors such as the binding affinity, MW, charge potential and ionic strength of the protein. As primary coagulants, the strength and regrowth of both SB and KE flocs were larger than OK due to their low lipid contents. The strength of OK flocs deteriorated due to weak inter-particle bonds resulting from the impact of the lipid as primary coagulant. The work reported here observed a direct relationship between floc strength factor and floc recovery factor in both OCE and KCE. It would appear that poor floc strength led to limited floc recovery, whereas stronger flocs exhibited a level of significant floc re-growth irrespective of the coagulation condition.

The impact of low pH coagulation on floc growth, breakage and re-growth ability of SCE and KCE indicated a relative increase in floc size while during the same growth period, the d_{50}

floc size of OCE decreased significantly. The change in floc size experienced across the extracts at low pH was due to a change in protein conformation and charge with pH. The electrostatic interaction of the ionisation of COOH groups is pH dependent when in contact with other ions in a solution (Yu and Somasundaran, 1996, Kwaambwa and Maikokera, 2007, Kwaambwa and Rennie, 2012). An assessment of floc strength formed at low pH coagulation shows that a change in water pH significantly impacted the coagulation process. As seen in this work, the change in pH from neutral to acid level causes a notable increase in strength factor of OCE and SCE flocs because pH can influence the ionic strength of protein and particle charge. Under low pH coagulation, the ionic strength of the system improves by reducing the repulsive force and enabling more adsorption sites for tighter bonding. Earlier work by Cao et al. (2010) and Sun et al. (2011) reported that flocs formed in the acidic pH region were stronger and more recoverable than flocs generated in alkaline conditions.

Several polymeric substances are widely used to aid floc growth and improve the performance of water treatment processes. The results presented in this work showed a significant improvement in floc growth when POP was dosed in combination with AS. A low dose of POP+AS produced the largest floc size due to the purity and concentration of the protein obtained in the IEX. The reduction in coagulant aid dose requirement was due to an increase in the ionic strength and adsorption potential of POP. Overdosing of protein+AS resulted in insufficient adsorption sites. However, flocs produced by POP when used as primary coagulant were smaller in size because the coagulation mechanism was entirely by adsorption and bridging only. Although high coagulant aid dose led to saturation of sorption sites, the concentrated protein helped in fast tracking the coagulation process, resulting in a significant floc growth. Equally, the higher dose of POP caused a slight improvement in floc strength, producing stronger flocs with well-compact structure (Jarvis et al., 2008).

Similarly, PSP produced the largest floc size with the smallest dose in conjunction with AS where overdosing caused saturation of polymer bridging sites with a resultant decrease in size. La Mer (1966) postulated that optimum dosage should correspond to half of the particle surface coverage. Flocs generated with PSP assumed a different pattern of growth, where the absolute deviation of the median floc size about the mean value was found to be greater than in flocs generated by POP. This trend may have been caused by the thread-like flocs, which were visible to the naked eye under lamination, growing in length and circumference. It is clear that the ionic strength of PSP species was not sufficient to compress the double layer during coagulation as shown by its surface charge potential and the peptide chain of the purified protein was virtually ineffective. Furthermore, overdosing with coagulant aid dose fast-tracked the coagulation process resulting in a more rapid floc growth rate but smaller d_{50} size. The formation of smaller sized floc was due to saturation of charges with high collision efficiency because smaller flocs have high collision rate.

The impact of increased dosing was investigated when PKP was the coagulant. The combination of low coagulant aid dose with AS again produced the largest floc size but deteriorated when more dose was added. In this case, both the floc strength and recovery factors increased with every stepwise addition of coagulant aid dose. The behaviour of PKP clearly show some distinct properties after purification. These changes may be due to increasing ionic strength, charge, molecular size and a general change in its structural conformation, and similarly its affinity to bind to other molecules was enhanced. However, when the results of using PKP as coagulant aid was compared with its floc properties as primary coagulant, a notable improvement in floc size, strength and re-growth was observed. Under similar conditions, Aguilar et al. (2003) and Jarvis et al. (2008) showed that the clarification of water is better than in system with smaller size flocs.

