THE EFFECT OF METHANOL RHIZOME EXTRACT OF *Nymphaea lotus* LINN. (NYMPHAEACEAE) IN ANIMAL MODELS OF DIARRHOEA

BY

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Declaration

I declare that the work in this Dissertation entitled:'The effect of methanol rhizome extract of *Nymphaea lotus* Linn. (Nymphaeaceae) in animal models of diarrhoea' has being carried out by me in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertationwas previously presented for another degree or diploma at this or any other Institution.

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Certification

This dissertation entitled THE EFFECT OF METHANOL RHIZOME EXTRACT OF *Nymphaea lotus* LINN. (NYMPHAEACEAE) IN ANIMAL MODELS OF DIARRHOEAby Fatima HauwaBELLO meets the regulations governing the award of the degree of Masters in Pharmacology of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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Abstract

Nymphaea lotus is one of the foremost aquatic macrophytes that have been identified in Nigerian fresh water bodies. It finds applications in the management of circulatory system disorders, digestive system disorders, infectionsand inflammations. This study aims to evaluate the antidiarrhoeal activity of the methanol rhizome extract of *N. lotus* Linn plant in laboratory animals. Shade-dried rhizome of *N. lotus* was extracted with 80% methanol using Soxhlet apparatus. Preliminary phytochemical screening was carried out.This revealed the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and triterpenes. The oral median lethal dose (LD_{50}) was determined andacute toxicity test of the extract gave LD_{50} value greater than 5,000mg/kg *p.o.* in mice. The antidiarrhoeal activityof the methanol extract was determined in mice. The extract was screened for activity against castor oil-induced diarrhoea and magnesium sulphate-induced diarrhoea as well as effect on gastric transit time in mice. For castor oil-induced diarrhoea, the extract at doses of 200, 400 and 800mg/kg produced statistical significant reduction in the frequency of diarrhoea (at $p<0.001$, $p<0.001$ and $p<0.01$ respectively). The extract at 800 mg/kg produced a significant delay in onset of diarrhoea ($p<0.05$) comparable to loperamide (3mg/kg). The frequency of magnesium sulphate-induced diarrhoeawas also significantly reduced in the groups treated with 200, 400 and 800mg/kg of the extract at $p<0.001$, $p<0.001$ and $p<0.01$ respectively. At doses of 200mg/kg (76.5%) and 400mg/kg (72.6%), the protection produced comparable to loperamide, 3mg/kg (70.6%).All treated groups produced statistically significant reduction in the transit of charcoal meal along the intestinal tract at $p<0.001$. The standard antidiarrhoeal drug, atropine (5mg/kg) produced greater antimotility effect (56.83%)

compared to the extract. Effect of methanol rhizome extract of *N. lotus* on isolated rabbit jejunum and guinea pig ileum was determined. The methanol rhizome extract of*Nymphaea lotus* at low concentration (4×10^{-4} – 6.4×10^{-2}mg/ml) had contractile effect on the tone of contraction of the rabbit jejunum while at higher concentrations (8×10^{-2} -512×10^{-2} mg/ml) produced significant reduction in the tone and rate of spontaneous contraction of rabbit jejunum. Interaction of the extract,at concentration that causes relaxation, with acetylcholine,attenuated the effect of the latter in a manner similar to atropine.The methanol rhizome extract of *N. lotus* at lower concentrations (4×10^{-4} to 2×10^{-2} mg/ml) has no effect on contraction of the guinea pig ileum while higher concentrations (4×10^{-2} - 512×10^{-2} mg/ml) produced significant relaxant activity on guinea pig ileum. Interaction of the extract with histamine attenuated the effect of the latter which may be as a result of physiologic antagonism.This study has shown that the methanol rhizome extract of *N. lotus* has antidiarrhoeal properties thus justifying its use by the local population for this purpose.

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Abbreviations, Definitions, Glossary and Symbols

ad libitum	at liberty
AID	Acquired Immune Deficiency Syndrome
AMMCOP	Academy of Medicine of Malaysia and Malaysian Paediatric Association
ANOVA	Analysis of Variance
APCON	Advertising Practitioners Council of Nigeria
CDC	Center for Disease Control and Prevention
cm	centimeter
CT	Computed Tomography
DW	Distilled water
ECL	Enterochromaffin-like
EPEC	Enteropathogenic*Escherichia coli*
g	gram
GIT	Gastrointestinal tract
H	hour
HDC	Histidine decarboxylase
HIV	Human immunodeficiency virus
IBS	Irritable bowel syndrome

K^+	Potassium ion
kg	kilogram
l	Liter
LD_{50}	Median lethal dose
Lop	Loperamide
mg	milligram
ml	milliliter
mm	millimeter
MRSA	Methicillin resistant *Staphylococcus aureus*
mV	millivolt
Na^+	Sodium ion
NITR	National Institute for Trypanosomiasis (and Onchocerciasis) Research
NL	methanol rhizome extract of *Nymphaea lotus*
ORS	Oral rehydration salt
ORT	Oral rehydration therapy
p.o.	Oral administration
PLWHA	People Living With HIV/AIDS
SEM	Standard error of mean

Spp	Species
SPSS	Statistical package for social sciences
STEA	Standard telomerase elongation assay
UNICEF	United Nations Children Fund
VRSA	Vancomycinresistant *Staphylococcus aureus*
w/v	weight per volume
WGO	World Gastroenterology Organisation Global Guidelines
W.H.O.	World Health Organisation
wt	weight

CHAPTER ONE

1.0 INTRODUCTION

Diarrhoea is characterized by increased frequency of bowel movement, wet stool and abdominal pain (Ezekwesili *et al.,* 2004). Diarrhoeal disease account for nearly 1.3 million deaths a year among children under-five years of age, making it the second most common cause of child deaths worldwide, with half of the deaths occuring in just five countries: India, Nigeria, Afghanistan, Pakistan and Ethiopia (UNICEF, 2012).

Diarrhoea occurs most of the time when there is an imbalance between the absorption of and secretion in gastrointestinal tract. When the absorptive capacity of the intestine is exceeded and net secretion is greater than absorption,excess loss of fluid in faeces results (Nigro *et al.,* 2000; Guerrant *et al.,* 2001).

According to the World Health Organization (WHO), diarrhoea is one of the most common causes of morbidity and mortality in many developing countries affecting mainly infants and children (Fernando*et al.,* 2010). In Nigeria, it is the number one fatal outcome disease among children under five years of age (Ahmed *et al.,* 2007).

The WHO in 2004, introduceda programme for control of diarrhoea which involved and encouraged the use of traditional herbal medicine (Njume and Goduka, 2012). This was introduced keeping in mind centuries' old efficacy, experience, accessibility and cost (Yakubu*et al.,* 2012).

Traditional medicine is a widely used and a rapidly growing health system with high economic importance. Up to 80% of the population in Africa uses traditional medicine to help meet their health care needs (WHO, 2002). This is mainly due to the economic viability, accessibility and ancestral experience (Wendell *et al.,* 2008). Traditional Medicine remains an indispensable component of Nigeria's health services and practices, patronized by a large percentage of the population. It should therefore be integrated into the nation's health delivery system in a manner that protects users of traditional medicine and the general public (APCON, 2014). Local herbalists have depended on medicinal plants as a reliable means of treating diarrhoea. Hence the use of medicinal plants that possess anti-diarrhoeal activities has been explored as a measure that could be of benefit in combating widespread diarrhoea infections especially in third world countries (Adeyemi *et al.,* 2003). Numerous studies have validated the traditional use of antidiarrhoeal medicinal plants by investigating the biological activity of extracts of such plants, which have antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water absorption or reduce electrolyte secretion (Kambaska *et al.,* 2006).

In many rural communities in the developing world, medicinal remedies prepared from indigenous plants are almost always the only readily accessible and affordable therapies for the control of diarrhoea (Green *et al.,* 2010). In these communities, extracts, decoctions/concoctions or ashes of various plant parts (roots, rhizomes, tubers, aerial parts, stem barks and leaves) are used as remedies for diarrhoea and other illnesses (Njume *et al.,* 2009).

The anti-diarrhoeal activity of many of the plants has been found to be due to the presence of tannins, alkaloids, saponins, flavonoids, steroids and/or terpenoids (Teke *et al.,* 2010). However, only few of these compounds have eventually found themselves on pharmaceutical shelves as anti-diarrhoeal agents after several years of testing and clinical evaluation. These findings lend pharmacological credence to the anecdotal, ethno medicinal use of medicinal plants as remedies for diarrhoea and indicate the need for more research in this area (Njume and Goduka, 2012).

It is, therefore, important to identify and evaluate natural drugs that can be used as alternatives to commonly used antidiarrhoeal drugs, which are often accompanied by adverse effects such as addiction (e.g. diphenoxylate) and constipation (e.g. loperamide) (Hardman and Limbird, 2010). As the herbal products possess few side effects when compared to the synthetic drugs, the WHO has supported various studies for the control of diarrhoea which involve the usage of herbal medicines based on traditional medicinal practices (Jebunnessa *et al.,* 2009).

Nymphaea lotus Linn is a herbaceous aquatic plant whose leaves floats or submerges in water (Abu-Zaida *et al.,* 2008). It is generally wide spread in tropical Africa (Sowemimo *et al.,* 2007a). It is native to Egypt, Central and West Africa and Madagascar.*N. lotus* finds applications in the management of circulatory and digestive system disorders (such as diarrhoea), infections, inflammations and some other disease conditions (Burkill, 1997).

1.1 Statement of Research Problem

Diarrhoeal diseases are a major health concern in developing countries with an estimated1.8 million deaths per annum (WHO, 2004). Despite improvements in public health and economic well-being, it remains an important clinical problem in developed countries as well (Casburn-Jones and Farthing, 2004). Diarrhoea can be very serious in infants and elderly people because of the risk of severe, potentially fatal dehydration. About 90% of People Living with HIV/AIDS (PLWHA)are affected with diarrhoea, a very common symptom of HIV/AIDS and results in a significant morbidity and mortality. In Africa, diarrhoea is four times more common among children with HIV and seven times more common among adults with HIV than their HIV-negative household members (WHO,1993).Apart from cost of treatment, diarrhoeal pathogens are becoming distressingly resistant to antimicrobial agents and some of the major treatments of diarrhoea (oral rehydration solutions, ORS) may not reduce the volume of

stool or duration of illness (Bardhan, 2007). As a means of ensuring effective treatment, use of combination therapies in the management of diarrhoeal diseases is being practiced hence leading to increase pill burden, longer duration of therapy and possible adverse effects. Given the fact that hospitals may not be readily accessible in rural African communities, dependence on indigenous herbal medicines as remedies for diarrhoea is on the rise in these communities (Njume and Goduka, 2012).

The various treatments available for diarrhoea (e.g. loperamide, bismuth subsalicylate, racecadotril and many more) possess various side effects like abdominal discomfort, dry mouth, nausea, constipation and headache. Various herbal remedies are present that possess lesser side effects than the conventional drugs and thus are safer to use (Sarin and Bafna, 2012).

1.2 Justification

Vast majority of people in developing countries rely on herbal drugs for the management of diarrhoea, despite the availability of vast spectrum of approaches for diarrhoeal management. More than 80% of people in rural African communities still rely on indigenous medicine as a primary source of health care (Tchacondo *et al.,* 2011). This is partly due to the fact that the majority of the people are not able to meet the high cost associated with the western health care system and also the benevolent attachment to their culture and tradition (Maroyi, 2011). The World Health Organisation,with a view to exploit and identify compounds that could provide safe and effective remedies for ailments of both microbial and non-microbial originshave encouraged the interaction between western-based and indigenous-based medicines(WHO, 2004).WHO has also encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medical practices (Atta and Mouneir, 2004). A range of medicinal plants with antidiarrhoeal and antimicrobial properties have been widely used by traditional healers. It becomes very important to pursue measures to maximise the medicinal potential of indigenous plants (Njume and Goduka, 2012). However, therapeutic potentials of some of these medicines have not been scientifically evaluated (Havagiray *et al.*,2004). Among these plants, *Nymphaea lotus*,which is widely distributed throughout tropical Africa, enjoys a number of ethnomedical uses in Nigeria. Traditionally, the rhizomes of *N. lotus* are used to cure diarrhoea (Burkill, 1997). Therefore, it is important to establish the scientific basis for the ethnomedical claim of *N. lotus* as an antidiarrhoeal agent. This may serve asa source for developing more effective antidiarrhoeal drug. For this reason, the antidiarrhoeal effect of methanol rhizome extract of *N. lotus* is being studied.

1.3 Aim and Objectives

1.3.1 Aim

The aim of the study is to evaluate the antidiarrhoeal activity of the methanol rhizome extract of *Nymphaea lotus* Linn in laboratory animals.

1.3.2 Specific objectives

This study has the following specific objectives:

i. To determine the median lethal dose (LD_{50}) of the methanol rhizome extract of *Nymphaea lotus*
ii. To evaluate the effect of the extract on the perfused isolated tissue preparations
iii. To evaluate the effect of the methanol rhizome extract of *Nymphaea lotus*in some animal models of diarrhoea
iv. To evaluate the effect of the methanol rhizome extract of *Nymphaea lotus* on gastric transit time

1.4 Hypothesis

The methanol rhizome extract of *Nymphaea lotus* Linn possesses antidiarrhoeal activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diarrhoea

2.1.1 Definition

Diarrhoea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual). Frequent passing of formed stools is not diarrhoea, nor is the passing of loose, "pasty" stools by breastfed babies (WHO, 2013).

Normal bowel frequency ranges from three times a day to three times a week in the normal population. Increased stooling, with stool consistency less solid than normal, constitutes a satisfactory, if somewhat imprecise, definition of diarrhoea (Soffer, 2001).

Diarrhoea is not itself a disease, but can be a symptom of several diseases. It is one of the most common clinical signs of gastrointestinal disease, but also can reflect primary disorders outside the digestive system (Sarin and Bafna, 2012).

