

**TURBIDITY AND DISINFECTION EFFICIENCIES OF
Hibiscus Sabdariffa SEED EXTRACT IN WATER.**

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Hibiscus Sabdariffa SEED EXTRACT IN WATER**

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PGD CIVIL ENG.

**A DISSERTATION SUBMITTED TO THE
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(M. ENG)**

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DECLARATION

I hereby declare that this work is the product of my research efforts undertaken under the supervision of Engr. Hussaini A. Daura and has not been presented anywhere for the award of a degree or certificate. All sources have been duly acknowledged.

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CERTIFICATION

This is to certify that the research work for this dissertation and the subsequent write-up of Habiba Abdullahi, with reg. no. SPS/13/MCE/00041 was carried out under my supervision.

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APPROVAL

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ABSTRACT

The study assessed the potential of using *Hibiscus Sabdariffa* seed extract (HSLSE) as a coagulant and for disinfectant in water treatment. It is aimed at determining its efficiency in turbidity removal and disinfecting of water. The seeds were winnowed, ground and sieved to 175 μ m. A stock solution was prepared from the seed powder and Jar test were run on surface water samples obtained from Challawa gorge dam, Kano River, Tomas and Kussalla dam to determine the required dose of HSLSE to meet acceptable drinking water standard. The pH and turbidity of both raw and treated water were noted. The decanted samples from the Jar test were used to carry out a bacteriological test using most probable number (MPN) and total coliform methods. The results obtained showed that the optimum content is between 2ml to 8ml / 1L of raw water sample is required to treat water to an acceptable quality. The pH of treated water with HSLSE was within the range of 6.2-7.9. The study also revealed that *Hibiscus Sabdariffa* seed extract was relatively good in turbidity removal with percentage efficiency of 62.80 and performs better in highly turbid water at an optimum of 6mg/ 1L. Furthermore, the result obtained for the MPN test were 9, 14, 9, and 9 MPN/100ml for a dose of 4ml of HSLSE while a dose of 6 ml yielded 2, 9, 4 and 2 MPN/100ml for Challawa Gorge Dam, Kano River, Tomas Dam and Kussalla Dam respectively. The result of the total coliform tests ranged between 81 to 86 percent efficiency for a dose of 4ml. A dose of 6ml yielded 99.6 % for Challawa gorge dam while the remaining samples were 97% efficient. Therefore, it can be concluded that *Hibiscus Sabdariffa* seed extract is a good turbidity removal and best in terms of disinfection.

CHAPTER ONE

INTRODUCTION

1.1 GENERAL

Water covers more than 70% of the earth surface. Organisms survive longer without food than without water. Water is an incredibly important resource in our daily lives. Everyday water is used for cooking, bathing, and many other activities are involving water. With all of the importance of water in our lives, many of us know very little about the water we use each day. We drink tap water, enjoying the convenience and cost-effectiveness of this practice, yet, we fail to recognize the serious threat this water may pose to our health if polluted or contaminated. It is one of the most valuable resources for life and source of socio- economic development for any community.

The demand for water is increasing rapidly with our growing population. Already there is acute shortage of both surface and groundwater in many parts of the world. Pollution and contamination of the available water source has greatly affected water quality. Pollution might have resulted from indiscriminate disposal of wastes or unsafe discharges into the environment. Globally the provision of safe drinking water remained a great challenge. About eighty million (80 000 000) people have no access to safe drinking water, more especially in rural areas and semi urban settlement in under developed countries of Sub-Sahara region. Only about 16% have access to safe and adequate drinking water via improved pipe system. (WHO and UNICEF, 2012).

Rapid population growth in Sub-Saharan Africa has brought about a tremendous increase in urbanization with great increase in the volume of domestic and industrial waste-water and solid waste. Water pollution is primarily associated with domestic and industrial wastes. Both types of waste-water pose threats to water quality which may be classified into health hazards and sanitary nuisances. In most parts of Sub-Saharan Africa, people have no access to potable water, consequently, raw water from rivers and streams are the major sources of water supply (UNICEF / WHO, 2000). Experts pointed out that the way people secure their drinking water has a direct impact on their health and on the economic status of the family units. Water secured from a remote and unprotected source can jeopardize the health of a household (UNICEF/ WHO, 2004; UNDP, 2006). Water is considered a renewable resource but in many parts of the world, water resources have become so depleted or contaminated that they are unable to meet the ever-increasing demands (USEPA, 2010).

It is estimated that over 1.2 billion people (one in every four people in the developing world) lack access to a safe water supply and “insufficient water quantity, poor water quality, inadequate sanitation, and poor personal hygiene practices are the direct cause for majority of diarrhoeal diseases which result in death of over 2 million children annually (USAID, 2005).

According to United Nations estimates, by the year 2025, up to 20% of the world's population could live in countries where water is in short supply (WWDR, 2003). Steep increase in population, rise in consumption and desire for a better living has placed a greater strain on the security of fresh water supply. Freshwater

scarcity, manifest in the form of rising demands, water depletion and water pollution. It is estimated that water used for human purposes has increased six-fold in the past 100 years (UNEP, 2004). Not surprising, reports indicated that the water problem is worse in poor countries, more especially in sub-Saharan Africa (Population Reports, 1998; UNICEF/WHO, 2000).

Nigeria is placed at 116th position out of 147 countries. The Nigeria's 62% water availability was below the global weighted average of 79% (UNICEF/WHO 2000). The United Nations Children Fund and World Health Organisation also in a joint report raised an alarm over water contamination in Nigeria. As it was put, it, "more than 70% of water sources in Nigeria are contaminated and injurious to health" (UNICEF/WHO, 2005). It went further to report that "the country's health will be in jeopardy if the quality of water continued to decline and if no effort is made towards improving the quality of technologies and application of best management."

Nigeria has a population of 140 million people (David, 2000) and has poor state of infrastructure; there is need to adopt cost-effective and proactive measures to boost her drinking water supply. A good step towards boosting water supply is the involvement of stakeholders in the design and implementation of a safe and affordable drinking water supply that can be effectively incorporate into overall water resource management (Amatobi, 2010).

Water supply system needs to be based on convenient and adequate supply of drinking water to the populace. Good water supplies reflect on the health and economic life of people, by reducing water-borne disease. The source of water

supply is very essential in the design and implementation of safe drinking water for all. It also reduces the chances of contamination, thereby reducing the cost of treatment.

In a survey conducted by the Federal Ministry of Health, only 15% of villages have access to adequate and portable drinking water (Ibiam, 2008). Problems associated with poor water supply in rural areas include; lack of clear policies, lack of attainable goals and objectives and poor coordination (WSSRP, 2006). David (2000), "Water supply services where they exist (in Nigeria), is unreliable and of low quality and are not sustainable because of the difficulties in management, operation, pricing and failure to recover costs. Many water supply systems show extensive deterioration and poor utilization of existing capacities due to under maintenance and lack of funds for operation".

Consequently, for most people, the only available source of water is the contaminated source with disease and harmful bacteria, yielding illness to millions of children and families, which may lead to death (Potters without borders, 2012).

Depending on their nature, impurities in water can be classified as; physical, chemical and bacteriological. The impurities are not only from the source of the water; as such pure water does not exist practically. Obviously not all substances present in water are harmful. Safe water for drinking should be free from germs that cause waterborne diseases, free from toxic substances and contain the required amount of minerals. Water becomes impure if it contains one or more substances in

excess concentrations, or toxic chemicals that may have adverse effect on the user (Venugopala, 2002).

Cheesbrough (2000), estimated that about 80% of ill health in developing countries are water and sanitation related. In the year 2000, the estimated global burden of diseases associated with poor water supply equalled more than 2 billion cases with an annual death toll of 2.2 million.

World Health Organisation (WHO, 2000) guidelines are general standard followed worldwide for drinking water quality requirement; although, each country can have their own guidelines.

1.2 BACKGROUND OF THE STUDY

In Kano city which is located on Latitude $12^{\circ}02'N$, Longitude $08^{\circ}30'E$, in the Northern part of Nigeria, water pollution comes more from domestic and industrial activities in which volumes of waste-waters are discharged into the water courses.

Kano State is not exempted from the challenges of safe water for drinking; water supply situation in Kano state is inadequate and has been described as a “nightmare”. It is one of the most populous state in Nigeria with a population of 9,401,288 inhabitants spread over forty four local government areas (Ukaid, 2012). It is a flat city drained by the Jakara River running eastwards; the Chalawa River runs to the South of the city; all are seriously polluted by urban and industrial effluents. Climate is tropical wet and dry, mean annual rainfall is about 850 mm. There is

significant industrial activity, including textiles, tanneries, chemicals and iron/steel (Dan’Azumi and Bichi, 2010).

A large proportion of the population lives in low-income settlements, including very poor informal settlements. Kano city is one of the fastest growing cities in the world due to rapid uncontrolled rural and urban migration. Pipe borne water in the last few years in Kano metropolis is in short supply. Most of the developing areas of the metropolis are not connected to pipe borne water network. Therefore, majority of Kano inhabitants, regardless of social status, depend on hand-dug wells and boreholes for their daily water supply needs. The quality of ground water has received much attention at several points in time by different researchers as poor quality water has huge health and economic implications (Tanko, 2002). It is therefore essential that this water used for drinking and cooking purposes requires treatment because it is more vulnerable to contamination from activities occurring at the earth’s surface. This work focused on the treatment of drinking water using *Hibiscus sabdariffa* seed extract.

A large proportion of the populace that lack adequate drinking water and resort to various options such as ponds, springs, stream and hand dug wells. Apparently the water sources are mostly contaminated as such it is a major source of disease. The public health significance of water quality cannot be over emphasized. Many infectious diseases are transmitted by water through the fecal-oral route.

There were cases of established outbreak of fatal water related diseases in Kano in the past. For instance in 1996 / 1997 a cholera outbreak in the State affected 5000

people and resulted in 400 deaths. In the same period cases of gastroenteritis involving 136,348 people and 2,773 lost their lives as a result (WUP, 2003).

Despite the availability of synthetic chemicals used in the purification of water, its acceptability and environmental safety has to be ensured. However, the use of natural biodegradable materials of plant origin to purify turbid surface waters, if successful, can be promoted (Subramaniametal, 2011).

1.3 STATEMENT OF THE PROBLEM

Water is an indispensable resource for life as such essential for survival. The need for its cleanliness cannot be over emphasised. The quest for clean water for drinking has become a global challenge. Available means of purifying water in Nigeria is expensive and not affordable especially by individuals. The uses of chemicals as disinfectants like chlorine are not only expensive but have general health effects and environmental problems. Its usage generally resulted in production of trihalomethane, a cancer precursor, while alum was linked to Alzheimer disease (Zhang et al, 2006). In view of the above, quite a number of natural materials of plant origin have long been used by local communities in many developing countries in water treatment. Properly protected water source are critical component of growth, poverty reduction and equity. The livelihoods of the populace are critically associated with access to water services. With higher rate of urbanization, there is increasing demand for clean water and good sanitation facilities which in turn affect the water are seriously lacking in Kano. The study will therefore help bring an alternative means of treating water in terms of turbidity and disinfection for individual and

communities. *Hibiscus sabdariffa* is a plant that is available, cheap and has less health risk. It is not only medicinal but also contains tannin, which has antioxidant and antimicrobial properties. This research work will assist in purifying water for the purpose of man and animal consumption.