The extraction of the lipid via IEX column increase the strength of POP and PSP compared to AS as primary coagulants because the lipid can inhibit inter-particle bonding. However, the only clear benefit of the small floc produced, as shown by Jarvis et al. (2005a), is that the resistance of smaller flocs in turbulent flow regions is higher than that of larger, weaker flocs. The addition of more coagulant aid dose did not improve floc strength, except in the case of PKP, which almost doubled the strength of AS flocs. The behaviour of PKP was coincidently similar to AS in work reported by (Yu et al., 2014), who indicated that an increase in AS dose during coagulation resulted in increased floc strength.

- **Impact of NOM addition in treated water using Hibiscus plant seeds**

The impact of NOM addition on OCE, SCE and KCE performance was in agreement with previous investigations where NOM loading in water was seen as a significant challenge to treated water quality. The results show excessive DOC addition, more than 3-fold in OCE and 2-fold in SCE and KCE treated water. Apart from protein, crude Hibiscus seed extracts contain multiple biomolecules, and other organic compounds and its application as coagulant led to increased DOC in the treated water. The release of NOM from the extracts could have adverse effects on treated water quality. Technically, the use of natural Hibiscus extract may not be a feasible alternative as coagulants in water clarification because of this challenge. However, all the three Hibiscus seeds are primary sources of food in most tropical countries, they are non-toxic and medicinal, and therefore drinking water which had been treated with the extract is unlikely to pose any health concern. Furthermore, the high organic loads may lead to deterioration of undesirable water quality (e.g. taste, odour and colour) due to decomposition of OM. Conventional practice requires that water suspected of any form of microbial activity to be disinfected, but chemical disinfectants such as chlorine may react with NOM to exacerbate the problem further due to DBPs formation (Bridgeman et al., 2011,

Liu et al., 2014). Hence, the use of natural extracts of Hibiscus origin should better be employed as POU water treatment option for low-income countries where treatment is encouraged to be consumed within 24 hours. Previously, Jahn (1986) and Ndabigengesere and Narasiah (1998a) proffered a solution to the NOM problem using MO extract, and they recommended that water be consumed within 24 hours of treatment. This is important especially in a situation where each hamlet collects their drinking water from a single scarce source daily for domestic activities. For large scale water production, however, where residencs time in pipe network could be days, the use of crude extract is not recommended due to deterioration in water quality caused by decomposed non-coagulant compounds.

In addressing the challenge posed by high DOC addition, Hibiscus seeds were purified in an IEX column and their performance evaluated. The performance of purified POP, PSP and PKP was found to be superior to the crude extracts at a reduced dosage due to increased adsorption ability. At optimal dose the purified proteins did not add DOC to the treated water and showing high adsorption capacity which is free of unwanted organic compounds.

The assessment of the impact of NOM in treated water was additionally performed using fluorescence matrices. Fluorescence EEM's of raw and treated water with either crude extracts or purified proteins show some clear, distinct features. The dominance of Peaks T and B in clarified water treated using crude samples was as a result of OM contribution from the extracts. The extracts consist of multiple macromolecules, and other than proteins, some compounds gave rise to fluorescence emission of Peak A and C, humic-like substances especially in KCE, which has low protein concentration as seen in Chapter 5. Kwaambwa and Maikokera (2007) had shown a direct relationship between fluorescence intensity and concentration in MO protein, but other factors such as inner filtering effect will undoubtedly affect this relationship. The high fluorescence intensities of the protein-like Peaks by the

extracts could give rise to deterioration in water quality (Ndabigengesere and Narasiah, 1998a). Previously, Baker (2002) and Baker et al. (2008) related the presence of Peak T to microbial activity. Hence, the increase in Peak T signal could significantly cause increased microbial activity as substrates for microbial growth. Henderson et al. (2009) postulated that Peak T could be used to monitor contamination in water. Therefore, this work has found a positive link between measured DOC concentration and fluorescence intensity as a powerful tool for evaluating water quality. Although the correlation was not linear, there was evidence of increased DOC and fluorescence intensity in treated water compared to raw water characteristics. Again due to the impact of protein purification, water treated with the purified proteins showed a decrease in fluorescence intensities and emission peaks. The decrease in intensity was an indication that the proteins may have lost both the secondary and tertiary structures. Also, the reduced emission led to blue shift emission, suggesting denaturation. Thus, Peaks T and C intensity and emission wavelength can provide useful information regarding DOC presence in water (Gone et al., 2009, Bieroza et al., 2009, Henderson et al., 2009).