2.1.2 Aetiology

Most of the time diarrhoea will take place when there is an imbalance between absorption and secretion, when the absorptive capacity of the intestine is exceeded and net secretion is greater than absorption (Nigro *et al.,* 2000). Diarrhoea may result when there is a minimal change in normal intestinal fluid and electrolyte balance. Such changes may be caused by infectious agents, toxins and other noxious agents present in the gut causing disruption of normal fluid secretion and stimulating the gut to expel its contents. This response is protective against acute irritations of the gut but becomes a problem when chronically present and no longer serving a physiological role (Payne *et al.,* 2006). Due to failures in the regulation of ionic balance, differences in fluid absorption and secretion may result in very large changes in stool consistency and volume (Njume and Goduka, 2012).

The causes of diarrhoea are wide and varied; the majority of them are related to poor sanitary conditions and low socio-economic status (Aremu *et al.,* 2011). Infectious diarrhoea, the most common form of diarrhoea worldwide may be caused by viruses, bacteria or protozoa (Palombo, 2006; WHO, 2013). The contribution of the various pathogens to diarrhoea may differ substantially between regions depending on local meteorological, geographic, and socio-economic conditions (Reither *et. al*., 2007).

Diarrhoea-causing pathogens are usually transmitted through the faecal-oral route. Multiple host factors that determine the level of illness once exposure to infectious agents has occurred include: age, personal hygiene, gastric acidity and other barriers, intestinal motility, enteric microflora, immunity and intestinal receptors (Soffer, 2001).Diarrhoea can also be the initial sign of non-gastrointestinal tract illness, including meningitis, bacterial pneumonia, otitis media, intussusception and urinary tract infection (CDC, 2003). Diarrhoea is an etiologically diverse condition caused by a variety of enteric pathogens (Bhattacharya, 2000).

Examples of viral causes of diarrhoea include Rotavirus, Enteric Adenovirus, Norovirus, Enteroviruses, Caliciviruses and Astroviruses (Cooke, 2010). Worldwide, Rotavirus infection is responsible for the most severe forms of diarrhoea (WHO, 2013), especially in children andmayaccount for up to 40% of cases in the developed countries and 25% in the developing world (Aremu*et al.*, 2011; Cooke 2010). The most common viruses that cause diarrhoea in adults are norovirus (Soffer, 2001).

Escherichia coli, *Clostridium difficile*,*Campylobacter*, *Salmonella* and *Shigella* spp. are common bacterial causes of diarrhoea. *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, *Salmonella* spp. and others may cause food poisoning (Soffer, 2001). *E. coli* is recognized to be a common cause of gastroenteritis (WHO, 2013) and accounts for nearly 30% of total diarrhoeal pathogens in some regions (Moyenuddin *et al.*, 1987). The enteropathogenic *E. coli* (EPEC) is an important category which is a leading cause of infantile diarrhoea in developing countries (Nataro and Kaper, 1998).

Entamoeba histolytica,*Giardiaintestinalis*, *Cryptosporidiumparvum* and *Cyclosporacayetanensis* are parasitic or protozoal agents that cause diarrhoea (Soffer, 2001).*Candida albicans* has also been known to cause diarrhoea in humans (Robert *et al.*, 2001). These protozoal agentshave also been incriminated as serious causes of diarrhoea in Africa and other parts of the developing world (Haque *et al.*, 2009; Nkrumah and Nguah, 2011). Diarrhoea caused by parasitic agentsis uncommon in the developed world and is usually restricted to travelers (WGO, 2012).

Causes of non-infectious diarrhoea include certain medications; plant and animal toxins;inflammatory and dysmotility problems of the gastro-intestinal tract such as inflammatory bowel disease, irritable bowel syndrome, ischaemic bowel disease, partial small bowel obstruction, pelvic abscess in the rectosigmoid area, faecal impaction, carcinoid syndrome;ingestion of poorly absorbable materials (such as lactulose)and substances that increase gastrointestinal tract secretions; food allergies (Ahlquist, 2001) and acute alcohol ingestion (Soffer, 2001).

Treatment-related diarrhoea can be caused by chemotherapy, hormone therapy, radiation therapy, biological response modifiers (drugs that improve the patient's immune system) or surgery (Rowland, 2004).

Diarrhoea is one of the most frequent adverse effects of prescription medications. It is important to note that drug-related diarrhoea usually occurs after a new drug is initiated or the dosage increased (Soffer, 2001). Prolonged use of antibiotics resulting in the disruption of gut microflora may cause diarrhoea and at times pseudomembranous colitis resulting from *Clostridiumdifficile* infection. Reactions to some magnesium-containing drugs (e.g., antacids) may cause maldigestion or malabsorption which may stimulate the expulsion of gut contents (Njume and Goduka, 2012). Drug-related diarrhoea is the most common form of diarrhoea in the elderly probably due to the fact that major organs of drug clearance such as the kidneys and liver are affected by age (Nigro *et al.*,2000).

2.1.2.1 Causes of acute diarrhoea (short term diarrhoea)

This is usually a symptom of a bowel infection when the stomach and the intestines become inflamed (gastroenteritis). This may be caused by a virus, most commonlynorovirus or rotavirus but also by a hepatitis virusor the herpes simplex virus.Bacteria as well as antibiotic,can disturb the natural balance of bacteria in the GIT. Anxiety, consumption of too much alcohol, coffee or some medications apart from antibiotics e.g. laxatives, antacids etc. are other causes of acute diarrhoea(Nordqvist, 2012).

2.1.2.2 Causes of chronic diarrhoea (persistent, longer term diarrhoea)

Bacteria,virus, habitual use of laxatives and dietary habits (such as chronic alcohol, coffee or sweet consumption) may cause persistent diarrhoea. Many sugar-free chewing gums containing a sweetener called sorbitol can cause chronic diarrhoea.Disease conditions such as celiac disease, Crohn's disease, diabetes, irritable bowel syndrome (IBS), lactose intolerance, pancreatitis, ulcerative colitiscan also cause chronic diarrhoea (Nordqvist, 2012).

2.1.3 Epidemiology

Diarrhoea kills 1.8 million children under five in developing countries. This figure, however, is an improvement from 4.5 million deaths 20 years ago.Annual incidence of diarrhoeal disease episodes in children less than five years old in developing countries currently stands at 2 billion diarrhoeal episodes globally with a median incidence rate of 3.2 episodes per child (Mandal, 2015). Diarrhoea rank high as a global public health problem causing considerable morbidity and mortality among infants and children

especially in the developing countries (Udoh and Uyah, 2010) and also among old and immunocompromised individuals (Soffer, 2001).Diarrhoea is responsible for annual global deaths of about 2.6 million people mostly among African children under the age of 5 years (WHO, 2010). In 2011, diarrhoea accounted for 700,000 deaths in children under five years of age worldwide making it the second leading cause of child mortality (Bhutta *et al.*, 2013). According to WHO (2013), it is the second leading cause of death in children under five years of age, and is responsible for killing around 760,000 children every year worldwide. It kills more young children than AIDS, malaria and measles combined (UNICEF/WHO, 2009).The United Nations Children Fund (UNICEF) has said about 194,000 children under five die annually as a result of diarrhoea in Nigeria (UNICEF, 2012). Diarrhoea is a special problem for travellers, hospitalized patients, homosexual males, persons with underlying immunosuppressed conditions (such as HIV patients), children in day-care centers as well as those living in unhygienic environments and having exposure to contaminated water and foods (Itah and Ben, 2004).Women are more susceptible to travel-related diarrhoea than men (Soffer, 2001).There isa high prevalence of diarrhoea among children whose mothers did not attend school and/or among children living in poorest households particularly in sub-Saharan Africa and South Asia (UNICEF and WHO, 2009).

According to the WHO and UNICEF, there are about two billion cases of diarrhoeal disease worldwide every year, and 1.9 million children younger than 5 years of age die from diarrhoea each year, mostly in developing countries. This amounts to 18% of all the deaths of children under the age of five and means that more than 5000 children are dying every day as a result of diarrheal diseases. Of all child deaths from diarrhoea, 78% occur in the African and South-East Asian regions (WGO, 2012).

The majority of diarrhoeal deaths occur in rural African communities where health care facilities are inadequate and the majority of the people lack access to clean and safe water, a major vehicle for transmission of diarrhoeal diseases (Forsberg *et al.*, 2007; Mwambete and Joseph, 2010). In some rural parts of the developing world, the mother's knowledge on the predisposing factors of diarrhoea is poor.The frequent occurrence of childhood diarrhoea is wrongly perceived as a developmental stage of the child and which results in mortality (Mwambete and Joseph, 2010).

According to the WHO, although diarrhoea episode/child/year has declined from an estimated 3.4 in1990 to 2.9 in 2010, the highest burden of the disease has remained consistent with respect to age. The incidence is highest among children under 5 years of age living in low- and middle-income countries (Mandal, 2015).

2.1.4 Pathophysiology / Pathological disorders

Diarrhoea is the mechanism by which the body rids itself of pathogenic organisms, with excessive stimulation of intestinal motility, leaving insufficient time for absorption of

intestinal fluid (Keusch *et al.*, 2006). Approximately 8-9 liters of fluid enters the intestines daily – 1-2 liters represents food and liquid intake, and the rest is from endogenous sources such as salivary, gastric, pancreatic, biliary, and intestinal secretions. The small and large intestines have the ability to reabsorb that and even more fluid when functioning normally (Kent and Banks *et. al.*, 2010). Most of the fluid, about 6-7 litres, is absorbed in the small intestine, and only about 1-2 liters is presented to the colon. Most of this is absorbed as it passes through the colon, leaving a stool output of about 100-200 g/day (Soffer, 2001).This normal gut physiology relies on a functioning enteric nervous system that coordinates the gut ion transport and motor activity (Binder, 2006). When any of these pathways are disrupted, diarrhoea can result. These disruptions result in osmotic, secretory, motility or mixed diarrhoea (Bliss *et. al.*, 2006).

2.1.4.1 Motility disorders

During normal functioning of the intestines, solids and fluid are moved through the gut with peristaltic waves of the smooth muscles within the intestines. This movement is slow and may take 3-5 hours for the mass to move from the pyloric valve at the proximal point of the small intestine to the large intestine. It may take as long as 24+ hours for the mass to move from the small intestine to the rectum to be expelled during defecation (Guyton and Hall, 2000). When the intestines are not functioning normally, motility can be either increased or decreased and both can lead to diarrhoea. Increased motility can be caused by infectious agents, changes within the bowel by inflammatory bowel disease or by irritable bowel syndrome (Bliss *et. al.*, 2006). This increased motility results in faster transport of stool through the bowel so there is less chance for reabsorption of fluid from the large intestine.

2.1.4.2 Mixed disorders

As with most disease, there are usually multiple physiological changes within the body that cause diarrhoea. Rarely is diarrhoea caused only by osmotic, secretory or motility problems. Most diarrhoeal states have more than one component. As an example, the bacteria *Clostridium difficile* produce toxins that are secretagogues promoting secretory diarrhoea. However, the bacteria also produce a pseudomembrane that alters theabsorptive ability of the gut, promoting osmotic diarrhoea (Bliss *et. al.*, 2006).Medications causing diarrhoea also fall under the category of mixed disorders.There are a number of drugs that are known to cause diarrhoea either as a side effect oras the desired effect of the drug. The mechanism of causing diarrhoea can vary fromdrug to drug. Some offending drug categories include: antibiotics, magnesium andphosphate containing antacids, osteoarthritis medications, cardiac medications,chemotherapeutic medications, Alzheimer's disease medications and oralhyperglycemic drugs (Zarowitz, 2009).

2.1.5 Types of diarrhoea

On the basis of its duration, diarrhoea can be classified as acute (<14 days), persistent (14 to 29 days), or chronic (≥30 days) (Herbert and DuPont, 2014). According to W.H.O (2005), four clinical types of diarrhoea can be recognized, each reflecting the basic underlying pathology and altered physiology. *Acute watery diarrhoea*lasts for several hours or days andthe main danger is dehydration as well as weight loss may also occur if feeding is not continued e.g. cholera. In acute bloody diarrhoea, the main dangers here are damage of the intestinal mucosa, sepsis and malnutrition. Other complications, including dehydration, may also occur (e.g., dysentery). Persistent diarrhoea lasts 14 days or longer with the main danger being malnutrition and serious non-intestinal infection. Dehydration may also occur. In diarrhoea with severe malnutrition,the main dangers are severe systemic infection, dehydration, heart failure and vitamin and mineral deficiency e.g. marasmus or kwashiorkor.

2.1.6 Other types of diarrhoea

2.1.6.1 Secretory diarrhoea

Secretory diarrhoea, an increase in the active secretion or an inhibition of absorption may bedue to enterotoxins, bile acids orover secretion of gastrointestinal hormones. There is little to no structural damage to the gastrointestinal tract and the most common cause of this type of diarrhoea is a cholera toxin that stimulates the secretion of anions, especially chloride ions (Nordqvist, 2012).

2.1.6.2 Osmotic diarrhoea

Osmotic diarrhoea occurs when too much water is drawn into the bowels. If a person drinks solutions with excessive sugar or excessive salt, these can draw water from the body into the bowel and cause osmotic diarrhoea (WHO, 2005). The ingestion of poorly absorbable low molecular weight aqueous solutes may create an osmotic pressure which pulls water and ions into the intestinal lumen leading to osmotic diarrhoea (Moser *et al.*, 2007). This kind of diarrhoea may occur when individuals with congenital lactase deficiency consume lactose-rich diets (Casburn-Jones and Farthing, 2004). Certain laxatives such as lactulose and citrate of magnesia or maldigestion of certain food substances such as milk are common causes of osmotic diarrhoea. An increased osmotic load can be measured in the stool. This type of diarrhoea ceases with fasting (Woods, 1990).

2.1.6.3 Exudative diarrhoea

Exudative diarrhoea results from extensive injury of the small bowel or colon mucosa as a result of inflammation or ulceration, leading to a loss of mucus, serum proteins, and blood into the bowel lumen. Increased faecal water and electrolyte excretion results from impaired water and electrolyte absorption by the inflamed intestine rather than from secretion of water and electrolytes into the exudates (Soffer, 2001).