1.4 AIM AND OBJECTIVES

1.4.1 Aim

This research is aimed at evaluating the efficiency of *Hibiscus sabdariffa* seed extract (Roselle in English or yakuwa in Hausa) in treating turbidity and disinfection in water.

1.4.2 Objectives

The aim of this study will be achieved through the following objectives;

- I. To determine the efficiency of *H. Sabdariffa* seed extracts in treating the turbidity and disinfection of raw water.
- II. To determine the optimum amount of *H. Sabdariffa* seed extract that would yield minimum acceptable quality of water with respect to turbidity and disinfection.
- III. To determine the variation of pH on the treated water samples.

1.5 SCOPE AND LIMITATION

1.5.1 Scope

The investigation will focus on laboratory analysis of the water sample in terms of turbidity and disinfection and also locating water sources that are used as sample sources. Water was collected from Kano River, Kussalla dam, Chalawa gorge dam and Tomas dam. In order to carry out turbidity, jar test and bacteriological analysis on the aforementioned samples was made. *Hibiscus sabdariffa* seed extract was used in treatment of raw water in terms of turbidity and disinfection. The investigation gave a comprehensive report on information to be used to describe the optimum quantity of *Hibiscus sabdariffa* seed extract that can clear and disinfect a particular quantity of water.

1.5.2 LIMITATION

The investigation was limited to selected water sources. This implied that the results obtained may be peculiar to surface water from river and dams and may not be completely used to generalize for all type of water. The research was limited to the ability of *Hibiscus sabdariffa* seeds extract in the purification of water turbidity and disinfection. Furthermore this research did not determine the optimum pH in which the best water quality is attained.

1.6 CONTRIBUTION TO KNOWLEDGE

The study provided an alternative means of water purification in terms of turbidity and disinfection using *Hibiscus Sabdariffa* seed extract which is cheap and readily available.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Water is typically referred to as polluted when it is impaired by anthropogenic contaminants and either does not support a human use, such as drinking, and/or undergo a marked shift in its ability to support its constituent biotic communities. Water pollution affects the health of the waterway, the health of the organisms living in and around the water bodies, and, eventually, the health of humans. The effects of water pollution can range from aquatic deformities to contaminated fish to 'dead' lakes. Toxic pollutants can also alter the genetic makeup of an organism, resulting in either death or extreme deformities. (USEPA, 2010)

One measure of good governance is good public health; Nigeria has an unenviable public health record. Mortality and morbidity rates are high due to the absence of clean water and adequate sanitation and, where potable water is available, the potential gains are frequently negated by contamination of the water after delivery due to poor sanitation practices. It is estimated that 150,000 to 200,000 children are lost to diarrhoea related diseases each year in Nigeria. Cholera, typhoid, paratyphoid, guinea worm, bilharzias, shistosomiasis are all too common. Every one of these diseases is preventable with good public health. (Atkins, 2006)

Water, in rivers, boreholes and wells, acquires chemicals from a variety of sources and these chemicals are accumulated as dissolved and or suspended constituents.

The composition of surface water and groundwater changes on time scales of minutes to years. Natural waters occur at or near the surface of the earth that comes in contact with sedimentary and igneous rocks promoting metal levels in water. Groundwater is obtained from holes drilled in the ground and water is usually saturated with rock chemicals found at different depths. The chemicals present in groundwater can be due to natural origins and anthropogenic sources such as nitrate from fertilizers and bacteriological contamination from sewage. The groundwater intakes are susceptible to seasonal fluctuations. The rainwater is also used during times of drought. Rainwater systems, particularly those involving storage tanks, can be a relatively safe supply of water. Surface water used for drinking and cooking purposes requires treatment because it is more vulnerable to contamination from activities occurring at the earth's surface. These are contamination from human waste, livestock and other hazards. Groundwater is prone to contamination if soil conditions are sandy and the water tables are shallow. (Subramaniam et al, 2011).

For many developing countries, coagulation, flocculation sedimentation and disinfection are expensive processes of water treatment because of high cost involved to import these chemicals in hard currency, leading to high pricing for treated water and the difficulties in accessing the chemicals. Chemical disinfectants like chlorine are not only expensive but have general health effects and environmental problems. Its usage generally results in production of trihalomethane, a cancer precursor while alum was linked to Alzheimer disease (Yongabi, 2011). In view of the above, quite a number of natural materials of plant origin have long been used by local communities in many developing countries in water treatment. Some

of the effective coagulants have been identified: Nirmali, Okra, Red beans, Sugar and Red maize and also *Moringaoleifera* (Jahn, 1988). Several extensive studies have been done on the use of these indigenous natural coagulants. *Moringaoleifera* and *J.curcas* lowered the turbidity of the water and the coliform count. These interrelativity are so complex that at the present time is almost impossible to predict theoretically the actual optimum coagulant for a particular water.

The use of natural extract is envisaged to be safe, effective and cheaper. Tannin, a type of bimolecular, is an astringent, bitter plant polyphenolic compound that binds to and precipitates proteins and various other organic compounds including amino acids and alkanoids. Tannin compounds are found in many species of plants.

2.2 TREATMENT PROCESS

2.2.1 Coagulation

The coagulation process involves adding iron or aluminum salts, such as aluminum sulphate, ferric sulphate, ferric chloride or polymers, to the water. These chemicals are called coagulants, and have a positive charge. The positive charge of the coagulant neutralizes the negative charge of dissolved and suspended particles in the water. When these reactions occur, the particles bind together, or coagulate. The larger particles, or floc, are heavy and quickly settle to the bottom of the water. This settling process is called sedimentation (Amatobi, 2010).

2.2.2 Factors Influencing Coagulation Process

Optimum coagulation treatment of raw water represents the attainment of a very complex equilibrium involving many variables. There is interrelativity

conditions such as pH, turbidity and chemical composition. The limited knowledge on the exact dosage and mechanism for usage renders them ineffective to compete favourably with the widely known synthetic chemicals. (Yongabi et al. 2011) Therefore it must be determined experimentally for each sample. (AWWA, 1971)

2.2.3 Coagulation Dosage and pH

The pH of water is important in coagulation and is controlled by coagulation dosage. It is possible however, for the properties of raw water to be such that optimum treatment is activated at a pH significantly different from that obtained from the coagulant alone. Lime is often used to adjust the pH of water to a high value. The use of acid to adjust the pH is seldom feasible because of the increased need to add alkali later for corrosion control purposes. Most coagulants are acidic and thus cause the pH value of water to fall on addition (AWWA, 1971).

2.2.4 Coagulant Aids

Difficulties in coagulation occur as a result of slow settling precipitate or fragile flocs that can easily fragment under hydraulic forces. Coagulate aids increase flocs formation by improving settling and toughness of flocs. The most widely used materials are polyelectrolyte, activated silica, adsorbent weighing agents and oxidants (Samaila, 2011).

2.2.5 Sedimentation

Sedimentation is the physical water treatment process used to settle out suspended solids in water under the influence of gravity. The heavy particles move to the bottom and clear water moves to filtration unit (Amatobi, 2010).

2.2.6 Filtration

Filtration is the process of removing suspended solids from water by passing the water through a permeable membrane or porous bed. Ground water is naturally filtered as it flows through porous layers of soil. Ground water may be subjected to contamination from any source and poses threat to human life, filtration is one of the oldest and simplest methods of removing them (Amatobi, 2010).

2.2.7 Disinfection

Disinfection is the elimination or inhibition of pathogenic microorganisms in an object so that they no longer pose a threat. Hence the water must be safe by public health standard. Conventionally chlorine is added to kill the bacteria or micro-organism. A disinfectant is a chemical agent used to disinfect inanimate objects such as work surfaces and floor. Disinfectants are incapable of killing spores within a reasonable time period, and are generally effective against a narrower range of organisms than physical means (Stuart, 2005). Figure 2.1 is a flow chart showing steps involved in treating water.

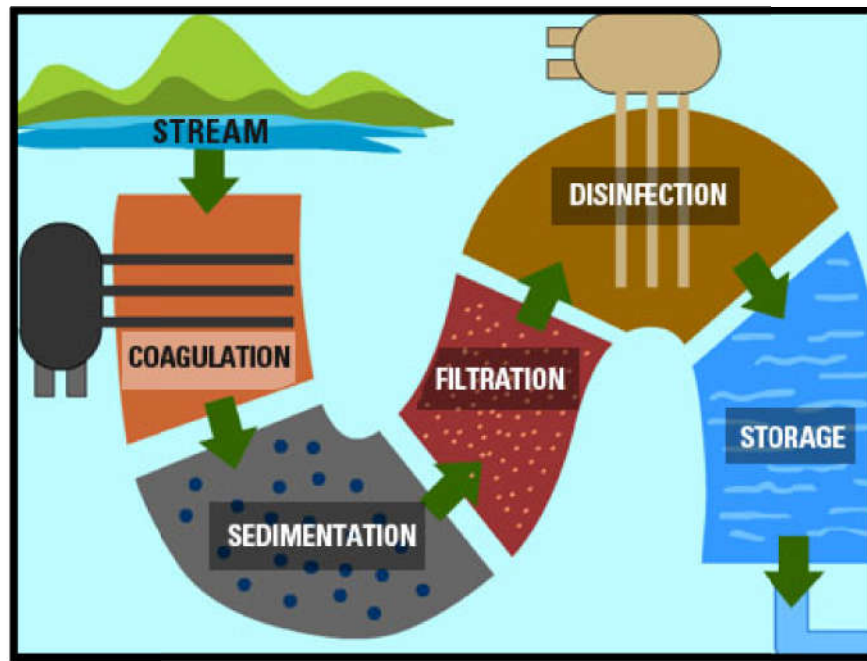


Figure2.1: A flow chart of water treatment processes

Source: file:///G:/how-water-is-treated-for-drinking.html

2.3 HIBISCUS FAMILY(MESTA)

Mesta is common word used for both *Hibiscus cannabinus* and *H. sabdariffa* which produces good fibre for commerce. These two species belong to the family *Malvaceae* with chromosome number $n=36$ and $2n=72$ respectively. *Hibiscus cannabinus* is popular in the Western world as kenaf. *Hibiscus cannabinus* is known by various names in India such as Bimli, Deccan hemp, Gogu, Channa, Ambadi, Gongkura, Sunkura, and Sunbeeja. While *Hibiscus sabdariffa* is known as roselle in English, shapala in Yoruba, and yakuwa in Hausa. Besides India the Mesta is grown mainly in Argentina, China, Cuba, Egypt, Hewti, Guatamala, Italy, Iran, Indonesia, Mozambique, North Africa, New Guinea, Peru, Spain, South Africa, Southern Part of Zimbabwe, Thailand, U.S.A, Nigeria and Russia.