The EEMs of the coagulant protein suspension present the likely spectra of the coagulant protein in Hibiscus seeds. After purification, the results show the most likely coagulant proteins in the region of tryptophan-like protein; Peak T_2 was dominant in all the suspensions. This Peak disappeared post-coagulation, indicating its binding ability to the particle which settled out with the other NOM. Residual tryptophan is reported to have higher adsorption ability than tyrosine (Chen and Kenny, 2007).

- **Bacterial inactivation potential of crude and purified Hibiscus protein**

The antibacterial activity results of OCE, SCE and KCE, and the purified proteins POP, PSP and PKP, and AS on contaminated river water are presented in Chapter 8. The results show

the trend of *faecal coliform* inactivation by the crude extracts to be dose dependent and none of the extracts achieved the WHO zero count for *faecal bacteria* after the treatment. However, it is evident that the seeds possess active antimicrobial agents. The presence of saponin, alkaloids and tannin in the seeds are responsible for the *faecal bacteria* inactivation (Mungole and Chaturvedi, 2011, Okasha et al., 2014, Cheng et al., 2016). Zhang et al. (2012) used alkaloids from Berberine to treat diarrheal disease while Ahn et al. (1998) and Min et al. (2007) demonstrated significant inhibition potential of tannin on *CI. Perfrigens, CI. Paraputrificum, E-coli* and other bacteria species. The presence of saponins in Luffa cylindrical seed extract tested against *total coliform*, and *faecal coliform* has also been acknowledged (Shaheed et al., 2009).

Furthermore, although, the LRV by the extracts were less than 1Log (90%) when *E-coli* was investigated, the trend of inactivation was inconsistent with coagulant dose across the samples. The relationship between inactivation and dosage is important in KCE as it revealed the sensitivity of its active compounds, and this was not surprising as it contains high bioactive compounds (e.g. saponin, alkaloids, tannin, essential oil, steroids) (Mohamed et al., 1995, Cheng et al., 2016). At the end of the treatment process, *total coliform* count remain unaffected, indicating the presence of many other microbes in the water sample that are not sensitive to the active agents in the extracts.

A major conclusion of this work is that the antimicrobial investigation conducted using crude seed proteins show some inhibition properties in the extracts. However, the major setback of using the extract as disinfectants is that they did not show a complete inactivation of the microbial population that would warrant them to be considered as possible disinfectant alternatives. However, to address this shortcoming, the seed proteins were purified to eliminate essentially some of the OM and the non-coagulant protein that might affect its inactivation performance.

For the first time, Hibiscus plant species were purified and tested to determine their antimicrobial potential on *total coliform, faecal* coliform and *E-coli* count in raw water. As anticipated, the protein purification process increased the bacteria inactivation performance of POP, PSP and PKP at a reduced dosage. Complete inactivation of *total coliform* was observed due to the removal of organic compounds in the seeds. Such compounds which may have been added to the original OM in the system with high negative tendencies of neutralising the active compounds and shielding of microorganisms are removed. In this work, PKP and PSP have shown stronger antimicrobial potential than POP and 15 mg/l of AS dose under the same coagulation condition, indicating their disinfection potential. It is believed here that most of the bacterial colonies present in the raw water had shown significant sensitivity to the purified active antimicrobial agents compared with the crude extracts. However, it is unclear at this point to explain which of the pathogens found in the raw water source are more sensitive to the antimicrobial agents, since the inactivation was based on *total colony* count. Examination of the two most significant subsets of total coliform, popularly considered as indicator organisms as in the case of the natural extracts e.g. *faecal coliform* and *E-coli* revealed excellent performance. The complete inactivation of *faecal coliform* and *E-coli* by all the purified protein further solidifies the earlier argument that the presence of saponin, tannin, alkaloids and RIP in the seeds is responsible for the disinfection activity. It is clear that the two indicator organisms are highly sensitive and were part of the microbes that were eliminated during the treatment.