If the intestinal epithelium's barrier function is compromised by loss of epithelial cells or disruption of tight junctions, hydrostatic pressure in blood vessels and lymphatics will cause water and electrolytes, mucus, protein, and sometimes even red and white cells to accumulate in the lumen leading to watery stools (Nighot *et al.*, 2010). This kind of diarrhoea is common in bacterial infection, especially *Shigella* (Field, 2003). Other organisms associated with exudative diarrhoea include *Salmonella*, *Yersinia*, *Campylobacter*, *Aeromonas*, Entero-invasive *E. coli* and *Rotavirus* infection (Thapar and Sanderson, 2004). These organisms invade the epithelium and multiply, damaging the surface epithelium and causing inflammation (Field, 2003). Diarrhoea is due to both the epithelial damage (exudation and decreased absorptive capacity) and the action of inflammatory mediators (Njume and Goduka, 2012).

Diseases associated with large quantities of inflammatory exudate, that is, blood, pus, and proteinaceous material, can produce diarrhoea. These inflammatory products in themselves can cause increased stool volume and frequency, but altered absorption of fluid and electrolytes also plays an important role. Mucosal inflammation can occur with diverticulitis, inflammatory bowel disease, or invasive enteric infections such as Shigella, Salmonella, or Campylobacter. The aetiology of the inflammatory response in ulcerative colitis and Crohn's disease remains poorly understood (Woods, 1990).

2.1.6.4 Inflammatory diarrhoea

Inflammatory diarrhoea occurs when there is damage to the mucosal lining or brush border, which leads to a passive loss of protein-rich fluids and a decreased ability to absorb these lost fluids. Features of all three of the other types of diarrhoea can be found in this type of diarrhoea. It can be caused by bacterial, viral or parasitic infections as well as autoimmune problems such as inflammatory bowel diseases. It can also be caused by tuberculosis, colon cancer and enteritis (Nordqvist, 2012).

2.1.6.5 Dysentery

Dysenteryis defined as diarrhoea in which is characterized by mucous or bloody stools, is generally caused by bacteria or viral infection or infestation by protozoa. Symptoms of dysentery include abdominal pain, tenesmus, and stools with blood or mucous. The

presence of blood in the stools is usually a sign of dysentery, rather than diarrhoea. Dysentery is one of the symptoms of *Shigella, Entamoeba histolytica*, and *Salmonella* (Nordqvist, 2012).

2.1.6.6 Motility-related diarrhoea

Food moves too quickly through the intestines(hypermotility). Hypermotility does not allow enough time for sufficient nutrients and water to be absorbed by the GIT. Patients who had a vagotomy as well as those with diabetic neuropathy are susceptible to this type of diarrhoea (Nordqvist, 2012).

2.1.6.7 Traveller's diarrhoea

Traveller's diarrhoea is a general term applied to the common problem of diarrhoeal illness experienced by travellers, usually in the first week or two of a stay in a foreign environment. It remains a major public health problem with significant morbidity.It particularly affects those who travel from industrialized countries to developing countries, especially tropical and semi-tropical destinations (DuPont, 2006).

2.1.7 Signs and symptoms

Diarrhoea caused by small intestine disease is typically high volume, watery, and often associated with malabsorption as well as frequent dehydration. Diarrhoea caused by colonic involvement is more often associated with frequent small-volume stools, the presence of blood, and a sensation of urgency. Important factors in evaluating diarrhoea include travel and animal exposure history, sources of water (e.g. well water), recent food intake, history of profuse diarrhoeal episodes, history of recent antibiotic treatment, dehydration, fever, hematochezia, nausea, vomiting, and abdominal pain (Soffer, 2001).

Diarrhoea of any duration may cause dehydration, which means the body lacks enough fluid and electrolytes, affects the muscle activity, and other important functions. Signs of dehydration in adults are thirst, fatigue, dry skin and tongue, dark coloured urine, dizziness, sunken eyeballs and so on and in infants and young children these are high fever, absence of tears when crying, dry mouth and tongue, sunken eyes, checks or soft spot in the skull and no wet diaper for 3 hours or more (Sarin and Bafna, 2012).

2.1.8 Risk factors

Factors that are relevant to the cause of diarrhoea include previous international travel; treatment with antibiotics, chemotherapy, or proton-pump inhibitors; unsafe sexual practices; work at a day-care center, and the presence of a known immunosuppressive

disorder. When vomiting is the predominant finding, viral gastroenteritis or food poisoning with a preformed toxin is probably the cause (Soffer, 2001; Herbert and DuPont, 2014).

2.1.9 Complications of diarrhoea

2.1.9.1 Dehydration

The most severe threat posed by diarrhoea is dehydration. During a diarrhoeal episode, water and electrolytes (sodium, chloride, potassium and bicarbonate) are lost through liquid stools, emesis, sweat, urine and breathing. Dehydration occurs when these losses are not replaced. The degree of dehydration is rated on a scale of three: Early dehydration (no signs or symptoms); Moderate dehydration (thirst, restless or irritable behavior, decreased skin elasticity and sunken eyes); or Severe dehydration (symptoms become more severe, shock with diminished consciousness, lack of urine output, cool, moist extremities, a rapid and feeble pulse, low or undetectable blood pressure, and pale skin) (WHO 2013).

Death can follow severe dehydration if body fluids and electrolytes are not replenished, either through the use of oral rehydration salts (ORS) solution, or through an intravenous drip (WHO, 2013).Dehydration and electrolyte losses associated with untreated diarrhoea are the main causes of morbidity and mortality of childhood acute gastroenteritis (AMMCOP, 2011).

2.1.9.2 Malnutrition

Bloody diarrhoea (dysentery) and persistent diarrhoea with malnutrition are also important causes of death (WHO, 2005). Repeated attacks of diarrhoea contribute to malnutrition, and diarrhoeal diseases are more likely to cause death in children who are malnourished. Undernourished children, in turn, have compromised immune systems and are at higher risk of developing pneumonia – which also contribute to high children mortality in the country (UNICEF, 2012).Research has shown, however, that the adverse effects of diarrhoea on a child's nutritional status can be lessened or prevented by continuing feeding during the illness (WHO, 2005).

2.1.9.3 Cardiovascular complications

Severe diarrhoea can affect the health of the heart. Dehydration can lead to orthostatic hypotension, wherein fainting upon standing is experienced. It occurs as the volume of blood gets reduced. Low volume of blood causes a drop in blood pressure levels upon standing (Palande, 2012).

2.1.9.3 Mineral defficiency

In case of prolonged diarrhoea, body loses electrolytes along with water. Mineral deficiencies, especially sodium or potassium deficiencies can lead to life-threatening health complications.Also abnormal levels of chloride and bicarbonate can be experienced (Palande, 2012).

2.1.9.4 Kidney failure

Prolonged and severe diarrhoea can lead to haemolytic uraemic syndrome (Cooke, 2010). This can result in kidney failure, diminished urine output, shock, and acidosis which if not promptly treated, can eventually lead to coma (Palande, 2012).

2.1.9.5 Other complications

Diarrhoea can affect the function of the brain, if not promptly treated. Impaired cognitive development may occur (WGO, 2012) in addition to excessive thirst and dry mouth. Moderate to severe abdominal pain or tenderness is quite common. In pregnant women, it can affect the health of the foetus. Anal irritation,nausea and vomiting can be experienced.There is loss of energy and weakness. Chronic diarrhea can lead to excessive fatigue (Palande, 2012) and growth faltering (WGO, 2012).

2.1.10 Diagnosis

The determination of the precise cause of diarrhoea is costly, and in most cases of non-severe diarrhoea it is not necessary (Herbert and DuPont, 2014).On the other hand, evaluation is indicated if symptoms are severe or prolonged, the patient appears toxic, there is evidence of colitis (occult or gross blood in the stools, severe abdominal pain or tenderness, and fever), or empirical therapy has failed. Passage of many small-volume stools containing blood and mucus, a temperature reading higher than 38.5° C (101.3° F), passage of more than 6 unformed stools in 24 hours, or a duration of illness longer than 48 hours, diarrhoea with severe abdominal pain in a patient older than 50 years, diarrhoea in older adults (>70 years) or in the immunocompromised patient (e.g., those with AIDS, after transplantation, or undergoing cancer chemotherapy) are all indications for a thorough medical and bacteriologic evaluation (Soffer, 2001).

2.1.10.1 History and physical examination

The physical examination in acute diarrhoea is helpful in determining the severity of the disease and hydration status. A directed physical examination may lead to a more focused evaluation. Vital signs (including temperature and orthostatic pulse and blood

pressure) and signs of volume depletion (including dry mucous membranes, decreased skin turgor, and confusion) should be carefully evaluated. A careful abdominal examination, to evaluate for tenderness and distention, and a stool examination to evaluate grossly bloody stools are warranted (Soffer, 2001).

History should include place of residence, drinking water (treated city water or well water), rural conditions, consumption of raw milk, consumption of raw meat or fish, and exposure to farm animals that may spread *Salmonella* or *Brucella* organisms. Sexual history is important because specific organisms can cause diarrhea in homosexual men and HIV-infected patients. The history and physical examination can help lead to a diagnosis but, for treatment of some organisms, a specific diagnosis is required, which will lead to more specific therapy and prevention of unneeded interventions (Soffer, 2001).

2.1.10.2 Blood Studies

Levels of electrolytes and serum creatinine should be measured in cases of systemic toxicity or dehydration, especially in elderly or infirm patients. A complete blood count may be indicated in patients with severe diarrhoea accompanied by fever or toxicity, in which leukocytosis or a shift to the left in neutrophils may indicate an inflammatory bacterial pathogen having prognostic significance(e.g., *C. difficile*–associated diarrhoea). Eosinophilia may be seen in parasitic infections with an extraintestinal migration phase (e.g., strongyloidiasis) (Herbert and DuPont, 2014).

2.1.10.3 Stool examination

Faecal testing should be performed in patients with a history of diarrhoea longer than 1 day who have the following symptoms: fever, bloody stools, systemic illness, recent or remote antibiotic treatment, hospital admission, or signs of dehydration (Soffer, 2001). Assessment of a stool sample to determine the cause of illness should be reserved for patients at high risk of diagnosable diarrhoea or cases in which identification of the pathogen would be important. Stool samples should be obtained from patients with any of the following conditions: acute diarrhoea that is severe or associated with fever (≥38.5°C), diarrhoea associated with a severe coexisting condition in a hospitalized patient who is receiving antibiotics (with testing only for *C. difficile* toxins), persistent diarrhoea (≥14 days' duration), profuse cholera-like watery diarrhoea, dehydration, and dysentery (Herbert and DuPont, 2014).

2.1.10.4 Polymerase Chain Reaction (PCR)-Based Diagnostic Tests

Laboratories throughout the industrialized world are now using PCR-based diagnostic tests, which are often combined in a single test to detect multiple enteropathogens (Liu

et al. 2012). PCR offers the advantage of improved sensitivity, but it focuses on genes rather than on virulence factors. Also, PCR methods may detect DNA in patients with transient colonization by organisms containing targeted genes who are ill from another cause. PCR for the diagnosis of *C. difficile*–associated diarrhoea has high sensitivity but lower positive predictive value when the rate of *C. difficile* infection is 10% or less among stools screened, with higher rates of asymptomatic infection in the general population (Deshpande *et al.*,2011).Genome analysis (Forgetta *et al.,* 2011),testing for messenger RNA as a measure of protein expression or quantitative PCR (Matsuda *et al.*,2012), more sensitive functional toxin assays (Darkoh *et al.,* 2011), or — in the case of colitis — subsequent identification of faecal inflammatory markers in PCR-positive cases of diarrhea (LaSala *et al.,* 2013) may improve the diagnostic value of nucleic acid–based diagnostic tests (Herbert and DuPont, 2014).

2.1.10.5 Endoscopy and Abdominal Computed Tomography

Flexible sigmoidoscopy or colonoscopy has limited value in the routine evaluation of patients with acute diarrhoea (Shen *et al.,* 2010). Flexible sigmoidoscopy is a useful diagnostic procedure in cases of persistent diarrhoea and in selected cases of acute diarrhoea with clinical colitis in which the diagnosis is not clear, such as cases of suspected *C. difficile*–associated diarrhea with toxin-negative stool. Indications for endoscopy include suspected *C. difficile*–associated diarrhoea and dysenteric diarrhoea with negative results of stool toxin and microbiologic tests. Bowel preparation before endoscopy should be selected to minimize mucosal changes, and in patients with severe diarrhoea, bowel preparation may be omitted. Esophagogastroduodenoscopy may be useful in patients with persistent diarrhoea if standard stool and serologic studies are not diagnostic (Donowitz *et al.,* 1995). Abdominal computed tomography (CT) may detect mucosal thickening or other changes of ischaemic, haemorrhagic, or inflammatory colitis, and it is the preferred diagnostic study when both intra-abdominal disease and intestinal disease are included in the differential diagnosis (Horton *et al.*, 2000).CT is particularly valuable for the detection of colonic mucosal thickening and pericolonic stranding, which may occur in cases of fulminant *C. difficile*–associated diarrhoea (Herbert and DuPont, 2014).

2.1.11 Prevention

Majority of diarrhoeal diseases can be prevented by implementing water, sanitation and hygiene (WASH) programmes, which all aim at interrupting faecal–oral transmission pathways, commonly referred to as the five “F” (fluids, fields, flies, fingers and food) (Wagner and Lanoix, 1958). According to WHO (2013), the key measures to prevent diarrhoea include access to safe drinking-water; use of improved sanitation; hand washing with soap; exclusive breastfeeding for the first six months of life; good personal and food hygiene; health education about how infections spread; and rotavirus vaccination. As diarrhoea diseases are basically faecal-oral in nature, one of the simplest and most cost effective barriers to infectious diarrhoea is hand washing with soap or ash

at critical times, such as before handling food and after defecation or changing a diaper (UNICEF,2012). The Centers for Disease Control and Prevention (CDC) informed that vaccinating babies against rotavirus can significantly reduce health care use and treatment costs for diarrhea-related diseases in American infants and young children (Nordqvist, 2012).

2.1.12 Management and treatment

Orthodox and indigenous systems are two major health care systems used in the treatment of diarrhoea in the developing world (Singh and Sharma, 2011). the indigenous systems are poorly organized and virtually unregulated while the orthodox system is well structured and highly developed (Tchacondo *et al.,* 2011). The latter makes use of informal methods of treatment, some of which are considered remote and inefficient (Sharma *et al.*, 2009).