2.3.1 *Hibiscus Cannabinus* (Kenaf)

The species *cannabinus* has been found to grow so far North and South of equator in Africa and undoubtedly varies in the same way whenever the plant is found growing wild. Generally, the species grows best in the warmer regions between the latitude of 300°N and 300°S . It does, however, grow wild or is successfully cultivated at latitudes much farther from the equator for instance on the southern shores of Caspian sea in Southern Russia, in Manchuria and Korea.

Since the *cannabinus* is cultivated at some distance North and South of equator, it is clear that the plant is capable of withstanding a considerable degree of cold. In the equatorial range of Africa, the wild plants are found growing best in places where the temperature ranged from 15°C to 30°C .

Variations exist in different species of *Mesta* and even in different varieties of same species. For example in case of *Hibiscus kitaibelifolius*, the plants possess a short flowering period which covers all the activities including reproduction. From the appearance of first flower on the plant, the plant practically ceases to grow. Another variety of *cannabinus* collected from Java was found to have a longer flowering period and the plants continued to grow even after the initiation of flower. Many exotic collections have been found to be very much the same in their flowering and growth behaviour. The plant is hermaphrodite, annually, producing large cream coloured flowers characterised by a reddish purple or scarlet throat. The flowers are short lived opening in the early hours of morning before sunrise and closing by noon of the same day. *Cannabinus* is generally considered as self-

pollinated, but a small amount of cross pollination may be affected by bees and other insects (Singh, 1993).

The colour of the stem is generally green. However, some types with reddish stem are also found. The reddish colour generally appears after the plants attain a certain age in some of the types. This has been attributed due to sunlight rather than a varietals character. The plants when mature may attain a height of four meters with a base diameter nearly 1.5 to 2.5 cm.

Most variable character in Mesta is the leaf. Both compound and simple leaves may be found on the same plant or same type may have all of one leaf shapes. Leaf shapes also vary with the type of variety. Generally variety '*viridis*' possesses entire leaves while the variety "*vulgaris*" has divided leaves. The variety '*simplex*' has red stem with divided leaves while the variety '*purpureus*' possesses purple stem and divided leaves.

The shape of the leaves also arise on the same stem. The entire shapes of leaves at the bottom may be triangular at slightly higher point and five to seven lobed at the top of the stem. The arrangements of leaves also differ in the varieties. In some varieties the petiole is at right angle to the stem while in others it is having an angle of 30° or 60°.

The varieties of *cannabinus* also differ in their flowering behaviour. This difference depends on the type of variety used for different purposes. For normal growth a day length of 13 ½ hours is good for cultivating variety for fibre purpose. The harvesting of crop depends generally on the formation of buds or opening of flowers on the plant.

Photoperiodic plays an important role in the cultivation of *cannabinus* in the world. The *cannabinus* types do not flower in Cuba until the day length is shortened to about 12 to 12½ hours. The plants will not flower till September-October. For the purpose of fibre production it is desirable to obtain plants with longer stems without branches of fruiting stalk, since these interrupt the continuity of the fibres and sowing must begin when the days are longer to coincide with the proper amount of rainfall. *Hibiscuscannabinus* is capable of adapting itself in variety of soils and climatic conditions. The crop cannot, however, tolerate frost. The crop can be grown up to the height of 3000 ft above sea level at latitude of 450⁰N to 480⁰ N in Russia and to latitude of 300⁰S in South Africa. For its good growth, a well-drained sandy loam soil is most suited. Mesta does not grow in waterlogged conditions. Its however, requires a rainfall of 50 to 65 cm during its growth period. The seed capsules are cylindrical, pubescent bearing from 18 to 20 seeds per capsule. The seeds are grey in colour. The seed is badly affected by the temperature of soil. A soil temperature of 15⁰ to 20°C is most suitable for the germination of seeds (Singh, 1993).

2.3.2 *Hibiscus Sabdariffa* (Roselle locally called yakuwa in Nigeria)

The flowers of *Hibiscus sabdariffa* are comparatively smaller than those of *Hibiscuscannabinus*. The predominant types have cream to light yellow flowers, having a scarlet to magenta throat and a green or slightly reddish stem depending on the variety. In Nigeria *Hibiscussabdariffa* is called 'Roselle' and all its types are classified into four main groups according to the extent of pigmentation present on the stem. They are full green, green pigmented, green to light red and red. The leaves

in 'Roselle' are deeply lobed and alternately borne on the stem. The plants are normally non-branching and attain a height of nearly 3 to 3.5m with a basal diameter of 1.0 to 2.0 cm (singh, 1993).

Many wild forms of *sabdariffa* are found in Uganda which closely resembles *H. machowii* and is considered as the immediate wild progenitor of *sabdariffa*. The tall fibre yielding type was isolated and described as *Hibiscus sabdariffa* from some seeds received from Gold Coast (W. Africa). This variety was accidentally introduced into India as a single seed in admixture with some of other consignment of seeds from Java (Indonesia). It was first described as a new type of Roselle hemp. It is clear that the species of *sabdariffa* consists of two distinct types, wild and cultivated. The calyces of the wild types are fleshy and are used for making jellies and jams and produce fibre of inferior quality. The tall types with fewer branches are cultivated for fibre purpose and they belong to *Hibiscus sabdariffa*.

Sabdariffa types are best cultivated in between 10⁰N and 30⁰S, where the temperature is not less than 10⁰C and not more than 30⁰C together a rainfall sufficient to give at least 10 inches during each month of the growing period. The air too must be still and humid to bring about a rapid and healthy growth. Strong winds, prolonged period of cool foggy weather interrupt and check its growth. The *Sabdariffa*(Roselle) types in general are not responsive to photoperiodic, although the plants of *sabdariffa* (Roselle) types tend to be best in short day. These types can be grown in soils having a pH of 4.4 to 7.8.

Both *H. cannabinus* and *H. sabdariffa* types require the following factors for their proper growth (singh, 1993).

1. Enough moisture in soil during the growing period.
2. Rainfall should be at least 100 mm or more per month during the crop cycle with a fairly uniform temperature.

The two species of Mesta resemble more or less in most of the characters as described above. However, they differ in some characters which help in the identification of these two species. The following characters may help in this direction;

- I. The apex of the epicalyx (bracteole) is entire in *cannabinus* and inconspicuously channelled in *sabdariffa*.
- II. The sepal nectary (gland) in *cannabinus* is prominent (swollen) while it is much less conspicuous (shrunken) in *sabdariffa*.
- III. The stem of *sabdariffa* is flexible while it is more or less rigid in *cannabinu* (Singh, 1993).

It is an economically important plant, particularly in the Sahel zone of West Africa. The leaves, seeds and calyces are valued for its nutritional and medicinal uses. The most exploited part of Roselle plant is its calyces which may be green, red or dark red (Schippers, 2000). The green calyces are used for making vegetable stew; while red and dark red ones are utilized in producing drinks, jellies, sauces, chutneys, wines, preserves and tea. The calyces drink, which is receiving industrial attention internationally, is a readily available and inexpensive source of vitamin C. Therefore, calyces of Roselle contain nine times more vitamin C than citrus (*Citrus sinensis*). The seed of Roselle is a valuable food resource on account of its protein, calorie, fat and also substantial amount of fibre and valuable micro-nutrients (Babalola, 2000). The seeds are subjected to a solid-state fermentation process to produce a meat substitute condiment called “Furudu” in Sudan, “mungzantusa” in Nigeria, “bi-kalga” in Central Burkina and datou in Mali. In Niger, this condiment is called “dawadawa-botso” or “mari mi”, respectively in

Hausa and Djerma language (Bengaly et al, 2006).

2.4PHYTOCHEMISTRY

The leaf was reported to contain protein, fat, carbohydrate, fibre, ash, calcium, phosphorus, iron, thiamine, B-carotene, riboflavin, niacin and ascorbic acid. The flower yields a yellow dye; the major pigment identified is daphniphylline. The plant flavonoids such as hibistrin and hibiscetin and dried calyces contain the flavonoids gossypetine, hibiscetine and sabdaretine. It also contains alkaloids, B – sitosterol, anthocyanin, citric acid, cynaidin-3-rutinoside, delphinidin, galactose, pectin, protocatechuic acid, quercetin, stearic acid and wax. Small amount of delphindin-3-monoglucoside, cyandin-3- monoglucoside (chrysanthenin) and delphindin are also present. Three water soluble polysaccharides have been isolated from flower buds; neutral arabinogalactans.

The calyces are rich in acid and pectin. Analysis of calyces has shown the presence of crude protein and minerals such as iron, phosphorus, calcium, manganese, aluminum, magnesium, sodium and potassium. Mucilage, calcium, citrate, ascorbic acid, gossypetin and hibscin chloride are also present in calyces. The seeds contain protein (18.8 – 22.3%), fat (19.1 – 22.8%) and dietary fibre (39.5 – 42.6%) content were found to be high. The seeds were found to be a good source of minerals like phosphorus, calcium, magnesium, lysine and tryptophan contents. Seed oil is rich in unsaturated fatty acids (70%), while linoleic acid constituted 44%. Seeds contain nitrogen, fatty oil, cellulose, pentosans and starch. Steroids and tocopherols have been reported in the seed oil. Kaempferol-3-O-rutinoside, kaempferol -3-O-

glucopyranside, quercetin, 3-O-rutinoside, citrusin C, 2,3-dihydro-2-(4'-hydroxy-3'-methoxyphenyl)-3-B-Dglucopyranosylmethyl-7-hydroxy-5-benzofuranpropanol, corchoionoside C and trans-carveol-6-O-glucopyranoside were isolated from 70% aqueous ethanol extract of leaves. The physicochemical analysis of the fresh calyces and leaves are given in Table 2.1 and phytochemicals present in the various parts of the plant are presented in Table 2.2. Tables 2.3 and 2.4 show the content of both physiochemical and phytochemical constituent of the dry *Hibiscus sabdariffa* (Mahandevan et al, 2007).