Interestingly, after 3-day storage, the results showed a robust performance against *faecal coliform* and *E-coli* bacteria. Even after re-suspension of the sludge, complete inactivation of these organisms was still seen, indicating that both *E-coli* and faecal *coliform* are highly sensitive to the antioxidants. It is also likely that the residual protein in the treated water was able to disinfect secondary microbes that have contacts with the treated water during the 72-

hr storage. *Total coliform* consist of multiple microbial colonies and so, there is the need to identify further and investigate the various species as done with the indicator organisms.

9.2 Summary

Multiple presence of biomolecules in Hibiscus extracts is essential and responsible for effective coagulation and flocculation activity in water treatment. Proteins are amphoteric substances, and thus offer considerable advantage over AS, as the pH of the treated water remains unaffected with high colloidal interaction at acidic pH. Interestingly, denaturation by storage or heating eliminates contaminants, resulting in a definitive improvement in coagulation efficiency. Additionally, the prevailing coagulants are more stable after heat treatment. The zeta potential window shows a two-way scenario where charge increased charge was seen in kaolin water whereas in river water where residual DOC played a significant role during the coagulation process zeta potential remain unchanged. Most importantly, the application of Hibiscus extracts produces small sludge volume. Flocs produced using Hibiscus seed in combination with metal salts are more resistant to breakage, and are bigger than flocs produced by AS alone. The extension and uncoiling of random carbon chain promote bridging through patchwise effect of AS. Furthermore, organic nutrients in final water disqualifies Hibiscus extract as a safe water treatment candidate as it encourages microbial growth. However, purified proteins rendered Hibiscus a perfect candidate showing minimal OM addition in treated water. The coagulant protein in Hibiscus seeds were tryptophan-like proteins based on Fluorescence EEMs analysis. The EEMs results also present Peak T and C intensities and emission as possible tools for monitoring DOC presence in water. It is obvious that many biological activities and particulates interfered with the disinfection process with the extracts, but the purified proteins were capable of, and demonstrated, complete bacterial inactivation with no evidence of regrowth over 72-hr post-treatment.

CHAPTER 10 – CONCLUSSIONS AND RECOMMENDATIONS

10.1 Background

The results of this investigation on the potential of using three Hibiscus plant species in drinking water treatment have been presented in preceding chapters. The main conclusions arising from the research are presented below and recommendations made as to how they could be employed further to evaluate other areas beneficial to sustainable development in clean water production.

10.2 Conclusions

- In this work, the efficacy of Hibiscus plant seeds as a coagulant or coagulant aid in both crude and purified form to provide drinking water supply comparable to drinking water quality obtain with the traditional water treatment coagulants has been demonstrated. The coagulant protein was identified in the purified proteins as a low MW protein around 39 kDa. Characterisation by fluorescence EEMs revealed that the active coagulant compound is a tryptophan-like protein which possesses anionic characteristics based on surface charge.
- All three species investigated in the study can be used to facilitate removal of colloidal impurities in contaminated water, and are capable of substituting the use of metal salts in developing countries. However, the crude samples are poor in treating water with low solid concentrations. The presence of multiple compounds in the extract makes it a potential coagulant for drinking water treatment which is not harmful to human health.
- Use of Hibiscus extracts for water treatment did not affect the pH of the clarified water because proteins are amphoteric substances and provide a buffer during the

treatment, thus, eliminating the requirement for additives to adjust the pH of the final water.