Relatively few patients die from diarrhoea in industrialized countries, but it continues to be an important cause of morbidity that is associated with substantial health-care costs in under developed countries (WGO, 2012).Factors such as the widespread availability and use of oral rehydration salts (ORS), improved rate of breastfeeding, improved nutrition, better sanitation and hygiene, and increased coverage of measles immunization are believed to have contributed to a decline in the mortality rate in developing countries (WGO, 2012).

2.1.12.1Rehydration in adults and children

The objective of any anti-diarrhoeal treatment is to replace or minimize fluid and electrolyte loss, reduce stool frequency and any other symptoms such as abdominal pain, reduce faecal losses and ultimately reduce duration and severity of illness. The administration of oral rehydration solutions (ORS) to replace fluid and electrolyte loss in diarrhoeic patients is therefore *sine qua non* to effective treatment (Casburn-Jones and Farthing, 2004).

Oral rehydration therapy (ORT) is the administration of appropriate solutions by mouth to prevent or correct diarrhoeal dehydration. ORT is a cost-effective method of managing acute gastroenteritis and it reduces hospitalization requirements in both developed and developing countries.Oral rehydration salts (ORS) used in ORT, contain specific amounts of important salts that are lost in diarrhoea stool. The new lower-osmolarity ORS (recommended by WHO and UNICEF) has reduced concentrations of sodium and glucose and is associated with less vomiting, less stool output, lesser chance of hypernatremia, and a reduced need for intravenous infusions in comparison with standard ORS.The formulation is recommended irrespective of age and the type of diarrhoea including cholera (WGO,2012). Different formulations of these solutions exist but the basic ingredients are water, electrolytes (e.g., sodium) and glucose. Their

mechanism of action lies in the fact that sodium/glucose co-transport proteins on the brush boarder cells of the intestinal lumen pull sodium and glucose from the gut into the cells (Forsberg *et al.*, 2007). As the cellular osmotic pressure increases, water is reabsorbed out of the gut into the body. This action reverses electrolyte imbalances and re-hydrates the patient.

Rice starch and other concentrated carbohydrates are also being used in the newer formulations of ORS with the advantage that an increased amount of cellular substrate will also drive active sodium absorption bringing about relief of symptoms (Atia and Buchman, 2009). Combined administration of ORS and zinc has also been reported to alleviate diarrhoeal symptoms and expedite recovery of many patients in different parts of the world (Santosham*et al.*, 2010). This treatment is being encouraged because it may be a way to avoid unnecessary use of antibiotics, especially in children. Despite the relief obtained with ORS, lack of parental knowledge concerning their application is among the major factors that limit their usage in rural and semi-urban areas of the developing world. It is also difficult to administer the therapy successfully to patients whose purging episodes are accompanied by vomiting (Casburn-Jones and Farthing, 2004). In this case, intravenous fluid replacement by a professional medical staff may be required.

2.1.12.2 Supplemental zinc therapy, multivitamins, and minerals in children

Zinc deficiency is widespread among children in developing countries. Routine zinctherapy, as an adjunct to ORT, is useful in modest reduction of the severity but moreimportantly reduce diarrhoea episodes in children. Therecommended dose for children above two months with diarrhoea is 20 mg of zinc per day for 10 days.Infants aged two months or younger should receive 10 mg per day for 10 days.Supplementation with zinc sulfate in recommended doses reduces the incidence ofdiarrhoea during the following three months, and reduces non-accidental deaths by as manyas 50%. It is more important in the management of diarrhoea in malnourished children and persistent diarrhoea. The WHO and UNICEF recommend routine zinc therapy for children with diarrhoea, irrespective of the types (WGO, 2012).Other supplementation treatment includes folate, vitamin A, magnesium and copper, which reduce the incidence of diarrhoea. These are also found to reduce mortality rates among children with diarrhoea (WHO, 2005).

2.1.12.3 Anti-motility and anti-secretory agents

Anti-motility agents (such asloperamide and diphenoxylate-atropine combinations) act by increasing intestinal transit time and enhancing the potential for re-absorption of fluids and electrolytes (Casburn-Jones and Farthing, 2004). However, these groups of drugs are usually not recommended for children and young infants due to the potential of central nervous system side-effects (Dupont, 2005). The antimotility drug loperamide

is helpful in decreasing the passage of diarrhoeal stools in persons who are traveling or on a tight schedule. However, this class of drugs usually will not shorten the total duration of the illness. The maximum initial dose is 4 mg, followed by 2 mg after each unformed stool, with a total maximum dose of 8 mg per day for 48 hours. Loperamide should not be used in patients with febrile or dysenteric diarrhoea. If it is used, the lowest effective dose should be administered to avoid constipation after diarrhoea; often the initial 4-mg loading dose is sufficient (WGO, 2012).

The anti-secretory properties of bismuth salicylate have been shown to be effective in reducing the number of unformed stools by approximately 50% in patients with travellers' diarrhoea (DuPont, 2005). Apart from its anti-secretory properties, bismuth salicylate also has antibacterial and anti-inflammatory properties which make it a good candidate for the treatment of diarrhoea. However, this drug is not a very popular choice because of its high pill burden, delayed onset of action and the presence of unpleasant side-effects such as tinnitus and black tongue (Manyi-Loh*et al.*, 2010). Antisecretory drugs are in development but remain untested in most forms of diarrhoea. Racecadotril is an enkephalinase inhibitor (nonopiate) with antisecretory activity. It is not useful in adults with cholera. It has been found useful in children with diarrhoea, and is now licensed in many countries in the world for such indication (WGO, 2012).

2.1.12.4 Antimicrobial therapy

Antimicrobial therapy shortens the duration of diarrhoea, prevents development of its complications and reduces the severity of associated symptoms such as fever and abdominal pain (Daniz-Santos *et al.*, 2006). It also decreases secondary cases by halting person-to-person spread of diarrhoeic pathogens. However, the use of antibiotics in the treatment of diarrhoea is being approached with caution due to potential problems of drug-resistance, side-effects and cost of treatment (Njume *et al.*, 2011). There is also the fear that antibiotic therapy may worsen the clinical state of the patients because of its effect on gut microflora. In most cases, antibiotic treatment is only recommended in the treatment of acute bloody diarrhoea in children (Cooke, 2010).

With only a few exceptions, antibiotic therapy should not be given routinely to children with diarrhoea. Such therapy is ineffective and may be harmful. Majority of gastroenteritis cases in children are viral in origin. Thus, antibiotics are only needed for specific pathogens or defined clinical settings (CDC, 2003; WHO, 2005). Empirical antibiotic therapy is recommended for sporadic cases of febrile dysentery, especially those associated with toxicity that suggests the possibility of systemic infection, as well as for severe cases of traveller's diarrhoea or hospital-associated or antibiotic-associated diarrhoea. In selecting specific therapy for most cases of acute diarrhoea, an aetiologic diagnosis must be established. Antimicrobial therapy can be lifesaving in the case of bacteraemic salmonellosis and *C. difficile* infection in the elderly (Herbertand DuPont,

2014). Anti protozoal drugs e.g., quinolones, tinidazole and azithromycin can also be effective for diarrhoea in children (Sharma and Sharma, 2007).

2.1.12.5 Probiotics for the treatment of acute diarrhoea

Probiotics are live microorganisms, such as *Lactobacillus* GG (ATCC 53103), with demonstrated beneficial health effects in humans. *L. reuteri* ATCC 55730, *L. rhamnosus* GG, *L. casei* DN-114 001, and *Saccharomyces cerevisiae (boulardii)* are useful in reducing the severity and duration of acute infectious diarrhoea in children. The oral administration of probiotics shortens the duration of acute diarrhoeal illness in children by approximately 1 day (WGO, 2012). Probiotics have limited value for the treatment and prevention of specific forms of diarrhoea, although they have some value in preventing antibiotic-associated diarrhoea (Herbert and DuPont, 2014).

2.1.12.6 Nonspecific antidiarrhoeal treatment

None of these drugs addresses the underlying causes or effects of diarrhoea such as loss of water, electrolytes, and nutrients (WGO, 2012). Crofelemer (Fulyzaq), a chloride-channel blocker, has been shown to reduce the number of stools in patients with travelers' diarrhoea (DiCesare *et al.,* 2002) and is approved for use in patients with human immunodeficiency virus infection complicated by diarrhoea (MacArthur and DuPont, 2012). These drugs should neverbe given to children below 5 years (WHO, 1990). Several adsorbents like kaolin, pectin, and activated charcoal are also given in the treatment of diarrhoea (Sarin and Bafna, 2012).

2.1.12.7 W.H.O. key measures to treat diarrhoea

It is most practical to base treatment of diarrhoea on the clinical typeof the illness, which can easily be determined when a child is first examined. The management of each type of diarrhoea should prevent or treat the main danger(s) that each presents (WHO 2005). According to WHO(2013), key measures to treat diarrhoea include rehydration with oral rehydration salts (ORS) solution; zinc supplementation;rehydration with intravenous fluids in case of severe dehydration or shock; giving nutrient-rich foodsduring and after diarrhoea(WHO, 2005).

Two recent advances in managing diarrhoeal disease–newly formulated oral rehydration salts (ORS) containing lower concentrations of glucose and salt, and zinc supplementation as part of the treatment and rotavirus vaccines – can drastically reduce the number of child deaths. These new methods, used in addition to prevention and treatment with appropriate fluids, breastfeeding, continued feeding and selective use of antibiotics will reduce the duration and severity of diarrhoeal episodes and lower their incidence (UNICEF, 2012 and WHO, 2013).

Differentiating between the various types of diarrhoea may make choosing the appropriate intervention easier. It is important to remember that oral rehydration therapy is the main stay for treatment of acute diarrhoea. Caregivers and patients need to be made aware of the signs and symptoms of dehydration and when to seek medical treatment. Persons with diarrhoea lasting longer than four weeks should be referred to their primary care practitioners to ascertain the cause of the symptom, even though there are over-the-counter drugs available to assist with control of diarrhoea (Sisson, 2011). Consulting a health professional, in particular for management of persistent diarrhoea or when there is blood in stool or if there are signs of dehydration.

2.1.13 Antidiarrhoeal drugs in recent use

2.1.13.1 Opioids

Loperamide is effective for the treatment of differenttypes of diarrhoea (Hanauer, 2008). It is a synthetic opiate agonist activating the μ receptors in the myenteric plexus of the large intestine. Activation of μ receptors by loperamide inhibits release of acetylcholine and thus relaxes smooth muscular tone in the gut wall (Regnard *et al.,* 2011). This is to enhance phasic colonic segmentation and inhibit peristalsis, thus increasing intestinal transit time (Chen *et al.,* 2012). Inhibition by loperamide of acetylcholine release has an antisecretory activity (Wood and Galligan 2004).Loperamide is a nonprescription opioid agonist that does not cross the blood-brain barrier and has no analgesic properties or potential for addiction. The usual adult dose is 4 mg initially followed by 2 mg after each subsequent loose stool, up to 16 mg per day.

Diphenoxylate is another opioid agonist that has no analgesic properties in standard doses. However, higher doses have central nervous system effects and prolonged use can lead to opioid dependence. Commercial preparations commonly contain small amounts of atropine to discourage overdosage (2.5 mg diphenoxylate with 0.025 mg atropine). The anticholinergic properties of atropine may contribute to the antidiarrheal action(Kenneth, 2007).

2.1.13.2 Antispasmodic/Antimotility agents

Anticholinergic drugs (e.g. atropine) are often characterized as antispasmodics, but have important antimotility effects as well. These drugs work like opiates to improve net absorption and reduce stool volumeby inhibiting gastric emptying and prolonging intestinal transit. The antidiarrhoeal effect is less profound than opiates. Dry mouth and bloating are side effects that limit patient's acceptance (Glende*et al.,* 2002).

Clonidine is an α_2-adrenergic agonist agent ordinarily used for treating hypertension. It has antimotility effects like opiates and also stimulates the rate of intestinal absorption;

both of these effects work to mitigate diarrhoea.It has been studied as an antidiarrheal drug mostly in patients with diabetic diarrhoea (Schiller *et al.*, 1985).

2.1.13.3 Bulking agents/Adsorbents

Kaolin is a naturally occurring hydrated magnesium aluminum silicate and pectin is an indigestible carbohydrate derived from apples. Both appear to act as absorbents of bacteria, toxins and fluid, thereby decreasing frequency of diarrhoea and stool liquidity. They may be useful in acute diarrhoea but are seldom used on a chronic basis. Kaolin-pectin formulations are not absorbed and have no significant adverse effects except constipation. A common commercial preparation is Kaopectate. The usual dose is 1.2-1.5 g after each loose bowel movement (maximum: 9 g/d) (Kenneth, 2007). Methylcellulose is also an example of bulking agent as well as adsorbent.

The bile acid sequestrants,cholestyramine or colestipol, may decrease diarrhoea caused by excess faecal bile acids. The usual dose is 4-5 g one to three times daily before meals. Adverse effects include bloating, flatulence, constipation, and fecal impaction. In patients with diminished circulating bile acid pools, further removal of bile acids may lead to an exacerbation of fat malabsorption. These agents bind a number of drugs and reduce their absorption; hence, they should not be given within 2 hours of other drugs (Walters and Pattni, 2010).

2.1.13.4 Somatostatin analog

Octreotide is an octapeptide analog of somatostatin that is effective in inhibiting the severe secretory diarrhoea or persistent refractory diarrhoea (Farthing, 1994) brought about by hormone-secreting tumors of the pancreas and the gastrointestinal tract. Its mechanism of action appears to involve inhibition of hormone secretion, including serotonin and various other GI peptides (*e.g.*, gastrin, vasoactive intestinal polypeptide, insulin, secretin*etc.*). Octreotide has been used, with varying success, in other forms of secretory diarrhoea such as chemotherapy-induced diarrhoea, diarrhoea associated with human immunodeficiency virus (HIV), and diabetes-associated diarrhoea (Fried, 1999).