Table 2.1: Physicochemical constituents of the fresh calyces and leaves of *H. sabdariffa* (g and mg/100g)

Constituents	Calyces (fresh)	Leaves (fresh) (%)
Moisture	9.2g	86.2
Protein	1.145g	1.7-3.2
Fat	2.61g	1.1
Fiber	12.0g	10
Ash	6.90g	1
Calcium	12.63mg	0.18
Phosphorus	273.2mg	0.04
Iron	8.98mg	0.005
Carotene	0.029mg	-
Thiamine	0.117mg	-
Riboflavin	0.277mg	-
Niacin	3.765mg	-
Ascorbic Acid	6.7mg	-

(Mahandevan et al,2007)

Table 2.2: Phytochemical of *Hibiscus Sabdariffa*

Part of the plant	Chemical Constituents
Flower	Carbohydrates, arabinans, mannose, sucrose, thiamin, xylose, mucilage, niacin, pectin, proteins, fat, arabinoglactans, rhamnogalacturans, riboflavin, β -carotene, phytosterols, citric acid, ascorbic acid, fruit acids, maleic acid, malic acid, hibisci acid, oxalic acid, tartaric acid, (+)-alloxycitronic acid-lactone, allohydroxycitric-acid, glycolic acid, utalonic acid, protocatechuic acid, cynidin-3-glucoside, cynidin-3sambubioside, cynidin-3xyloglucoside, delphinidin, delphinidin-3-glucoside, delphinidin-3-xyloglucoside, delphini, gossypetin, gossypetin-3-glucoside, hibiscetin, hibiscin, hibiscitrin, sabdaretin, sabdaritrin, fiber (crude), resin, fibre (dietary), minerals and ash.
Seed	Starch, cholesterol, cellulose, carbohydrates, campesterol, β -sitosterol, ergosterol, propionic acid, pentosans, palargonic acid, palmitoleic acid, palmitici acid, oleic acid, myristic acid, methanol, malvalic acid, linoleic acid, sterculic acid, caprylic acid, formic acid, stearic acid, cis-12, 13-epoxy-cis-9-octadecenoic acid, isopropyl alchcohol, isamyl alcohol, ethanol, 3-methyl-1-butanol, fibre and minerals.
Leaf	α -Terpinyl acetate, anisaldehyde, β -carotene, β -sitosterol, β -sitosterol, benzoate, niacin, fat, isoamyl alcohol, iso-propyl alcohol, methanol, 3-methyl-1-butanol, benzyl alcohol, ethanol, malic acid, fibre and ash.
Fruit	α -Terpinyl acetate, pectin, anisaldehyde, ascorbic acid, calcium oxalate, caprylic acid, citric acid, acetic acid, ethanol, formic acid, pelargonic acid, propionic acid, isopropyl alcohol, methanol, benzyl alcohol, 3-methyl-1-butanol, benzaldehyde and minerals.
Root	Tartaric acid and saponin.

Table 2.3: Physicochemical constituents of the dry calyces of *Hibiscus Sabdariffa* (g or mg/100g)

Constituent	Dry calyces of <i>Hibiscus Sabdariffa</i>
Dry matter	89.81%
Moisture content	84.55%
Carbohydrate	2.21g
Protein	1.87g
Fat	0.13g
Fibre	2.27g
Ash	3.97g
Calcium	2.67mg
Phosphorus	60.68mg
Magnesium	4.40mg
Vitamin C	13.79mg

Table 2.4: Phytochemical constituents of *Hibiscus Sabdariffa*(antinutrients) %

Constituent	Dry calyces of <i>Hibiscus Sabdariffa</i> (%)
Alkaloid	0.27
Tannin	0.158
Flavonoid	0.43
Phenol	0.26
Saponin	0.009

2.5 PHARMACOLOGICAL USE OF *Hibiscus Sabdariffa*

2.5.1 Antihypertensive

Aqueous extract of petals exhibited antihypertensive and cardio protective effects in rats. Infusion was also found to lower both systolic and diastolic pressure significantly in spontaneously hypertensive and normotensive rats. Tea of calyces showed 11.2% reduction in the systolic blood pressure and 10.7% decrease in diastolic pressure. Effectiveness and tolerability of a standardized extract was studied in patients with mild to moderate hypertension which revealed a reduction in systolic and diastolic blood pressure by more than 10 percent.

The aqueous extracts of the calyx showed a dose-dependent decrease in mean arterial pressure of the rats. The extract has a vasodilator effect in the isolated aortic rings of hypertensive rats. These effects are probably mediated through the endothelium-derived nitric oxide-cGMP-relaxant pathway and inhibition of calcium influx into

vascular smooth muscle cells. Daily consumption of tea lowers blood pressure in pre and mildly hypertensive adults and may prove an effective component of the dietary changes recommended for people at risk of developing hypertension. A standardized extract has shown effective blood pressure lowering activity in hypertensive humans. A recent double blind, reference-controlled trial demonstrated significant reduction in blood pressure in the hibiscus group when compared with lisinopril (Mahadevan et al, 2007).

2.5.2 Hepatoprotective

Protective effects of dried flower extracts against oxidative stress in rat primary hepatocytes were demonstrated. Protocatechuic acid, a simple phenolic compound isolated from *Hibiscus. Sabdariffa* showed protective effects against cytotoxicity and genotoxicity of hepatocytes induced by t-BHP. One of the mechanisms may be associated with its property of scavenging free radicals.

The extract of its petals protected rats against cadmium induced liver, prostate and testis lipoperoxidation. The extract offers hepatoprotection by influencing the levels of lipid peroxidation products and liver marker enzymes in experimental hyperammonemia and this could be due to the free radical scavenging property of natural antioxidants present in the plant.

The protective effect of aqueous extract and anthocyanins on paracetamol-induced hepatotoxicity in rats has also been reported. Aqueous-ethanol (1:1) extract of the calyx showed a significant decrease in the level of lipid peroxidation in carbon

tetrachloride induced liver damage. However, a study showed that prolonged usage of aqueous-methanol extract of the calyces could cause liver injury (Mahandevan et al, 2007).

2.5.3 Antioxidant activity

The antioxidant and free radical scavenging effects of two fractions of the ethanol extract (chloroform soluble fraction and ethyl acetate soluble fraction) obtained from its dried flowers were investigated and found that both the fractions scavenge hydrogen peroxide (79-94%) at the dose of 500µg. Similarly, the extracts showed inhibitory (70-80%) effects on superoxide anions radicals (O_2^-) at a dose of 1000µg. The antioxidant activities of three varieties using liposome system have also been reported. Methanol and ethyl acetate extracts showed higher COX-1 enzyme inhibition than COX-2 inhibition (Mahandevan,et al, 2007).

2.5.4 Anticancer

Anthocyanins can cause cancer cell apoptosis, especially in HL-60 cells. Antioxidative activity of anthocyanins was evaluated by their effects on LDL oxidation in cell free system and anti-apoptotic abilities in RAW264.7 cells. The study showed that anthocyanins of this plant may be used to inhibit LDL oxidation and oxLDL-mediated macrophage apoptosis, serving as a chemopreventive agent. Inhibitory effect of protocatechuic acid on tumour promotion in mouse skin demonstrated that protocatechuic acid possesses potential as a cancer chemopreventive agent against tumour promotion (Mahandevan, et al , 2007)

2.5.5 Other activities

Ethanol and aqueous extracts of its calyces possess antipyretic activity in experimental animals. Ethanol extract of the plant reduces the extent of cisplatin-induced sperm abnormality and enhanced sperm motility in rats. Inhibition of intestinal motility by methanol extract in rats showed a significant dose dependent relaxant effect on rat which is comparable to the effect shown by nifedipin and papaverine as reference compounds. Investigation of the antispasmodic potential revealed that aqueous extract of calyces inhibited the tone of various isolated muscle preparations.

Effect of zobo drink (*H. sabdariffa* water extract) on the pharmacokinetics of acetaminophen in human volunteers was studied and the results showed no statistically significant changes in the absorption parameters after the administration of zobo.

Investigation of the anti-inflammatory activity showed that its extract had no effect on rat oedema but had an inhibitory effect on yeast induced pyrexia and a significant effect on the hot plate reaction time. Polysaccharides from its flowers can stimulate proliferation and differentiation of Human Keratinocytes. The study also showed that raw polysaccharides and all acidic fractions cause a strong induction of proliferation of human keratinocytes while the neutral polymers were ineffective. Neuropharmacological effects of the aqueous extract of calyx in rodents revealed that the extract produced a remarkable dose dependent decrease in spontaneous motor activity in mice and increased the duration of pentobarbital induced sleep in rats.

Antibacterial activity of gossypetin isolated from *Hibiscus. Sabdariffa* was carried out and results revealed that the activity may be due to polyphenolic nature of the flavonoid gossypetin. Investigation on non-tropic activity of its calyces in mice indicated that the extract of calyces might prove to be useful memory restorative agent in the treatment of dementia seen in elderly who may be due to its anti-acetylcholinesterase property'. The haemostatic effect of the leaves was evaluated to confirm its traditional use to arrest bleeding. The extract enhanced coagulation of blood, while causing precipitation of some blood material. The bleeding time was also decreased.

Tea made from dry Roselle calyces was given to human and analysed for uric acid and other chemical composition related to urinary stone risk factors, the results suggested the urocosuric effect of the tea in human (Mahandevanet al, 2007).

2.6 TREATING WATER USING *HIBISCUS* FAMILY

Jones (2013), in his study used *H. cannabinus* to treat the pH and turbidity of water sample that was obtained from an open pond used for irrigation. In the said study, powdered *cannabinus* was discovered to be more effective in removing high turbidity water, with an efficiency of 96.0% against the extract with 85.6%. However, in low turbidity water, the methanol extract performed better, with a turbidity removal efficiency of 89.4% compared to 84.4%, if the *cannabinus* powder was used. Another parameter studied was the pH of the water which remains unaffected after the treatment at 7.0. The outcome of the performance of this natural coagulant and alum when compared indicated that alum also performed better on high turbidity

water than low turbid water with a removal efficiency of 98.8% and 95.7% respectively (Jones, 2013).

Yongabi et al. (2011) studied the phytodisinfective and phytocoagulative activities of some plants in rural Cameroon which revealed that, *H. sabdariffa* seed and its calyx, *M. oleifera*, *J. curcas* and *Pleurotus tuberregium scherotum* lowered the turbidity of the water and the coliform count. The limited knowledge on the exact dosage and mechanism for usage renders them ineffective to compete favourably with the widely known synthetic chemicals. Also, the use of these plants in folk medicine and as a food makes them unlikely that they may contain any toxic substances (Jahn, et al 1979). Plant coagulants can perform well if fully explored. The relationship between the botanical type and a content of coagulants (chemotaxonomy) could be detected for several plants genera and families. One of such families is the *Hibiscus* (malvaceae) family and a member of this family is *hibiscus cannibinus Linn* (kenaf) (Jahn et al, 1979).

2.7 COLIFORM

Coliform bacteria were described and grouped, based on their common origin or characteristics, as either total or fecal coliform. The Total group includes Fecal Coliform bacteria such as *Escherichia coli* (*E. coli*), as well as other types of Coliform bacteria that are naturally found in the soil. Fecal coliform bacteria exist in the intestines of warm blooded animals and humans, and are found in bodily waste, animal droppings, and naturally in soil. The coliform organisms are the most commonly measured indicators of water quality, although experience has shown that

they are not completely satisfactory for this purpose. Total coliforms are defined as gram-negative bacteria that ferment lactose at 35° or 37° C, with the production of acid, gas, and aldehyde within 24-48 hours (WHO, 2011).