- Extreme temperature and storage conditions prompted the discrete release of flocculating compounds in the extracts with a corresponding increase in coagulation performance as a result of the removal of protein-protein complexes, which hinder coagulation activity. As such the extracts may be considered thermo-tolerant, not requiring any particular environment or storage facilities. This advantage was also notable with the raw, unprocessed seeds.
- Cost-saving opportunities are available via the use of Hibiscus seeds compared to the procurement of traditional water treatment materials, enabling the channelling of resources to other alternative infrastructures in the developing world.
- Several clear benefits have resulted from the use of Hibiscus plants as water treatment material with regard to improvement in floc properties. When used as a coagulant aid in combination with AS, there was a significant increase in solid/liquid separation process performance associated with the large agglomerates that were formed. The longer-term consequence, of this would be a reduction in volume of imported chemicals in low-income societies with resultant low sludge volume production. The sludge arising is non-toxic and could potentially be used as manure on farmlands.
- Water coagulated using crude Hibiscus seed extracts or its purified form produced stronger flocs than that of AS with excellent recovery ability, and giving it a distinct advantage over AS flocs.
- The results of this study show the potential of using fluorescence EEMs to monitor the level of organic matter contamination in water. Peak T and C intensities were used to evaluate the amount of DOC removal in final water. It was also observed that, in

the absence of protein sequence analysis, fluorescence fingerprints are a valuable tool to identify the character of coagulant protein in a seed sample.

- The antimicrobial action of Hibiscus seed protein was attributed to active compounds in the seeds. Most indicator organisms were fully inactivated and removed with no evidence of reactivation after considerable residence time using the purified samples. The crude seed extracts also showed partial inactivation of bacteria in water. However, the presence of particulates and other organic compounds in the water interfered with the disinfection process.
- The protein purification process is relatively simple and straightforward to scale up using locally available materials. As such it will encourage large-scale production of purified Hibiscus protein in order to make water accessible for rual community.

10.3 Study limitations

- Lack of isoelectric focusing apparatus to identify the IEP of the proteins affected the purification process because the buffer pH adopted in this work was taken based on the performance of the crude extracts across a range of pH values and not the IEP of an individual protein. The situation might have affected the degree of purity of the coagulant proteins obtained from the study as the buffer and column pH adopted might not be the correct pH.

- Many rural communities used locally made materials for processing the coagulants. However, the report presented here did not use locally made materials due to time constraint. Thus, it is recommended that locally made material be evaluated in water treatment using Hibiscus seeds.
- Lack of MALDI-TOF-MS/MS to conduct the protein sequence analysis to identify the coagulant protein and obtain information of the various amino acid peptides

responsible for the coagulation activity has affected the general results. A clear knowledge of the number of peptides would have aided more robust explanations regarding the behaviour and properties of the coagulated flocs.

10.4 Recommendations for further work

This research work showed the potential of using Hibiscus plant seeds as alternative materials in water treatment. Sustainable water treatment using the seed extracts would be feasible only if several steps needed to address some of the notable challenges are put in place. They include, but are not limited to, the following:

- Several studies have previously linked the presence of natural organic matter as a precursor for disinfection by-products formation during disinfection using chemicals such as chlorine. In many related studies, including this work, it was observed that the main setback of using natural extracts in water treatment is the release of organic compounds in the treated water. Therefore, water treated using Hibiscus extracts should be investigated for disinfection by-product formation if it is proposed for large scale water production where disinfection process will be incorporated.
- This study used NaCl concentration in both the extraction of the crude protein and in the elution of the purified samples. Thus, there is the need to examine whether the crude extraction process and elution with NaCl during protein purification may require desalting of the coagulant suspension before application in water treatment, by checking the salt contents and its impact on the overall water quality.
- MO seed oil has been exported from India to Europe and used as a lubricant in many industries. Therefore, the large volume of lipids extracted from the seeds could be advantageous; it is essential to investigate the chemical composition of Hibiscus seeds

oil to evaluate its applicability in other ventures such as lubricants in industrial application and as household cooking oil.

- Characterisation of protein fractions by conducting protein sequence of the amino acids peptides is important to understand the sequence of the coagulant proteins.
- The work primarily investigated turbidity and bacterial removal in water; as such the use of natural Hibiscus seeds in wastewater treatment and water with heavy metal compounds should be exploited. Additionally, the work reported used two indicator organisms to assess the impact of the crude extracts and purified proteins on those microbes; it is suggested that other microbial in water should be investigated individually to ascertain their resistivity to the extracts. Finally, investigating the mode of action and bacterial inactivation mechanism of the extracts is another important area that requires further evaluation. Incorporating processes such as solar disinfection together with the crude extracts is highly recommended for evaluation.

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