Other antidiarrhoeals include electrolyte solutions, intestinal anti-infective and anti-inflammatory agents. Bismuth subsalicylate is an over-the-counter preparation and has been used to treat variety of gastrointestinal diseases and symptoms for centuries, although their mechanism of action remains poorly understood. Bismuth is thought to have antisecretory, anti-inflammatory, and antimicrobial effects. Nausea and abdominal cramps also are relieved by bismuth.Bismuth subsalicylate has been used extensively for the prevention and treatment of traveler's diarrhoea, but it also is effective in other forms of episodic diarrhoea and in acute gastroenteritis (Pasricha, 2006).

2.2 The Plant: *Nymphaea lotus*

2.2.1 Taxonomy and nomenclature

According to Burkill (1985), *Nymphaea lotus*(Plate 1) falls under the following:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Nymphaeales

Family: Nymphaeaceae

Genus: *Nymphaea*

Species: *Nymphaea lotus* L. 1973

2.2.2 Synonyms

Castalia edulis Salisb., *Castalia lotus* Tratt., *Castalia mystica* Salisb., *Castalia pubescens* Wood., *Castalia sacra* Salisb., *Leuconymphaea lotus* Kuntze., *Nymphaea dentate* Schumach and Thonn., *Nymphaea liberiensis* A. Chev. (Burkill, 1997)

2.2.3 Common/English names

Water-lily; White water-lily; Lotus; White lotus; Egyptian lotus; Egyptian Water-Lily; Egyptian white lotus; Sacred lotus; Tiger Lotus; Tropical Night-Blooming Water Lily;White Egyptian Lotus; White Egyptian water-lily; Winter lotus (Burkill, 1997).

2.2.4 Vernacular names

Bödo (Kanuri), *Bâdo*(Hausa), *Osibata*(Yoruba), *Mkpokpome* (Igbo) (Burkill, 1997).

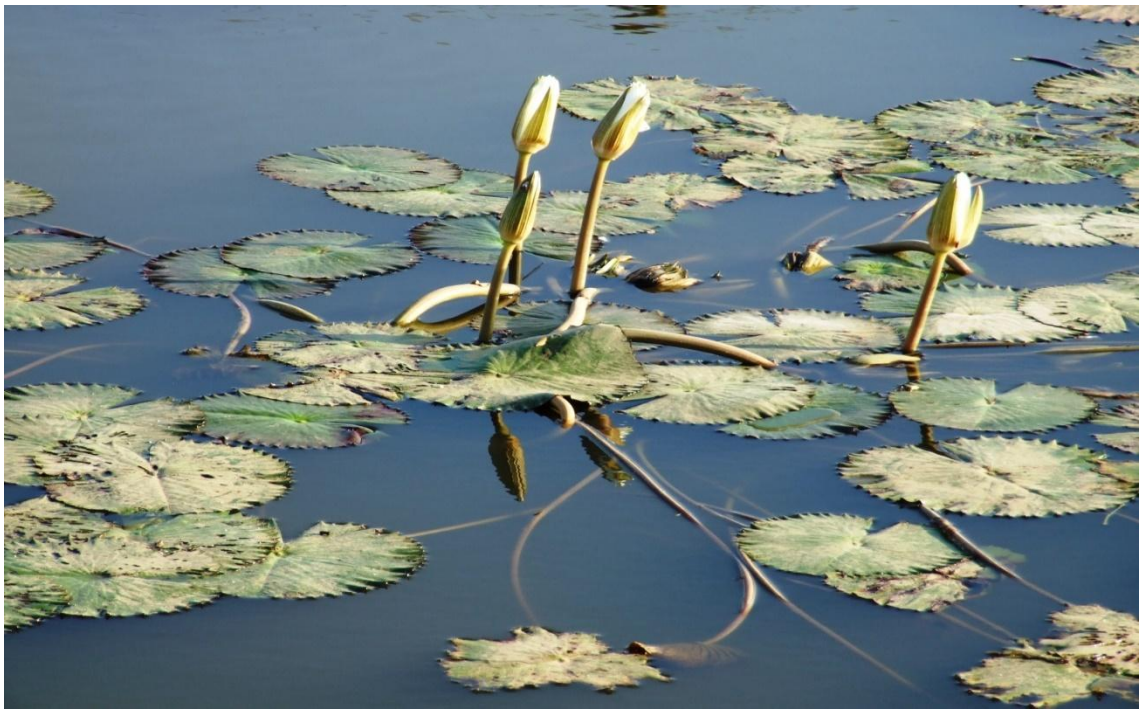


Plate I: *Nymphaea lotus* L. in its natural habitat along old Kano Road Dogarawa, Zaria, Kaduna State.



Plate II: *Nymphaea lotus* rhizome from a pond along old Kano road Dogarawa, Zaria, Kaduna State.

2.2.5 Botanical description / morphology

Nymphaea lotus is anannual or perennial aquatic herb with its rhizome submerged in mud. Leaves are ovate to suborbicular, deeply cordate with notch at the petiole, coarsely dentate, 15 – 20(-40cm) cm in diameter, green, floating; petiole cylindrical, glabrous and long. Submerged leaves are reddish brown. Flowers are solitary, large, 5-8cm across, with 4 outer green oblong, caducuous sepals; petals 12-14(-30), elliptic-oblong, obtuse, white (occasionally pink); stamens with yellow anthers; carpels united in single ovary and a stigma of 12-15(-20) rays. The fruit is globose, compressed, 3.5-5cm, fleshy and ripens under the water. The seeds are subglobose, 1-2mm and numerous (Lim, 2014).The fruit is a dry berry.N. lotus grows from tubers that can persist for several months in dormant state during dry seasons. N. lotus is occasionally viviparous, producing new plantlets from tubers that emerge from the flowers (Everett 1981).Rhizomesare stout branched or unbranched, erect,ovoid;stolons slender (Dukku, 2011).

2.2.6 Geographical distribution / habitat

Nymphaea lotusis an aquatic flowering plant prefers clear, warm, still and slightly acidic water and is localized to Central and Southern Europe, Southeast Asia, the Middle East, North, Central, parts of East Africa, tropical mountains in Africa and West Africa especially in Nigeria (Akinjogunla *et al.,* 2009; Lim, 2014). All permanent and temporary waters are suitable to waterlily growth (Burkill, 1997). It is frequently used as an aquarium plant or in water gardens (Lim, 2014).

2.2.7 Ethno medical Uses of *Nymphaea lotus*

Tradomedicinal uses of the plant include treatment of fever, skin diseases, cancer, gonorrhoea and bronchitis (Burkill, 1997).

Nymphaea lotus rhizome finds applications in the management of circulatory system disorders, digestive system disorders,genitourinary system disorders, mental disorders,hyposomnia, leprosy, infections and inflammations (Burkill, 1997). In Philippines, the rhizome is infused to make a refreshing drink as a remedy for fevers. The rhizome is also used to treat jaundice (Dragendorff, 1898; Burkill, 1997).The rhizome is used in making a refreshing tea, prescribed for cystitis, nephritis, metritis and enteritis, may very well be soporific in its effects (De Wildeman, 1948; Burkill, 1997).

The seeds are sweet, cooling, constipating, aphrodisiac, stomachic and restorative. It has found uses both as a culinary delight and starchy food staple as well as being used internally as a treatment for gastrointestinal disorders and jaundice. In Senegal, the fruit is employed as a vermifuge, for nausea, anxiety and nervous disorder. In Niger, powdered ripe fruit is used to treat urogenital infections (Lim, 2014).

The leaf is used in cutaneous, subcutaneous parasitic infection, eye treatments, and pregnancy. In south-western Nigeria, a leaf decoction of *Nymphaea lotus* is used topically for cancer; poultice of the leaves is applied to wounds and burns (Lim, 2014); the leaves are used as part of preparations for treatment of women reproductive problems to clear all womb blockages (Borokini *et al.*,2013).Syrup or decoction of the leaf has been used as an intra-uterine injection in the treatment of hysteria (De Wildeman, 1948). In Brazzaville, leaf juice is prescribed for tarchycardia, anxiety and nervous disorders. A decoction of leaf and bark from a selection of plants such as *Nymphaea lotus, Noronhia linocerioides, Vepris ampody, Zanthoxylum tsihanimposa, and Peddia involucrata* is used to relieve malarial symptoms, tiredness, muscular aches and pains and poisoning in eastern Madagascar (Lim, 2014).

In western Sudan, poultice of roots of *Nymphaea lotus* is used as a treatment for hangnail (Doka and Yagi, 2009).

A study conducted on ethno-botanical and socio-economic importance of Water lily to fishing communities in the Kainji Lake Basin revealed that the people do utilize the leaves, petiole, roots and seeds in preparation of concoction for different ailment and consumption (Mohammad *et al.*, 2008).

2.2.8 Pharmacological studies on *Nymphaea lotus* Linn plant

Many bioactive and pharmacologically important compounds have been obtained from *Nymphaea spp* (Siddhanta *et al.*, 1997). However, there is dearth of information on *N.*

lotus despite its presence on many of fresh water bodies in Nigeria (Mohammad and Awodoyin, 2008).

Research work done by Akinjogunla and co-workers (2009)demonstrated that the *Nymphaea lotus* plant has antimicrobial activity against Gram-negative and Gram-positive bacteria which supported the traditional application of the plant and suggests that the plant extracts possess compounds with antibacterial properties that can be used as potential drugs for the treatment of wound infections.According to Akinjogunla and co-workers (2010), ethanolic extracts of *N. lotus* leaf exhibited inhibitory activities against Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Staphylococcus aureus* (VRSA) bacteria.

Three novel flavonols, myricetin-3'-O-(6''-p-coumaroyl)glucoside and two epimeric macrocyclic derivatives, as well as myricetin-3-O-rhamnoside and pentagalloyl glucose, have been isolated from *Nymphaea lotus* L. and identified using 2D NMR (Elegami *et al.,* 2003). Amino acids (alanine, tyrosine, phenyl alanine, valine, threonine, arginine, leucine, D and L-isoleucine and aspartic acid), 2 alkanoic acids in form of butanoic acids and its α-hydroxyl isomer, a dipeptide (serine-arginine) as well as a rare compound named 2-amino-7-methyl octanoic acid were also isolated from *Nymphaea lotus* (Sowemimo *et al.,* 2007a).

Report on the toxicity, genotoxicity and mutagenic potential of the ethanolic extract of selected Nigerian medicinalplants using the brine shrimp lethality test, standard telomeraseelongation assay (STEA) as well as the observed inducedchromosomal aberrations in rat lymphocytes by Sowemimo and colleague (2007b) showed that *N. lotus*has some potential as anticancer agent. The whole plant of *N. lotus* is active in inhibiting polymerization of the human chromosomes and hence has the potential in inhibiting cancer cell formation (Sowemimo *et al.,* 2007b).Its antioxidant and antiviral potentials have also been reported (Saleem *et al.,* 2001; Esimone *et al.,* 2006).

2.2.9 Other medicinal plants used for management of diarrhoea

Various traditional medicinal plants showed antidiarrhoeal activity by reducing the intestinal transit, suppressing the gut motility and stimulating the water adsorption or by reducing the electrolyte secretion (Palombo, 2006).

Some indigenous medicinal plants whose ethnomedicinal use in the treatment of diarrhoea in rural and semi-urban areas of the developing world has been validated include:The aerial parts of *Alhagi maurorum, Mentha microphylla, Zygophyllum album* (Atta and Mouneir, 2004); bulb of *Eleutherina bulbosa* (Lin *et al.,* 1999); leaves of *Alchornea cordifolia* (Agbor *et al.,* 2004; Adeshina *et al.,* 2010), *Catharanthus roseus* (Hassan *et al.,*2011), *Conyza dioscoridis* (Atta and Mouneir 2004), *Dissotis rotundifolia* (Abere *et al.,* 2010), *Euphorbia hirta* (Galvez *et al.,* 1993; Kumar *et al.,* 2010), *Psidium guajava* (Ojewole *et al.,* 2008; Cowan, 1999; Lin *et al.,* 2002; Gutierrez *et al.,* 2008;

Birdi *et al.,* 2010), *Tithonia diversifolia* (Tona *et al.*, 1999; Chagas-Paula *et al.*, 2012), *Mormodica charantia* (Bakare *et al.*, 2011) root bark of *Heinsia pulchella* (Tona *et al.,* 1998); roots of *Hippocratea Africana* (Okokon *et al.,* 2011), *Ziziphus mauritiana* (Dahiru *et al.,* 2006); stem bark of *Bridelia micrantha* (Lin *et al.*, 1999), *Cylicodiscus gabunensis* (Mabeku *et al.,* 2006; Gutierrez *et al.*, 2007), *Stereospermum kunthianum* (Ching *et al.,* 2008), *Trilepisium madagascariense* (Teke *et al.,* 2010), *Carica papaya* (Tona *et al.,* 1999); roots, leaves and stem bark of *Securinega virosa* (Magaji *et al.,* 2007)etc.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant material

The rhizomes of *Nymphaea lotus* was obtained from Dogarawa, Zaria Kaduna state. The botanical identification and authentication of the plant was carried out by Mallam Musa Muhammad at the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria wherean identification number (894)already deposited was obtained.

3.1.2 Experimental animals

Four male New Zealand rabbits (2-3 kg) andfour male guinea pigs (300-500g)obtained from the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria as well asninetyadult Swiss albino mice(20-25g) of both sexesobtained from National Institute for Trypanosomiasis (and Onchocerciasis) Research (NITR), Kadunawereused for the experiments. The animalswere maintained under normal laboratory conditions of humidity, temperature and light for two weeks prior to the experimentso as to allow for their acclimatization.The animals were provided with commercial rodent diet and water *ad libitum*.