CHAPTER THREE

METHODOLOGY

3.1 SEED ACQUISITION

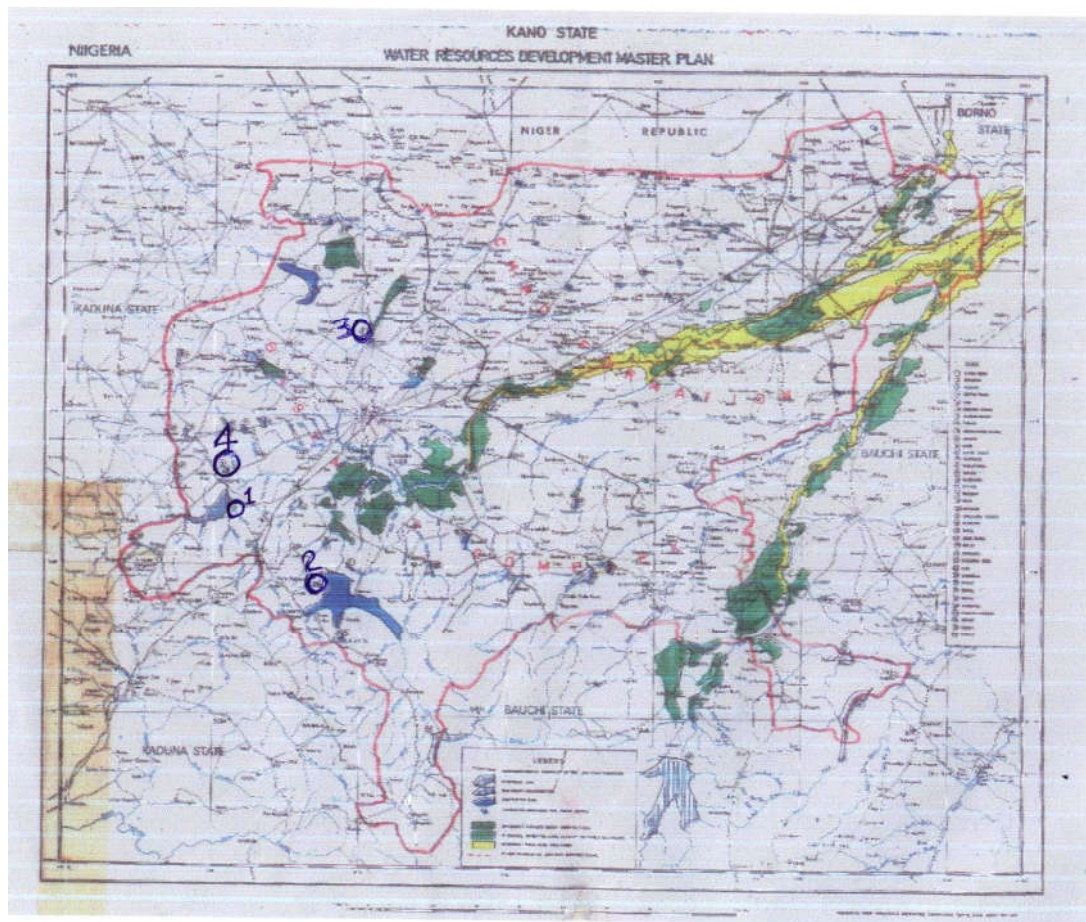
A large quantity of *Hibiscus Sabdariffa* seed (Roselle or locally call yakuwa) weighing 2kg was purchase from Rimi Market in Kano municipal, Kano state. Thedried *Hibiscus sabdariffa* seed was ground using mortar and pestle into powdered form and sieved with 175um sieve. 15 g of the powder was dissolved in 500ml of distilled water to prepare a stock solution to be used for jar test.

3.2 Raw Water Location and Collection

The samples were collected from four different locations and thedescriptions of the locations are as follows:

- (a) Challawa Gorge Dam is located at Karaye local Government area of Kano state. It is situated at latitude $11^{\circ} 43' 10''$ N and longitude $8^{\circ} 00' 48''$. It has an elevation of 1678ft with a catchment area of 3859km^2 .
- (b) Kano River – This is located at an elevation of 1749ft, it is on latitude $11^{\circ} 25' 06''$ N and on longitude $8^{\circ} 24' 41''$ E. Kano River empties its flows into Tiga Dam.
- (c) Tomas Dam- It is located at Dambatta Local Government area of Kano state. It is situated at latitude $12^{\circ} 18' 01''$ N and on longitude $8^{\circ} 31' 17''$ E with an elevation of 1411ft. It covers an area of 585km^2 .

(d) Kussalla Dam- This is situated at Karaye local government area of Kano State on latitude $11^{\circ} 49' 03''$ N and on longitude $8^{\circ} 01' 34''$ E. It is located on an elevation of 1708ft. Figure 3.1 shows the map indicating the location of the dams and river.



NB; 1-Challawa, 2- Kano River, 3- Tomas dam and 4- Kussalla dam

Figure 3.1 Map showing sampling locations. Source;Kano state Ministry of water resources

Raw water from Kussalla Dam, Kano River, Tomas Dam and Chalawa Gorge Dam were collected and stored in a 15 litres container (the container was rinsed with distilled water and again rinsed with raw water sample). The samples were collected

in the morning and analyzed same day. The pH, temperature, turbidity was measured on arrival at laboratory.

3.3. Laboratory Test

The basic tests carried out for the purpose of this research work included; turbidity, jar test and test for fecal and total coliform bacteria. The apparatus and procedures adopted for carrying out the test are stated and described below; the samples were tested at the Civil Engineering Laboratory, Bayero University, Kano and at National Research Institute for Chemical Technology, Zaria.

3.3.1 Determination of Turbidity (Nephelometric method)

The turbidity of the water used for this study was determined using the following apparatus;

I Turbidimeter

II Sample Cell

The turbidimeter was set for the operation by verifying the calibration before the water sample was tested. A clean sample cell was filled with 25ml of the raw water to a mark on the sample cell. The sample was placed in the instrument and covered with the white light shield. The turbidity was read directly in nephelometric turbidity units (NTU) on the machine. Same was repeated for all the samples and the results were recorded.

3.3.2 pH

The pH of the water samples for the purpose of this study was determined using;

- I pH meter (a pocket size Hanna instrument).
- II Distilled water
- III Wash bottle

The calibration of the pH meter was verified and the pH meter was rinsed with distilled water. The pH meter was then inserted into the raw water sample and the readings were noted. Three different readings were obtained and the average was recorded.

3.3.3 Jar Test

The optimum amount of *Hibiscus sabdariffa linn* seed extract required for the purpose of this study was determined through jar test using the following apparatus;

- I Volumetric flask (1,000 ml)
- II Analytical balance
- III Hibiscus sabdariffa Linn seed extract
- IV Magnetic stirrer (optional)
- V A stirring machine with six paddles capable of variable speeds from 0 to 100 revolutions per minute (RPM)
- VI Beakers (1,000 ml)
- VII Pipets (10 ml)

- VIII Watch or clock
- IX Turbidimeter and sample tube

A 30g of *Hibiscus sabdariffa* seed powder was weighed mixed, with 1L of distilled water to make a stock solution. The stock solution was placed on a magnetic stirrer for one hour so as to activate the active ingredient present there in and the solution was sieved using muslin cloth. The stock solution was added to six beakers containing 250ml of raw water sample in concentration of 2ml, 4ml, 6ml, 8ml, 10ml and 12ml using a pipet. The content in the beaker (raw water and stock solution) was stirred at 150rpm for 2minute for effective mixing and 50rpm for 15 minutes so as to effect flocculation. The mixture was left undisturbed for 30min, 1hour, 6hour and 24 hours. Fragile flocs formation was observed and the flocs settled gradually at the bottom of the beakers and leaving a brownish water sample. The water sample was allowed to settle and filtered by decanting into conical flask and refrigerate and stored for coliform analysis. The turbidity of the water samples and pH were noted both before and after the analysis.

3.3.4 Determination of Coliform Bacteria in Raw Water Samples

Two different methods were used for the purpose of this work to determine coliform bacteria; most probable number (MPN) and standard plate method. This further, ascertained the efficiency of *Hibiscus sabdariffa* Linn seed extract in disinfecting the water.

□ Presumptive stage.

a) Most probable number test

In carrying out the most probable number test on the water samples the following apparatus are required;

A	Autoclave
B	Incubator
C	Test tubes
D	Test tubes racks
E	Durham's tubes
F	Wire loops
G	Slides
H	Petri dishes
I	Distilled water
J	Foil paper
K	Cotton wool
L	Hot plate
M	Ethanol sterilization
N	Syringes
O	Spatula
P	Erlenmeyer flask 250mg
Q	Weighing balance

Reagents

- a. Lactose broth
- b. Gram's reagent

Preparation of Lactose broth.

The lactose broth was prepared in two different concentrations; single and double strength(UNEP/WHO 1996).

Double strength

250ml of lactose broth was prepared for double strength in accordance with the manufacture's instruction of 13g per litre of distilled water.

Therefore 13g of lactose broth was weighed on a weighing machine and dissolved in 1000ml of distilled water in an Erlenmeyer flask (250ml). The solution was heated on a hot plate. The medium was allowed to cool and then sterilized using an autoclaving machine at 121°C for 15 minutes. After autoclaving the medium was allowed to cool. Using a sterilized syringe, 10ml each of the dissolved lactose broth was measured and dispensed into 20 test tubes and was covered with a foil paper filled with cotton wool to avoid contamination. 10ml of each raw water sample was further inoculated into five test tubes which already contain 10ml of the dissolved lactose broth. Durham tubes were dropped in an inverted position into each test tube, this Durham tubes are for collection of gases. Each group was labelled and the concentration of lactose broth was also written for ease of identification. Same was done for the remaining fifteen in a group of five for each raw water sample. These brought the total to twenty for a group of five for four raw water samples. The test tubes were then incubated at 37°C for 24 hours.

Single Strength Preparation

In this preparation, 13g of lactose broth was dissolved in 1L of distilled water and was prepared according to manufacturer's instruction.

The whole process was repeated just as in double strength except that 1ml of raw water was inoculated into 5 test tubes (which contain 10ml of lactose broth and a durham tube) for a sample of raw water. Another 5 set of test tubes for same water sample as above was inoculated with 0.1m of raw water and covered. This brought the total number of 15 test tubes per sample and a grand total of 60 test tubes for the whole experiment. All the 60 test tubes were then incubated at 37⁰ c for 24 hours. The entire process is the presumptive stage.

□ Confirmatory test

The confirmatory and complete test was carried out using the following apparatus;

1. Eosine MerthyleneBlue(EMB Agar)
2. Distilled water
3. Autoclave Machine
4. Foil paper
5. Weighing balance
6. Spatular
7. Disposable petri dish
8. Cotton wool
9. Hot plate

Preparation of EMB Agar

EosineMenthylene Blue (EMB) agar was prepared according to manufacturer's instruction (37.5g/1liter of distilled water). In order to prepare 400ml needed for the study, 15g was weighed and dissolved into 400ml of distilled water in an Erlenmeyer flask and heated to dissolve via hot plate. The medium was further sterilized using an autoclave at 121⁰c for 15 minutes. After sterilization the medium was allowed to cool at room temperature and poured into thirty disposable petri dishes and were allowed to solidify before inoculation of raw water sample on it in the petri dish which will be incubated at 37⁰for 24hours.

□ Complete tests

The apparatus required for complete test are same as that of the confirmatory test. 30g of lactose broth in 500ml of distilled water was prepared as instructed by manufacture which was written on the side of the container. The mixture was heated to dissolve via hot plate and autoclave at 121⁰c for 15 minutes for sterilization, and then allowed to cool to room temperature. 10ml of the prepared lactose broth solution was dispensed into 20 test tubes containing inverted durham tubes (5 test tubes for each water sample). A wire loop was heated and used to transfer a colony from the confirmatory test above into the 20 test tubes and incubated for 24 hours at 37⁰C. Gas was observed in the durham tubes which signified the present of E-coli.