3.1.3 Equipment and other laboratory materials

Microdynamometer (Ugo Basile, Italy); Soxhlet apparatus (Quickfit, England); Water bath (HH-S Digital thermostatic water bath, China); Weighing balance (Lab tech. BL 20001 and Mettler P162, USA); Cages (locally made for mice); Dissecting kit (Gold Cross Dissecting Set, Malaysia); Data Capsule (Ugo Basile, Italy); Filter papers (Whattman filter papers size 1); Glass wares such as Pipette, Test tubes (Pyrex, France), Syringes (1ml, 2ml, 5ml and 10ml); Porcelain pestle and mortar; Stop watch

3.1.4 Chemicals and drugs

Acacia powder (Evans Medical Lt Speke, Liverpool); Acetylcholine (Sigma-Aldrich Inc., 3050 Spruce Street, St. Louis, USA) ; Atropine sulphate (Gland Pharma, India) ; Castor oil (Bell, Sons and Co Ltd, Southport PR9 9 AL, England); Histamine (Sigma-Aldrich Inc., 3050 Spruce Street, St. Louis, USA); Loperamide (Imodium® – Janssen Pharmaceutical, Pakistan); Magnesium sulphate (BDH Chemical Ltd, Poole England); Medicinal charcoal (Ultracarbon® tablets – Merck KGaA, Darmstadt, Germany)

3.2 Methods

3.2.1 Preparation of the plant extract

The rhizomes of *Nymphaea lotus* werecleaned and air dried under shade and reduced into a fine powder using mortar and pestle. The powdered rhizome weighing 1.2kgwas extracted using Soxhlet apparatus employing 80% aqueous methanol. The extract was later subjected to drying in flask evaporator under reduced pressure and controlled temperature (40°C) over a water bath. It was then stored in a leveled air tight container for future use.

3.2.2 Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening tests for the presence of carbohydrates, anthraquinones, flavonoids, alkaloids, saponins, steroids, tannins,triterpenes and cardiac glycosides using standard test proceduresoutlined by Evans, 2002; Sofowora, 1993 and Silver *et al.*, 2003.

3.2.2.1 Test for cardiac glycosides

Keller-Killiani test:One ml of aqueous solution of the extract was mixed with 1ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was transferred into a dry test tube and 1ml of concentrated sulphuric acid was added with a pipette down the side of the test tube to form a lower layer at the bottom. The interphase was observed carefully for a purple-brown ring. The ring indicated the presence of deoxy sugars and presence of a pale green colour in the upper acetic acid layer indicated the presence of cardiac glycosides.

Kedde's test:To 1ml of the aqueous extract solution, 1 ml of 2% solution of 3,5-dinitrobenzoic acid in 95% alcohol was added. The solution was made alkaline with 5% sodium hydroxide solution, appearance of a purple-blue colour, indicated the presence of cardenolides.

3.2.2.2 Test for saponins

Frothing test:About 10 ml of distilled water was added to 1ml of the extract solution which was shaken vigorously for 30 seconds and allowed to stand for 30 minutes in a vertical position. A honeycomb froth that persists for 15 minutes indicatedpresence of saponins.

3.2.2.3 Test for steroids and triterpenes

Lieberman-Burchard's test:To 1ml of the chloroform solution of extract,equal volume of acetic acid anhydride wasadded and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer.Changes were observed immediately and over a period of one hour. Blue to blue-green colour in the upper layer indicated the presence of steroids and a reddish, pink or purple colour indicated the presence of triterpene.

3.2.2.4 Test for flavonoids

Shinoda test:One hundred mg of the extract was dissolved in 2 ml of 50% methanol and warmed on a steam bath. Few pieces of metallic magnesium chips and a few drops of concentrated hydrochloric acid were added. Appearance ofa red colour indicated the presence of flavonoids.

Sodium hydroxide test:Few drops of 10% sodium hydroxide were added to aqueous solution of the extract. Yellow coloration indicated the presence of flavonoid.

3.2.2.5 Test for tannins

Ferric chloride test:To 1ml of aqueous extract solution, 3dropsof ferric chloride solution was added. A greenish-black precipitate indicatedthe presence of condensed tannins whilea blue or brownish-blue precipitate indicated the presence ofhydrolysable tannins.

Lead sub-acetate test:To 1ml aqueous solution of the extract, 3 drops of lead sub-acetate solution was added. A colored precipitate indicated the presence of tannins.

3.2.2.6 Test for alkaloids

Dragendoff's test:To 1ml of the aqueous solution of the extract, five drops of Dragendoff's reagent were added. Occurrence of orange red precipitate indicated the presence of alkaloids.

Wagner's test: Five drops of Wagner's reagent were added to 1ml solution of the extract, appearance of a reddish brown precipitate indicated the presence of alkaloids.

3.2.2.7 Test for anthraquinones

Bontrager's Test: To500 mg of the extract in a dry test tube, 5ml of chloroform was added and was shaken for at least 5 minutes.This was filtered and the filtrate was shaken with an equal volume of 10% ammonia solution. Abright pink colour indicated the presence of free anthraquinones.

Modified Bontrager's test:Five hundred mg of the extract in a test tube was boiled with 5ml of 10% hydrochloric acid for 3minutes. This was then filtered and the filtrate cooled and extracted with 5ml of benzene. The benzene layer was pipetted off and shaken gently in another test tube with half of its volume of ammonium hydroxide. The lower ammonia layer became rose pink to cherry red, indicating the presence of anthraquinones derivative.

3.2.2.8 Test for carbohydrates

Molisch'stest:To 1ml aqueous solution of the extract in a test tube, five drops of Molisch reagent was added and then concentrated sulphuric acid was added down the side of the test tube to form a lower layer, a reddish colored ring at the interphase indicated presence of carbohydrates.

Fehling's test:To 1ml aqueous solution of the extract in a test tube, 5ml of an equal mixture of Fehling's solutions A and B was added. This was boiled on a water bath and a brick red precipitate indicated presence of reducing sugar.

3.2.3 Acute Toxicity Studies

The median lethal dose (LD_{50}) of the extract was determined by Lorke's method (1983). The study was carried out in two phases and mice were deprived of food for 12 h prior to administration of the extract. In phase 1, three groups of three mice per group were used. The extract was administered orally in three graded doses (10, 100and 1000mg/kg). The treated mice were observed for 4 h post administration for signs of toxicity. After 24 h, no death was recorded, thus phase 2 was initiated. Based on the

result in phase 1, three micewere given the extract orally in doses of 1600mg/kg, 2900mg/kg and 5000mg/kg respectively. The mice were then observed for signs of toxicity for the first 4 h and mortality after 24h.

3.2.4 *In vitro* Studies

3.2.4.1 Effect of methanol rhizome extract of Nymphaea lotus on the isolated rabbit jejunum

A New Zealand adult male rabbit used for the study was starved of feed for about 18 h. It was then sacrificed by cervical dislocation. The abdomen was cut open and segments of the jejunum (about 3 cm long) removed and dissected free of adhering mesentery. The tissue was then suspended in a 25 ml organ bath containing tyrode solution and allowed to stabilize for 30 minutes (period of acclimatization). The effects of acetylcholine ($8 \times 10^{-7} – 512 \times 10^{-7}$ mg/ml)and the methanol rhizome extract of *N. lotus*($4 \times 10^{-4} – 512 \times 10^{-2}$ mg/ml) were then tested on the jejunum. The contact time for each concentration was 30 seconds, which was followed by washing three times with tyrode solution. The tissue was allowed a resting period before addition of the next concentration. The responses were recorded isometrically using a microdynamometer set at a sensitivity of 3.0 mV and a speed of 24 mm/min.

3.2.4.2 Effect of methanol rhizome extract of Nymphaea lotus on isolated guinea pig ileum

Similar protocol as for that of the effect of methanol rhizome extract of *N. lotus* on isolated rabbit jejunum was followed. The effects of histamine ($2 \times 10^{-6} – 128 \times 10^{-6}$mg/ml) and the methanol rhizome extract of *N. lotus*($4 \times 10^{-4} – 512 \times 10^{-2}$ mg/ml) were then tested on guinea pig ileum.

3.2.5 Antidiarrhoeal studies

3.2.5.1 Castor oil induced diarrhoea in mice

The method described by Shoba and Thomas (2001) was used for this study. The mice were screened initially by giving 0.5 ml of castor oil one week before the actual experiment. Only those that exhibit diarrhoea were selected for the experiment. The mice were fasted for 12 hours and divided into five groups of six mice each. Group I received deionized water at a dose of 10 ml/kg *p.o.* (negative control), Group II received the standard drug loperamide (3 mg/kg*p.o.*) and served as the positive control while Groups III, IV and V received the methanol rhizome extract of *Nymphaea lotus* at the doses of 200, 400 and 800 mg/kg *p.o.*, respectively. One hour after administration, all mice received 0.5 ml of castor oil *p.o*and then were placed individually in cages whose floors were lined with pre-weighed white filter paper. During the observation period of 4 h, the time of onset of diarrhoea, frequency of defecation and weight of faecesexcreted

by the animals were recorded. The percentage protection against diarrhoea was calculated with respect to the number of wet faeces using the formula below:

% inhibition = [(Number of WFC – Number of WFT) / Number of WFC] × 100%

Where;

WFC = wet faeces in negative control group

WFT = wet faeces in test group

3.2.5.2 Magnesium sulphate induced diarrhoea in mice

The method described by Afroz *et al.,* 2006 (similar protocol as for castor oil-induced diarrhoea) was used. The 30 mice were fasted for 12 hours and divided into five groups of six mice each. Group I received deionized water at a dose of 10 ml/kg *p.o.* (negative control), Group II received the standard drug loperamide (3 mg/kg *p.o.*) and served as positive control, while Groups III, IV and V received the methanol rhizome extract of *N. lotus* at the doses of 200, 400 and 800 mg/kg *p.o.* respectively. Diarrhoea was induced in the mice by oral administration of magnesium sulphate at the dose of 2 g/kg 60 min after pretreatmentwith the extract and loperamide. The mice were then individually placed in cages whose floors were lined with pre-weighed white filter paper. During an observation period of 4 h, the time of onset of diarrhoea, the total number of faecal output (frequency of defecation) and weight of faeces excreted by the animals were recorded. The percent protection against diarrhoea was calculated with respect to the number of wet faeces using the formula below:

% inhibition = [(Number of WFC – Number of WFT) / Number of WFC] × 100%

Where;

WFC =wet faeces in negative control group

WFT = wet faeces in test group

3.2.5.3 Effect of methanol rhizome extract on gastric transit time in mice

Intestinal motility test was done according to the method of Tafesse and Mekonnen (2012). The mice (30) were fasted for 12 hours and divided into five groups of six mice each. Group I received deionized water at a dose of 10 ml/kg *p.o.* (negative control), Group II received the standard drug atropine sulfate (5mg/kg *p.o.*) which served as positive control while Groups III, IV and V received the methanol rhizome extract of *Nymphaea lotus* at the doses of 200, 400 and 800 mg/kg *p.o.*, respectively.A charcoal

meal (10ml) was prepared by grinding 4 tablets of activated charcoal (250mg of activated charcoal each) with 0.5g of acacia. To this, 10ml of distilled water was added gradually with continuous stirring to give a slurry, charcoal meal (10%w/v charcoal suspension in 5%w/v suspension of acacia). This was then transferred into a bottle for final use. Each of the animals in Groups I-V was given 0.3ml charcoal mealas a marker by the oral route 30 minutes after drug treatments.All mice were sacrificed after 30 minof the charcoal meal by cervical dislocation. The stomach and small intestine werethen removed and extended on a clean surface. All care was taken to prevent any damage to the tissue. The distance moved by the charcoal meal from the pylorus was measured and then expressed as a percentage of the total distance from the pylorus to the caecum as follows:

% Movement of charcoal meal = (C / D)× 100%

Where:

C: distance travelled by charcoal meal

D: distance from the pylorus to the caecum.

3.2.6 Data Analysis

The data collected were expressed as mean ± SEM.Results were presented as graphs and tables where appropriate. Data were compared usingone way analysis of variance (ANOVA) followed by Dunnett's post hoctest using SPSS Version 20 software, where differences of$P \leq 0.05$ were considered statistically significant. The positive control and test groups were compared with the negative control.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage yield of crude rhizome extract of *Nymphaea lotus*

The weight of the extract obtained when 1.2 kg (1200 g) of the dried rhizome of *Nymphaea lotus* was extracted with methanol using Soxhlet's extraction technique was 44.5 g, representing a percentage yield of 3.7%.

4.2 Phytochemical constituents of methanol rhizome extract of *Nymphaea lotus*

Preliminary phytochemical screening of the methanol rhizome extract revealed the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and triterpenes as shown in table 4.1.

4.3 Acute toxicity study (LD_{50})

There were no lethality or toxic reactions observed at any of the doses of methanol rhizome extract of *Nymphaea lotus* used in the study upon oral administration. The oral median lethal dose of the rhizome extract was therefore estimated to be greater than 5000 mg/kg in mice.

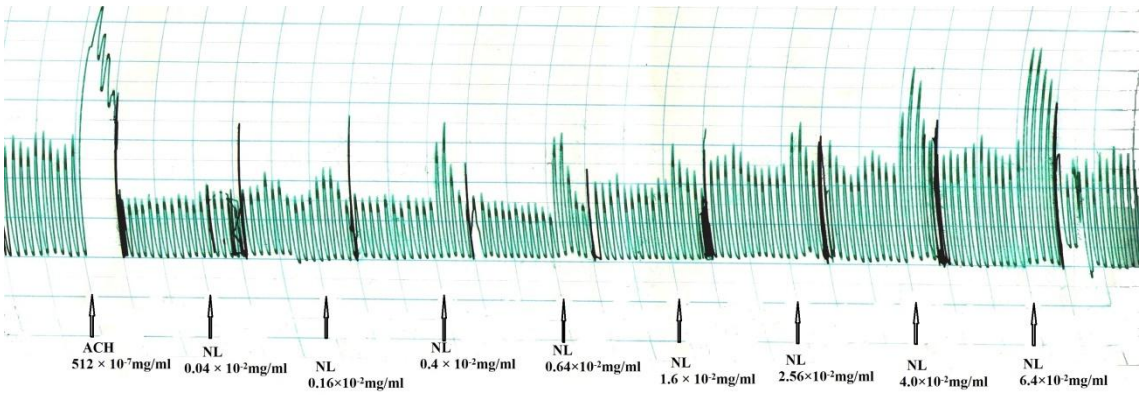
Table 4.1: Phytochemical constituents of methanol rhizome extract of *Nymphaea lotus* Linn

Phytochemical constituent	Test	Inference
Alkaloids	Dragendoff	Present
	Wagners	Present
Anthraquinones	Bontragers	Present
	Modified Bontragers	Present
Carbohydrates	Fehlings	Present
	Molisch	Present
Cardiac glycosides	Kadde	Present
	Kelle-Killiani	Present
Flavonoids	Shinoda	Present
	Sodium hydroxide	Present
Saponins	Frothing	Present
Steroids	Liberman Buchard	Present
Tannins	Lead acetate	Present
	Ferric chloride	Present
Triterpenes	Liberman Buchard	Present

4.4 In vitro Studies

4.4.1 Effect of methanol rhizome extract of *Nymphaea lotus* on isolated rabbit jejunum

The methanol rhizome extract of*N. lotus* (4×10^{-4} – 6.4×10^{-2}mg/ml) produced contractile effect on the tone of contraction of the rabbit jejunum, while at concentrations of 8×10^{-2}–512×10^{-2} mg/ml, it produced a concentration dependent significant reduction in the tone and rate of spontaneous contraction of rabbit jejunum (Plate III) indicating a biphasic activity (Figure 4.1). The extract (128×10^{-2} mg/ml) when interacted with acetylcholine (3.2×10^{-6} mg/ml)completely blocked the effect of the latter (Plate IV).