B) Total Coliform

To carry out the total coliform test, the following apparatus were required;

1. Nutrient agar
2. Petri dishes
3. Test tubes
4. Colony counter
5. Glass rack
6. Distilled water
7. Hand gloves

The cultured sample contained an appropriate number of discrete bacteria cell colonies, the original sample was subjected to serial dilution according to the method described by APHA *et al.*, (1998). Ten test tubes were serially arranged in a test tube rack containing 9ml of sterilized distilled water. Using a sterilized syringe 1ml of the raw water sample was inoculated into the first test tube, the mixture (total of 10ml) was shaken gently for proper mixing of the sample and thereafter, 1ml was then transferred to the second test tube and the same procedure was repeated up to the tenth test tube. A measure of 0.1ml from each of the test tube was cultured onto a solidified nutrient agar and a bent glass rod was used to evenly distribute the sample across the surface of the agar. The petri dishes were then incubated at 37°C for 24 hours. After the incubation period, the plates were removed from the incubator and petri dishes having an estimated count of between 30 to 360 colonies being counted and the values were multiplied by 0.1 followed by multiplying by 10 and finally by the reciprocal of the dilution factor. The values were then expressed as colony forming unit per millilitre (cfu/ml) as observed by Stuart, (2005).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 RESULT PRESENTATION

The presentation consists of data obtained from laboratory work (jar test, pH, turbidity and bacteriological examination) of raw water samples taken from three different dams and a River, namely; Chalawa Gorge Dam, Kano River, Tomas Dam and Kussalla Dam.

4.2 Jar Test

The result of the jar test was performed using *Hibiscus Sabdariffa* seed extract as a coagulating material. The study revealed that a dose of 4mg /L of raw water is a good amount for slightly turbid water, while 6mg/L of raw water yields a better result for highly turbid water. The result of the samples are presented and discussed in the sub sections below.

4.2.1 Chalawa Gorge Dam Sample

From Figure 4.1, it was observed that when Chalawa Gorge Dam raw water sample was treated with *Hibiscus sabdariffa* seed extract, as the doses of the extract increased from 2ml to 6ml, the turbidity removal increases. When the dose of *Hibiscus sabdariffa* seed was increased to 8ml and above, there was decrease in the turbidity removal. The highest removal efficiency was obtained at a longer settling time of 24 hours. Although for all the remaining studied duration the peak of

the turbidity removal was obtained at 6ml, while beyond 6ml of the dose of *Hibiscus sabdariffa* seed extract increases the turbidity of the water. Furthermore Figure 4.1 also depicts that settling time is significant in turbidity removal, hence the longer the settling time the more the turbidity removal. It could be inferred that *Hibiscus sabdariffa* seed extract coagulate relatively well on Challawa Gorge Dam water which has 546 NTU as initial turbidity. It was observed that the turbidity decreased when it was treated with *Hibiscus sabdariffa* seed extract as the settling time increased and also decreased when the dosage did not exceed 6mg/L otherwise the water became more turbid.

Findings from this study indicated that, *H. Sabdariffa* seed extract coagulated relatively well on high turbid water, moderate on medium and low turbid water with little floc formation. This was because the alkalinity of the treated samples was not enough to allow the formation of flocs. Bina (2009), in a study, viewed that the presence of bivalent cations such as Ca^{+2} and Mg^{+2} increases the ion strength of the solution and the destabilization of the colloidal particles. This was also in conformity with this study.

Furthermore from Appendix 1, it was also observed that, further increase of the extract decreased the pH of the water. Although the initial pH was 7.5, slight variation was noted at a settling time of 30 minutes and 24 hours for a dose of 8ml to 12mg/L, while at 1 hour and 6 hours for same dose, the pH remained slightly not constant. Thus, the pH decreased with respect to the dose and remained relatively unstable with respect to settling time. This was in accordance to study conducted by Mangale et al (2012), that after treatment with *Moringa oleifera* seed powder, the pH

decreased at 50 and 100mg/l dose but at 150mg/l it increased. The unstableness of the pH could be as a result of the variation in temperature. Although higher pH was obtained from the result, the study concurred with a study conducted by Madhavi (2013), which opined that optimum coagulation occurred at pH 8.

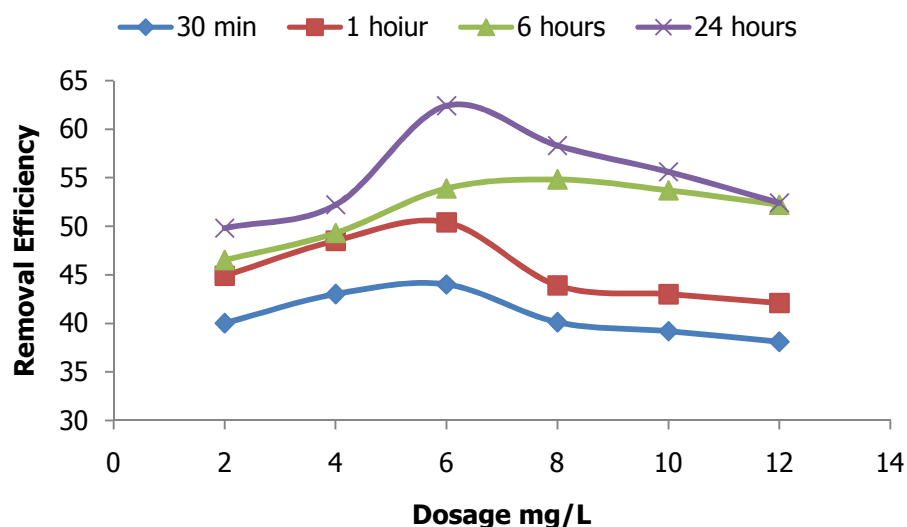


Figure 4.1: Turbidity removal efficiency of *Hibiscus sabdariffa* seed extracts on high turbid Water (546NTU) From Challawa Gorge Dam

It was clear that the turbidity of the sample is far above the WHO's recommended value for good quality drinking water. The dosages were varied from 2mg/L – 12mg/L for each sample treated.

A great difference in turbidity decrease was seen at the dose of 6mg/L of raw water which decreased the turbidity from 546NTU to 203 NTU. The removal efficiency was calculated using;

$$\text{Efficiency} = \left(\frac{C_1 - C_2}{C_1} \right) \times 100 \dots\dots\dots 4.1$$

where C_1 = initial turbidity

C_2 = final turbidity.

The maximum removal efficiency was recorded for Chalawa Gorge Dam with 62.80%, which occurred at a settling time of 24 hours, while the least occurred at a settling time of 1 hour at a dose of 12ml/ 1000L.

4.2.2 Kano River Sample

The reduction in turbidity was achieved at a dose just beyond 2ml, but above 4ml, the water became more turbid. From Figure 4.2, it was observed that *Hibiscus sabdariffa* seed extract performed slightly well on medium turbid water. It showed that the graph was at its peak after a dose of 2ml, while at 4ml the efficiency drops. This definitely increased the turbidity of the sample thus, as the dose increased, the turbidity of the water increased. This is in conformity with a study conducted by Jones (2013) in which he opined that, an increase in coagulant dose from 20mg/l reduces the removal efficiencies of both the Cannabis powder and the extract. It appeared that, an increase in cannabis dosage led to an increase in turbidity of the water. Kano River originated from the rocky area of plateau with low turbidity water, as such the water had less turbidity from source when compared to other samples used in this study.

Moreover, from Appendix 2, the use of *Hibiscus sabdariffa* seed extract on Kano River showed that the pH decreased as the dose increased. The pH at 30 minutes and 1 hour remained constant. At 6 hours and 24 hours there was slight variation in all the pH except for 6ml, both duration remained stable. This could be attributed to the fact that as the dose increased the pH tended to be stable towards acidity which is as

result of the presence of tannin and saponin in its phytochemical constituent. Consequently, the pH in this sample ranges between 6.2 to 7.9 which was within the recommended acceptable range for drinking water as specified by WHO (2006) is between 6.0 and 8.0. The observation on pH made in this present study were in accordance with previous studies on coagulation and flocculation ability of some seeds (Ndabigengesere et al., 1995).

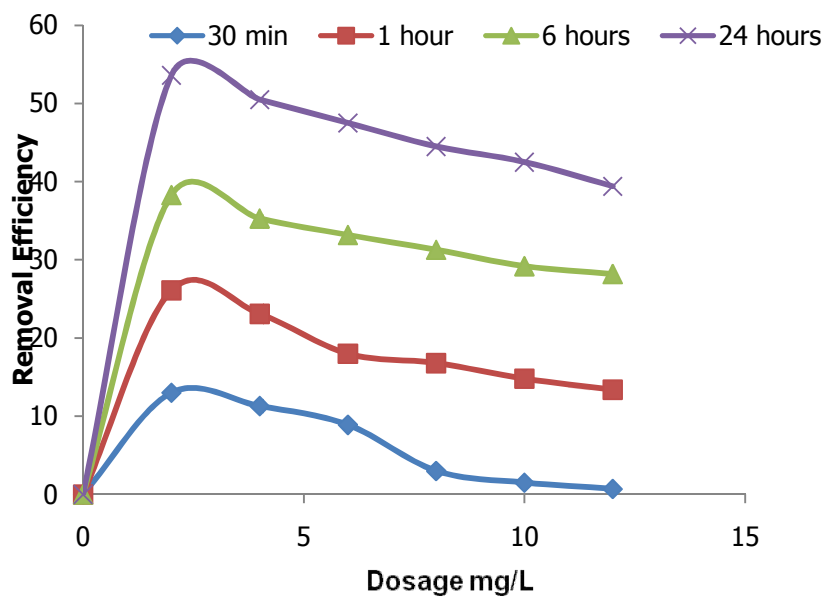


Figure 4.2: Turbidity removal efficiency of *Hibiscus sabdariffa* seed extract on high turbid Water (97.9NTU) From Kano River

4.2.3 Tomas Dam Sample

From Figure 4.3, it was observed that the optimum dose was 6mg/L which is the peak of the graph at 24 hours with removal efficiency of 63NTU. The least was obtained 30 minutes at a dose of 6ml/L with a removal efficiency of 43NTU. This turbidity value is still above the WHO recommended level of 5NTU. However, according to Arnoldsson et al.,(2008), the optimal dosage for specific water is

defined as the dosage which gives the lowest turbidity in the treated water. This is also associated to the fact that plant as coagulant and disinfectant performs better in high turbid water than in medium or low turbid water. From figure 4.3, it could be deduced that further increase in dose will increase the turbidity. This was because the particles are colloidal, as such do not settle easy and caused the water to appear more turbid. Figure 4.3 also showed that the best result was obtained at 6ml/L of raw water sample for all the durations. This implied that as the dose increased the water become more turbid resulting into destabilization of colloidal particles (Bina 2009). This is also in accordance with the findings by Jones (2013), an increase in coagulant dosage resulted in a reduction in the removal efficiency of the coagulants.

Furthermore, it can also be seen on Appendix 3 that as the doses of the extract increased from 2ml to 8ml, the pH decreased, but further increase in the extract decreased the pH of water. Although the initial pH was 6.7, slight variation was noted in the pH across the settling time but maintained a stable pattern with respect to the dose. It was also observed that further increase in the dose resulted into a stable pattern with slight difference. Furthermore, in Appendix 3, it was also deduced that at 2ml the pH was at its peak for all the duration with slight variation except for 6 hour in which the pH dropped below 6.5. This could be associated to the fact that temperature played a vital role thus, with low temperature the pH also drops.

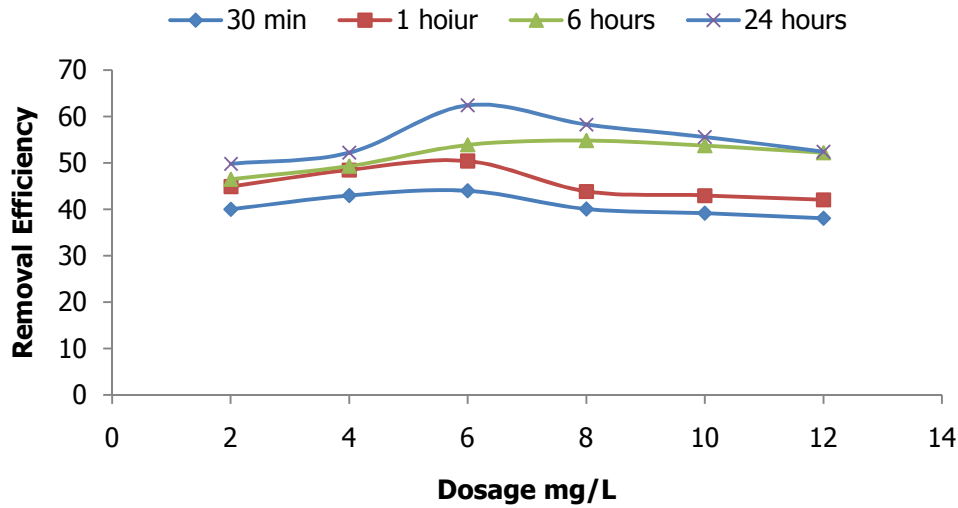


Figure 4.3: Turbidity removal efficiency of *Hibiscus sabdariffa* seed extract on medium turbid Water (81.1 NTU) From Tomas dam

4.2.4 Kussalla Dam Sample

The best result for turbidity removal for this sample was obtained at 4ml as further increase in dose yield increased turbidity. This was in conformity to the study conducted by Jones (2013), that natural coagulants performs better in high turbid water than medium and low turbid waters. The study also showed that higher dosages did not significantly increase pollutant removal and were not economically viable. The graph for Kussalla Dam was not plotted because computing for the efficiency gave a negative value and since efficiency cannot be in negative the graph for Kussalla dam was not plotted. This also signifies that the initial turbidity was very insignificant. Therefore the used of *Hibiscus sabdariffa* seed extract was more of a disinfectant, yielding 94% at 6ml of the extract from from figure 4.4.

With an initial pH of 6.7 as seen in Appendix 4, as the doses of the extract increased from 2mg to 4mg, the pH decreased, but further increase of the extract increased the

pH of water. Although slight variation was noted at a settling time of 30 minutes, while above that; 1 hour, 6 hour and 24 hours, the pH increased to 7 while a pH of 7.9 was recorded at a settling time of 24 hour for a dose of 2ml/L. The pH remains generally not constant. It could be inferred that the pH ranged between 6.2 and 6.4 at 30 minutes when *Hibiscus sabdariffa* seed extract was added to the raw water. As the settling time increased to 1 hour and 6 hours respectively, the pH was also constant with respect to the dose but with higher value of the pH at a dose of 4ml and 6ml. This could be associated to the fact that pH within the alkalinity range aids coagulation. Hence at doses of 2ml for a settling period of 24 hours a higher pH of 7.9 was obtained.

It was depicted from Appendix 4 that, at higher pH the turbidity removal was effective although the initial turbidity was 13.2. This study was in line with a study conducted by Ahamed (2010), opined that; pH was the most important parameter for proper coagulation.

In summary, for all the samples, the pH could either decrease or increase as the dose increased but were found to be within the range of 6.2 to 7.9, which was in accordance with WHO (2006), that recommended acceptable range of pH for drinking water to be between 6.0 and 8.0. It could be inferred that 2ml /L to 4ml /L of *Hibiscus sabdariffa* seed extract is a good amount when water is slightly turbid and 2ml /L to 6ml /L when water is highly turbid.

4.3 BACTERIOLOGICAL ANALYSIS

For the purpose of this study, test for bacteriological analysis was conducted in two different methods; most probable number (MPN) and standard plate count. This was to further ascertain the efficiency of *H. Sabdariffa* seed extract in disinfecting water.

4.3.1 Result for MPN Analysis

Table 4.1 presents the results of bacterial cell concentration before treatment with *Hibiscus sabdariffa* seed extract for water samples for three Dams and a River. From the presumptive test, the positive test tubes were counted and interpreted using the MPN chart on Appendix 5. The highest most probable number (MPN) of bacterial cell concentration before treatment was recorded from water sample collected from Kano River with 100 MPN / 100ml, while 26MPN/100ml was the least bacterial cell concentration recorded for Tomas Dam water sample.

Table 4.1: Concentration of bacteria cells before treatment with *Hibiscus sabdariffa* seeds in raw water.

Samples	10ml(no of +ve test tube)	1ml (no of +ve test tube)	0.1ml (no of +ve test tube)	MPN Index per 100ml
Challawa gorge Dam	4	3	5	33
Kano River	5	5	1	100
Tomas Dam	4	2	5	26
Kussalla Dam	4	5	5	34

The result of bacteria cell concentration after treatment with 4ml of *Hibiscus sabdariffa* seed was presented in Table 4.2. The highest value of bacteria concentration after treatment was found in Kano River sample with MPN of 14, from MPN chart, while Chalawa Gorge, Tomas and Kussalla Dams had 9 MPN.

Table 4. 2: Concentration of bacteria cell after treatment with 4ml of *Hibiscus sabdariffa* seeds extract.

Samples	10ml(no of +ve test tube)	1ml (no of +ve test tube)	0.1ml (no of +ve test tube)	MPN Index per 100ml
Challawa gorge Dam	2	2	0	9
Kano River	3	2	0	14
Tomas Dam	2	2	1	9
Kussalla Dam	2	2	2	9

From Table 4.3, when samples were treated with 6ml of *Hibiscus sabdariffa* seed extract, the highest value of bacteria concentration was found in Kano River sample with MPN of 9. This was attributed to the fact that, Kano River sample contained high concentration of bacteria cells before treatment than other samples. From the MPN chart which could be seen in Appendix 5, Chalawa Gorge Dam, Tomas Dam and Kussalla Dam had a cell concentration of 2, 4 and 2 respectively

4. 3: Concentration of bacteria cells after treatment with 6ml of *Hibiscus sabdariffa* seeds extract.

Samples	10ml (no of +ve test tube)	1ml (no of +ve test tube)	0.1ml (no of +ve test tube)	MPN Index per 100ml
Challawa Gorge Dam	1	0	0	2
Kano River	2	2	0	9
Tomas Dam	1	0	1	4
Kussalla Dam	1	0	0	2

4.3.2 Discussion of MPN result of the samples

The percentage disinfection efficiency of 4ml and 6ml concentration of *Hibiscus sabdariffa* seed extract in all the sample analyses in the study was calculated using the formulae

$$\text{Efficiency (\%)} = (((C_1 - C_2) / C_1) * 100) \dots\dots\dots 4.2$$

where C_1 = bacteria concentration before treatment

C_2 = bacteria concentration after treatment with 2ml or 4ml.

A bar chart was plotted for the dose of 4ml and 6ml of 100ml in Figure 4.4. It was observed that the highest disinfection efficiency recorded from Kussalla Dam was 94% after 24 hours at a dose of 6ml of the extract while at a dose of 4ml, it recorded 74%. Kano River recorded 86% and 91% at 4ml and 6ml respectively. The least was recorded in water sample collected from Tomas Dam with 65% for 4ml/100 MPN and 85%/100 MPN for 6ml of *Hibiscus Sabdariffa* seed extract. In this study, the

presence of *E. coli* was justified by the green metallic colonies formed when EMB agar was used at the confirmatory stage and gas production at the complete stage. It was also reported by Tyagi *et al.*, (2006), that the presence of *E. coli* could be regarded as a more representative of fecal pollution because it is present in higher numbers in fecal material and generally not elsewhere in the environment as recorded in this study. The reduction of the number of faecal coliform (*E. coli*) using *Hibiscus sabdariffa* seed extract in the study could be as a result of the presence of antibacterial activity of the plant which had been reported in previous research by Emelike *et al* (2014) to have bactericidal effect. In a similar study conducted by Harashit (2014) in the use of natural coagulant and fecal coliform counts were about 96%, 90.47%, and 89.52% using *Moringa oleifera*, *Cicerarietinum*, and *Dolichos lablab*. This further agrees with the success recorded in this study; the use of *Hibiscus sabdariffa* Linn seed extract in treating fecal coliform in water. Generally faecal coliforms bacteria are present in the environment and feces of all warm-blooded animals and humans. Faecal coliform bacteria are unlikely to cause illness; however, their presence in drinking water indicates the presence of disease-causing organisms (pathogens) such as *Salmonella typhi* (that causes typhoid); *Shigella spp*, causes Shigellosis; and *Klebsiella spp* could be in the water system. Most pathogens that can contaminate water supplies come from the feces of humans or animals. Testing drinking water for all possible pathogens is complex, time-consuming, and expensive. It is easy and inexpensive to test for faecal coliform bacteria because they can survive longer in water. This is the reason why WHO (2003), reported that faecal coliform should be absent in any drinking water.