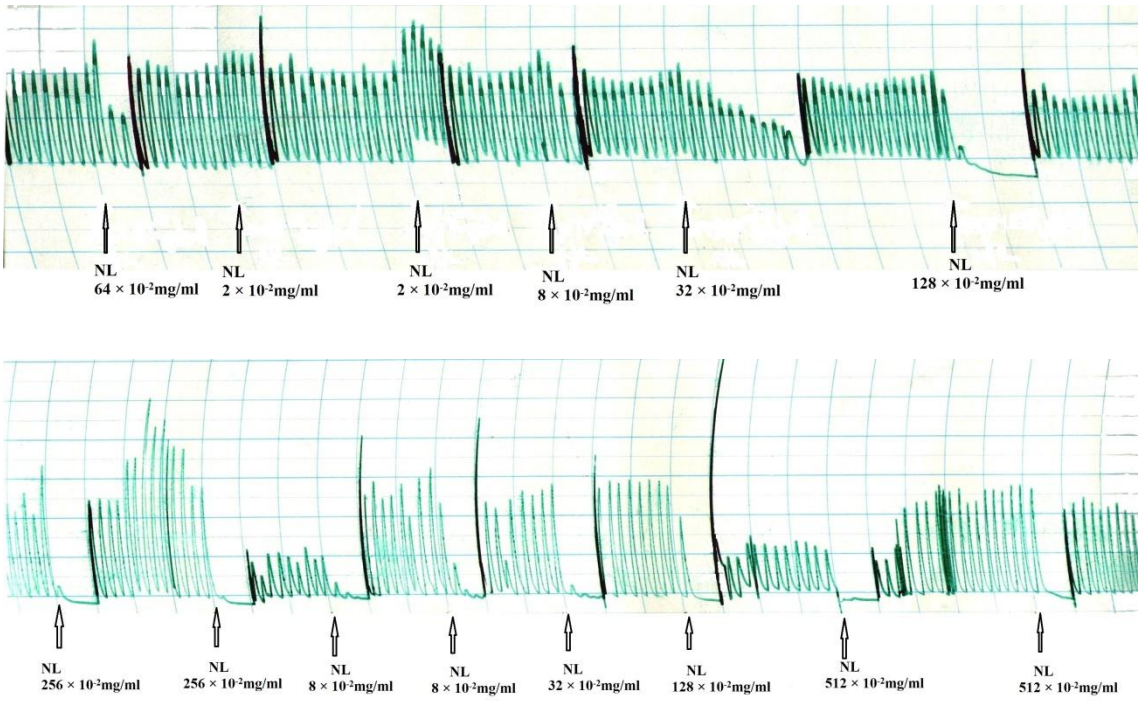


Plate III: Spasmogenic and spasmolytic effects of methanol rhizome extract of *Nymphaea lotus*(NL) 4×10^{-4} - 512×10^{-2}mg/ml on isolated rabbit jejunum

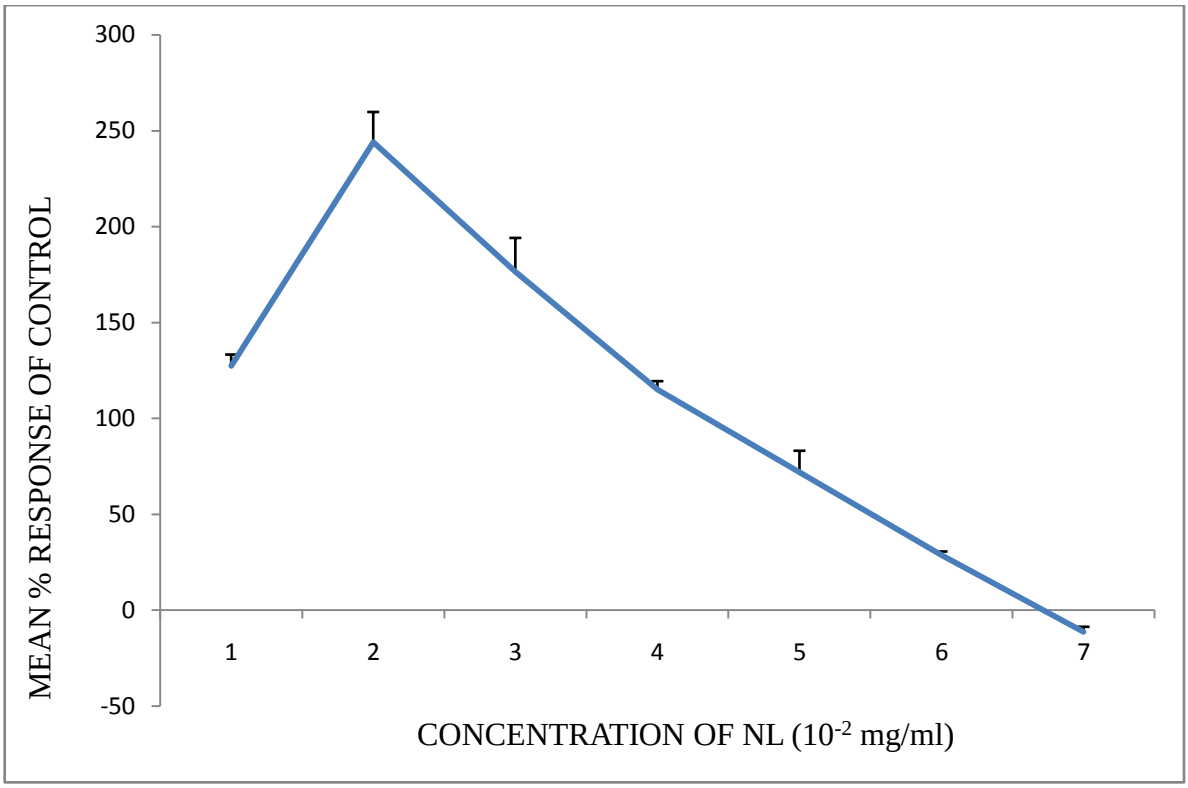


Figure 4.1: Effect of methanol rhizome extract of *Nymphaea lotus* (0.04-128×10^{-2}mg/ml) on isolated rabbit jejunum; n=3

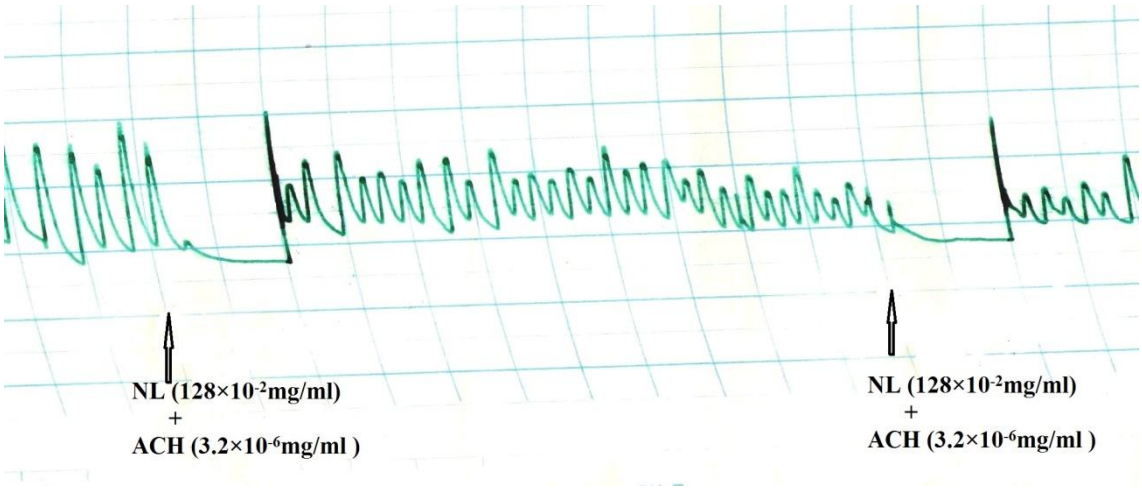


Plate IV: Effect of methanol rhizome extract of *Nymphaea lotus* (128×10^{-2} mg/ml)when interacted with acetylcholine (3.2×10^{-6} mg/ml) on isolated rabbit jejunum

4.4.2 Effect of methanol rhizome extract of *Nymphaea lotus* on isolated guinea pig ileum

At lower concentrations (4×10^{-4} - 2×10^{-2} mg/ml),the methanol rhizome extract of *Nymphaea lotus* has no effect on the tone of contraction of the guinea pig ileum while higher concentrations (4×10^{-2} -512×10^{-2} mg/ml) produced a concentration dependent significant reduction in the tone of contraction of guinea pig ileum (Plate V and Figure 4.2). When*Nymphaea lotus* extract (256×10^{-2} mg/ml) was interacted with histamine (64×10^{-6} mg/ml), the effect of histamine was completely blockedwith the extract still eliciting its relaxant activity(PlateVI).

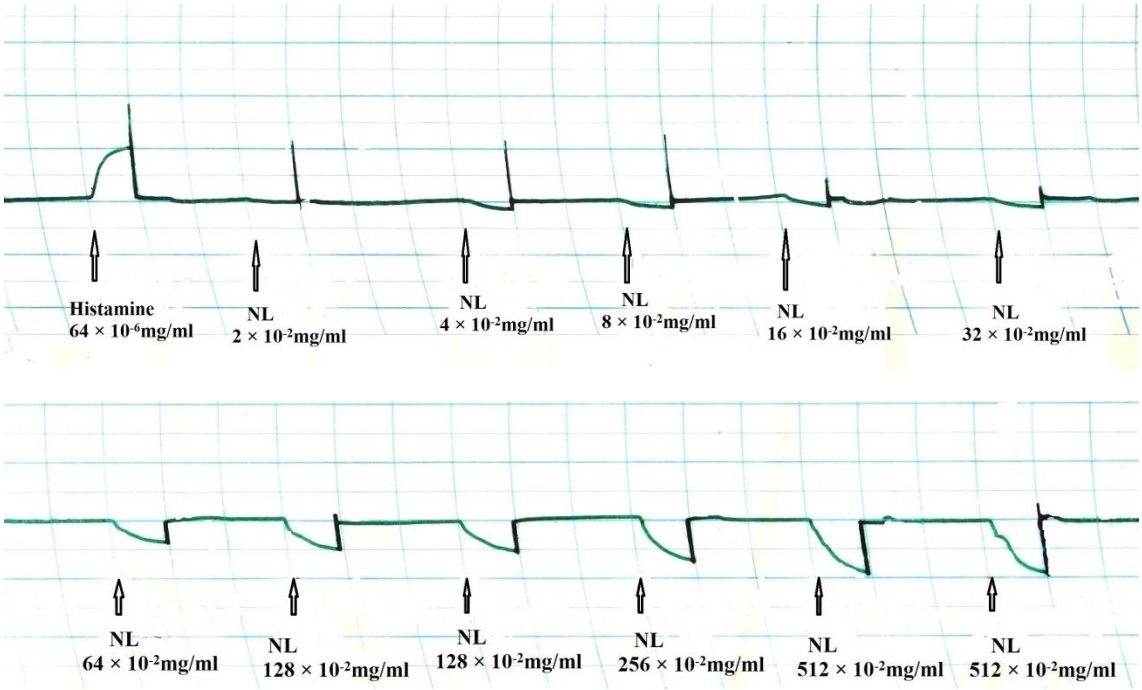


Plate V: Effect of methanol rhizome extract of *Nymphaea lotus*($2\times10^{-2} - 512\times10^{-2}$) on isolated guinea pig ileum