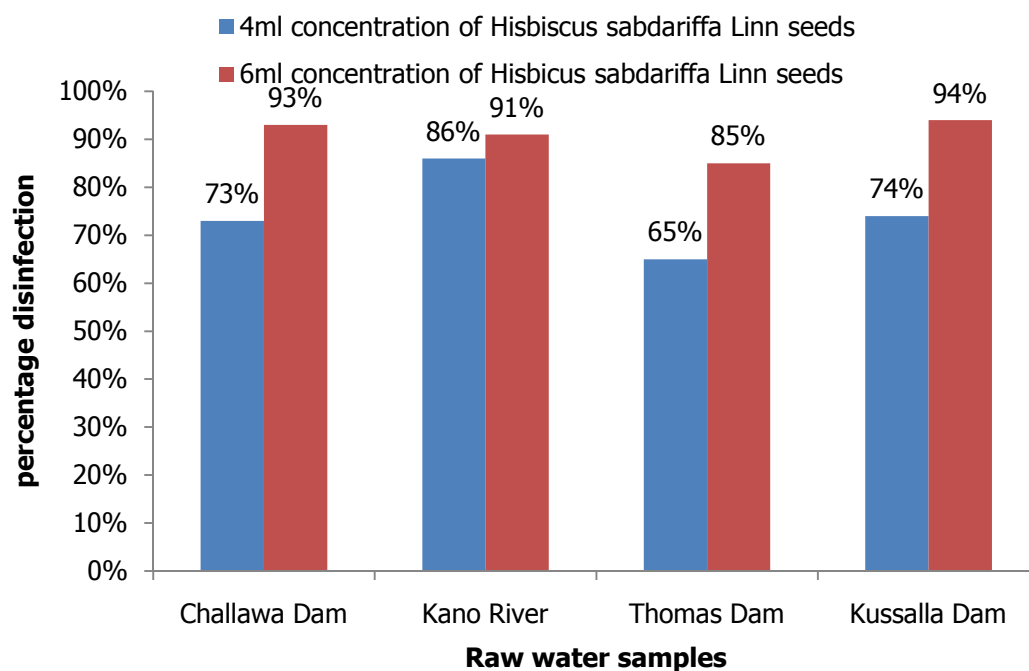


Figure 4.4: Efficiency of 4ml and 6ml Concentration of *Hibiscussabdariffa* seeds extract used in treatment of raw water sample

4.3.3 Results of standard plate count for all the samples

Results of the total coliform count of the raw water sample before treatment with *Hibiscus sabdariffa* seeds is presented in Table 4.4. Kano River and Kussalla Dam had the highest total coliform count of 4.3×10^4 cfu/ml, while Tomas Dam had the least total coliform count of 1.7×10^4 cfu/ml

Table 4.4: Total Coliform count of raw water sample before treatment with *Hibiscus sabdariffa* seeds extract.

Samples	Total coliform Count (cfu/ml)
Challawa Gorge Dam	1.9×10^4
Kano River	4.3×10^4
Tomas Dam	1.7×10^4
Kussalla Dam	4.3×10^4

After treatment with 4ml, the least total coliform count of 0.26×10^4 cfu/ml was recorded for water sample from Challawa Gorge Dam which represented 86.3% reduction while the highest total coliform count of 0.72×10^4 cfu/ml was recorded for water sample from Kano River which represented 83.3% as presented in Table 4.5.

Table 4.5: Total coliform count of treated water sample with 4ml of with *Hibiscus sabdariffa* seeds extract.

Samples	Total coliform Count (cfu/ml)	Percentage Efficiency Reduction
Challawa Gorge Dam	0.26×10^4	86.3%
Kano River	0.72×10^4	83.3%
Thomas Dam	0.31×10^4	81.3%
Kussalla Dam	0.58×10^4	81.3%

When 6ml of *Hibiscus sabdariffa* Seeds extract was used, the highest percentage reduction of 99.7 % was recorded in the entire water sample collected in this study except the water sample from Challawa dam which recorded 99.6%. The total coliform for the four water samples used in this study were 76, 15, 49 and 92 for Challawa Dam, Kano River, Tomas Dam and Kussalla Dam respectively as presented in Table 4.6.

Table 4.6: Total coliform count of treated water sample with 6ml of with *Hibiscus sabdariffa* Seeds extract.

Samples	Total Coliform Count (cfu/ml)	Percentage Efficiency Reduction
Challawa Gorge Dam	76	99.6%
Kano River	15	99.7%
Thomas Dam	49	99.7%
Kussalla Dam	92	99.7%

The result of the total coliform count of the water samples used in this study indicated that there were significant reductions in the level of the total coliform count after treatment with *Hibiscus sabdariffa* Seeds extract. Despite the success recorded in the reduction of the coliform count by varying the concentrations of *Hibiscus sabdariffa* seeds extract, more of the activities in terms of reduction of the coliform count was recorded when the concentration of antimicrobial activity of plants increased with the increase in concentration as previously reported by Stuart, (2005). The study further opined that efficient reduction (80% to 90%) for high turbid pathogenic surface water and produces an aesthetically supernatant, concurrently accompanied by 90.00% to 99.99% bacterial reduction. This may be an indication of bactericidal activity of these natural coagulants. This also conformed to this present study. Also the variations recorded with the water samples in the percentage reduction of the coliform could be attributed to the initial concentration of the coliform in the raw water samples before treatment with *Hibiscus sabdariffa* seeds extract as the raw water sample from Kano River and Kussalladam was highly contaminated with coliform (4.3×10^4 cfu/ml; Table 4.4) compared to the rest of the raw water samples used for the purpose of this study. Furthermore, it was found that

when the samples were stored for 24hours, there were no growths of the coliforms. Such findings have previously been reported by Bina (2009).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusions

- 1) Based on the turbidity result obtained from this study, it showed that *Hibiscus sabdariffa* seed extract is relatively effective in removing the turbidity of water.
- 2) From the findings of this study, the maximum amount of *Hibiscus sabdariffa* seeds extract that will yield acceptable water quality is within the range of 2mg to 6mg/1L of raw water sample.
- 3) The findings in this research also showed that the pH value of the treated water was within the specified range. However the pH maintained a stable value with longer settling time.

5.2 Recommendations

Based on the outcome of this study, the following recommendations are made;

1. *Hibiscus sabdariffa* seed extract should be used on other water sources such as waste water from industries and highly turbid water from other sources to remove turbidity and disinfect water.
2. Re-run of decanted jar test sample is also recommended, as this will give a possible maximum effective turbidity removal.
3. The optimum pH at which the best coagulation and disinfection is attained should be determined.

4. The use of *Hibiscus sabdariffa* seed extract as a coagulant with other plants so as to improve its coagulation ability.
5. More work should be done in disinfection with dose above 8ml to attain 100%.
6. It is recommended that more natural coagulant and disinfectant should be investigated.

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APPENDIX 1

Variation of turbidity / pH with respect to dosage for Challawa Gorge dam

Setting time	30 minute		1 hour		6 hour		24 hour	
Dosage(ml)	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH
0	546.0	7.5	544.0	7.4	542.0	7.4	540.0	7.5
2	328.0	7.4	300.0	7.1	290.0	7.9	271.0	7.2
4	310.0	7.3	280.0	6.6	275.0	7.7	258.0	7.4
6	305.0	7.2	270.0	6.6	250.0	7.4	203.0	7.3
8	325.0	7.2	305.0	6.3	245.0	7.3	225.0	7.2
10	330.0	7.2	310.0	6.2	251.0	7.2	240.0	7.2
12	338.0	7.1	315.0	6.2	259.0	6.9	257.0	7.2

APPENDIX 2

Variation of turbidity / pH with respect to dosage for Kano River

Setting time	30 minute		1 hour		6 hour		24 hour	
Dosage(ml)	Turbidity	pH	Turbidity	Ph	Turbidity	pH	Turbidity	pH
0	97.9	7.9	97.7	7.4	97.4	7.4	97.2	7.5
2	85.2	6.7	72.1	6.7	60.2	6.9	45.2	6.9
4	87.1	6.2	75.1	6.2	63.1	6.7	48.1	6.8
6	89.2	6.3	80.1	6.3	65.1	6.6	51.1	6.7
8	95.0	6.3	81.3	6.3	67.0	6.5	54.0	6.7
10	96.4	6.3	83.2	6.3	69.0	6.5	56.0	6.5
12	97.2	6.3	84.6	6.3	70.0	6.4	59.0	6.6

APPENDIX 3

Variation of turbidity / pH with respect to dosage for Tomas dam

Setting time	30 minute		1 hour		6 hour		24 hour	
Dosage(ml)	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH
0	81.1	6.7	81.0	6.5	79.9	6.4	81.0	6.2
2	80.0	7.4	78.0	7.3	71.0	6.6	60.5	7.4
4	79.1	7.3	70.1	7.2	68.0	6.5	48.2	7.3
6	65.0	7.2	60.0	7.2	55.0	6.5	43.0	7.3
8	67.0	7.2	65.0	7.2	65.0	6.5	51.0	7.3
10	67.2	7.1	78.0	7.1	67.0	6.4	54.0	7.2
12	68.0	7.1	80.0	7.1	70.0	6.5	55.0	7.2

APPENDIX 4

Variation of turbidity / pH with respect to dosage for Kussalla dam

Setting time	30 minute		1 hour		6 hour		24 hour	
Dosage(ml)	Turbidity	pH	Turbidity	pH	Turbidity	pH	Turbidity	pH
0	13.2	6.7	13.0	6.5	13.1	6.4	13.0	6.2
2	9.9	6.4	9.3	7.4	5.6	7.4	2.1	7.9
4	9.3	6.3	8.4	7.5	5.2	7.5	1.2	7.6
6	13.0	6.2	11.7	7.5	6.7	7.5	2.5	7.4
8	19.4	6.3	17.1	7.4	13.2	7.4	2.5	7.3
10	21.0	6.3	18.1	7.3	14.3	7.3	5.5	7.2
12	26.0	6.3	21.3	7.3	15.5	7.3	6.7	7.1

APPENDIX 5

Table 10.5 MPN index and 95 per cent confidence limits for various combinations of positive results when five tubes are used per dilution (10 ml, 1.0 ml, 0.1 ml portions of sample)

Combination of positives	MPN index per 100 ml	95 % confidence limits		Combination of positives	MPN index per 100 ml	95 % confidence limits	
		Upper	Lower			Upper	Lower
0-0-0	<2	-	-	4-2-0	22	9.0	56
0-0-1	2	1.0	10	4-2-1	26	12	65
0-1-0	2	1.0	10	4-3-0	27	12	67
0-2-0	4	1.0	13	4-3-1	33	15	77
				4-4-0	34	16	80
1-0-0	2	1.0	11	5-0-0	23	9.0	86
1-0-1	4	1.0	15	5-0-1	30	10	110
1-1-0	4	1.0	15	5-0-2	40	20	140
1-1-1	6	2.0	18	5-1-0	30	10	120
1-2-0	6	2.0	18	5-1-1	50	20	150
				5-1-2	60	30	180
2-0-0	4	1.0	17	5-2-0	50	20	170
2-0-1	7	2.0	20	5-2-1	70	30	210
2-1-0	7	2.0	21	5-2-2	90	40	250
2-1-1	9	3.0	24	5-3-0	80	30	250
2-2-0	9	3.0	25	5-3-1	110	40	300
2-3-0	12	5.0	29	5-3-2	140	60	360
3-0-0	8	3.0	24	5-3-3	170	80	410
3-0-1	11	4.0	29	5-4-0	130	50	390
3-1-0	11	4.0	29	5-4-1	170	70	480
3-1-1	14	6.0	35	5-4-2	220	100	580
3-2-0	14	6.0	35	5-4-3	280	120	690
3-2-1	17	7.0	40	5-4-4	350	160	820
4-0-0	13	5.0	38	5-5-0	240	100	940
4-0-1	17	7.0	45	5-5-1	300	100	1,300
4-1-0	17	7.0	46	5-5-2	500	200	2,000
4-1-1	21	9.0	55	5-5-3	900	300	2,900
4-1-2	26	12.0	63	5-5-4	1,600	600	5,300
				5-5-5	>1,600	-	-

Source: After APHA, 1992