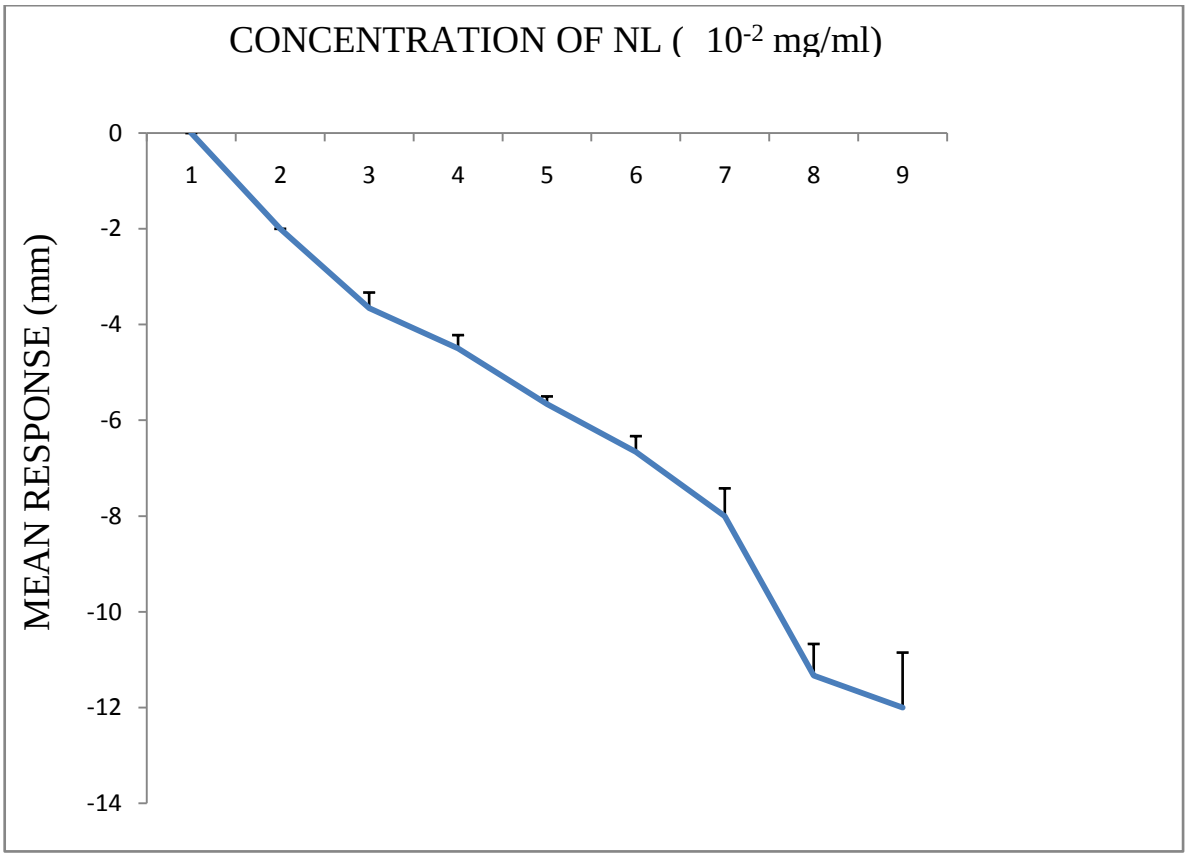


Figure 4.2: Effect of methanol rhizome extract of *Nymphaea lotus* (2-512 × 10^{-2} mg/ml) on isolated guinea pig ileum; n=3

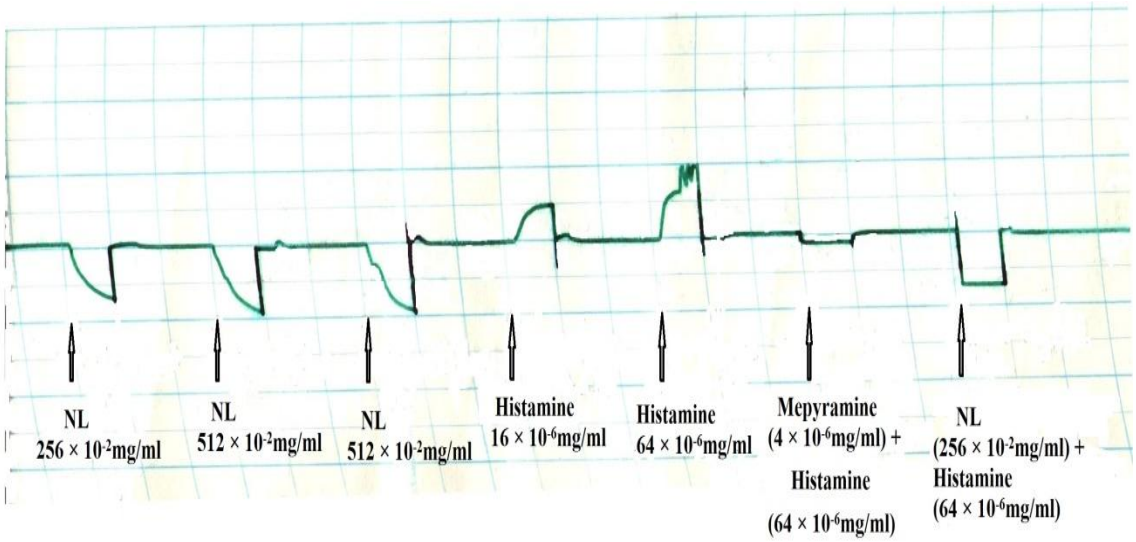


Plate VI: Effect of methanol rhizome extract of *Nymphaea lotus* (256×10^{-2} mg/ml) when interacted with histamine (64×10^{-6} mg/ml) on guinea pig ileum

4.5 Castor oil-induceddiarrhoea in mice

An hour after castor oil administration, most mice in the control group produced significant amount of wet mucoid faeces. Pretreatment of mice with methanol extract of *N. lotus*(800mg/kg) significantlydelayed the onset of diarrhoea ($p<0.05$). The methanol rhizome extract of *N. lotus* decreased the frequency of diarrhoea in a dose dependent manner. A statistically significant reduction in onset andfrequency of diarrhoea as well as weight of wet faeces was observed in the group treated with loperamide (3mg/kg) at $p<0.05$, $p<0.001$ and $p<0.05$ respectively. There was also a significant reduction in frequency of diarrhoea in groups treated with 200mg/kg, 400mg/kg and 800mg/kg of the extract at $p<0.05$, $p<0.01$ and $p<0.001$ respectively as shown in table 4.2. No significant decrease in the weight of faeces was observed in any of the groups treated with the extract.

Table 4.2: Effect of methanol rhizome extract of *Nymphaea lotus* on castor oil-induced diarrhoea in mice

Group (mg/kg)	Mean onset of diarrhoea±SEM(min)	Mean number of dry faeces ±SEM	Mean frequency of diarrhoea ±SEM	Mean total wt of faeces ±SEM (g)	Mean total wt of wet faeces ±SEM (g)	% Protection
DW 10ml/kg	77.16±7.48	2.66±0.88	6.83±0.79	0.34±0.05	0.31±0.05	-
Lop 3	160.25±18.50*	2.50±0.62	1.83±0.65***	0.23±0.07	0.12±0.06*	73.20
NL 200	133.17±18.88	1.66±0.80	4.00 ± 0.51*	0.36±0.03	0.33 ±0.02	41.43
NL 400	132.17 ± 6.96	2.00±0.89	3.50 ±0.42**	0.28±0.02	0.24 ±0.03	48.76
NL 800	154.60±26.98*	3.83±0.31	2.16±0.70***	0.24±0.05	0.16 ±0.05	68.37

Values are represented as Mean ± SEM; n = 6

*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$ – One-way ANOVA followed by Dunnett's post hoc test NL- *Nymphaea lotus* rhizome extract; DW – Distilled water; Lop – Loperamide; g – gram; min – minutes; wt – weight

4.6 Magnesium sulphate-induceddiarrhoea in mice

Approximately 2 hours after administration of magnesium sulphate, most of the mice in control group produced significant wet faeces without mucus. The methanol rhizome extract produced significant reduction in the frequency of diarrhoea with the lower doses producing better effect. A statistically significant reduction in frequency of diarrhoea was observed in the group treatment with loperamide (3mg/kg) at $p<0.001$. There was also statistically significant reduction in frequency of diarrhoea in the groups treated with 200, 400 and 800mg/kg of the extract at $p<0.01$, $p<0.001$ and $p<0.001$ respectively as shown in table 4.3. At doses of 200mg/kg (76.5%) and 400mg/kg (72.6%), the protection produced was comparable to that of the positive control, loperamide 3mg/kg (70.6%).

Table 4.3: Effect of methanol rhizome extract of *Nymphaea lotus* onmagnesium sulphate-induced diarrhoea in mice

Test group (mg/kg)	Mean onset of diarrhoea±SEM (min)	Mean number of dry feaces±SEM	Mean frequency of diarrhoea ± SEM	Mean total weight of faeces ± SEM (g)	Mean weight of wet faeces ± SEM (g)	% Prote-ction
DW10 (ml/kg)	115.83±16.22	6.83±1.16	8.50±0.92	0.15±0.01	0.08±0.02	-
Lop3	186.67±12.16**	4.50±0.62	2.50±0.67***	0.10±0.09	0.05±0.01	70.6
NL 200	156.60±11.60	3.16±0.48*	2.00±0.58***	0.06±0.01*	0.03±0.01	76.5
NL 400	161.25±13.09	6.50±0.99	2.33±0.76***	0.09±0.03	0.05±0.02	72.6
NL 800	159.25±18.69	7.33±1.35	3.50±1.48**	0.11±0.03	0.04±0.02	58.8

Values are represented as Mean ± SEM; n = 6

*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$ – One-way ANOVA followed by Dunnett's post hoc test

NL - *Nymphaea lotus* rhizome extract;Lop–Loperamide; g – gram; min – minutes; wt – weight

4.7 Effect of methanol rhizome extract of *Nymphaea lotus* on gastric transit time in mice

All treated groups produced statistically significant reduction in the propulsive movement and transit of charcoal meal in the gastrointestinal tract($p<0.001$). The standard antidiarrhoeal drug, atropine (5mg/kg) produced greater antimotility effect (56.83%) compared to the extract, as shown in table 4.4.

Table 4.4: Effect of methanol rhizome extract of *Nymphaea lotus* on gastric transit time in mice

Test group (mg/kg)	Mean length of intestine ± SEM (cm)	Mean movement of charcoal ± SEM (cm)	Mean % movement	% Protection
Deionized water (10ml/kg)	53.50 ± 0.65	45.66 ± 1.68	85.34 ± 2.96	-
Atropine 5	52.43 ± 1.45	19.03 ±2.66***	36.84 ± 5.52***	56.83
NL 200	56.40 ± 0.91	26.13 ± 2.50***	46.36 ± 4.45***	45.67
NL 400	52.43 ± 1.75	25.56 ± 2.79***	48.32 ± 3.98***	43.37
NL 800	56.03 ± 2.68	27.05 ± 1.71***	48.67 ± 3.60***	42.97

Values are represented as Mean ± SEM; n = 6

*** $p \leq 0.001$ – One-way ANOVA followed by Dunnett's post hoc test

NL - *Nymphaea lotus* rhizome extract; cm – centimeter; SEM – standard error of mean

CHAPTER FIVE

5.0 DISCUSSION

To restore personal comfort and convenience, many patients require antidiarrhoeal therapy and treatment is carried out to achieve, among other objectives, increased resistance to flow (segmental contraction, decreased propulsion and persistalsis) and increased mucosal absorption or decreased secretion (Akindele and Adeyemi, 2006).

The most common test of acute (short-term) toxicity is the median lethal dose, LD_{50} test. The oral LD_{50} of the methanol rhizome extract of *Nymphaea lotus* in mice was greater than 5000mg/kg body weight. This suggests that the extract is non-toxic orally according to Lorke's classification of chemicals(Lorke, 1983). Determination of LD_{50} is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. The larger the LD_{50} value, the lower the toxicity and vice versa. It is also used for determination of doses to be used for experimental work.The methanol rhizome extract of*N. lotus* may be considered potentially safe for oral use in the ethno-therapeutic management of diarrhoea.

Despite the multiplicity of aetiologies of diarrhoea, the four major mechanisms responsible for the pathophysiology in water and electrolytes transport are increased luminal osmolarity (osmotic diarrhoea), increased electrolytes secretion (secretory diarrhoea), decreased electrolytes absorption, and deranged intestinal motility causing a decreased transit time (Umer *et al.,* 2013). The antidiarrhoeal activity of methanol rhizome extract of *N. lotus* was evaluated by employing castor oil induced and magnesium sulphate-induced animal models of diarrhoea in additionto gastrointestinal motility test.

The effects of castor oil are mediated by ricinoleic acid, a hydroxylated fatty acid released from castor oil by intestinal lipases (Yoshio *et al*., 1999). The triglyceride present in castor oil is hydrolyzed in the small bowel by the action of lipases into glycerol and the active agent, ricinoleic acid (Semwal *et al.,* 2014). Ricinoleic acidinduces diarrhoea by hypersecretory response (Almeida *et al.,* 1995). Due to the polar nature of ricinoleic acid, it is poorly absorbed and its presence in the small intestine results inchanges in permeability of the intestinal mucosa to electrolytes and stimulates peristaltic activity in the intestine which in turn produces hypersecretion and fluid accumulationoccur (Yoshio *et al*., 1999; Dash *et al.,* 2013). The ricinoleic acid produces irritating and inflammatory actions on the intestinal mucosa leading to the release of prostaglandins (Yoshio *et al*., 1999) by colonic cells (Awouters*et al.,* 1978) or stimulates adenyl cyclase in the intestinal epithelial cells (Racusen and Binder, 1979).Prostaglandins thus released promote vasodilatation, smooth muscle contraction, and mucus secretion in the small intestines and thereby producing diarrhoea. Prostaglandins of the E series are considered to be good diarrheogenic agents in

experimental animals as well as in human beings. The inhibitors of prostaglandins biosynthesis are therefore considered to delay castor oil– induced diarrhoea (Brijesh *et al.,* 2009; Sunilson *et al.,* 2009).From the result obtained in this study, the extract was capable of inhibiting the castor oil induced diarrhoea in a dose dependent manner.*N. lotus*, therefore, may also have the ability to inhibit prostaglandin synthesis and possess anti-inflammatory activity. The effect of the extract at 800 mg/kg is comparable to loperamide at the dose used.

Loperamide, a well-known antidiarrhoeal agent (Hughes *et al.,* 1982) is an analog of meperidine, an opioid-receptor agonist. It acts on the μ-opioid receptors in the myenteric plexus of the large intestine.Similar to morphine, it works by decreasing the activity of the myenteric plexus, which in turn decreases the tone of the longitudinal and circular smooth muscles of the intestinal wall.It activates presynaptic opioid receptors in the enteric nervous system to inhibit acetylcholine release and decrease peristalsis (Lutterrodt, 1989). This increases the transit time allowing for more water to be absorbed from the faecal matter. Loperamide also decreases colonic mass movements and suppresses the gastrocolic reflex. The pharmacological effect of loperamide is due to its anti-motility and anti-secretory properties (Karim and Adeikan, 1977). From the result obtained, it is likely that the plant extract mediate its effect through similar mechanisms.

Magnesium sulphate produces diarrhoea by osmotic properties, preventing reabsorption of water molecules, thus leading to increase in the volume of the intestinal content. It promotes the liberation of cholecytokinin from the duodenal mucosa, which increases the secretion and motility of small intestine thereby preventing the reabsorption of sodium chloride and water (Galvez *et al.,* 1993; Parimala *et al.,* 2002). Since the extract was capable of inhibiting the magnesium sulphate-induced diarrhoea, *N. lotus* may also have the ability to inhibit production of diarrhoea by osmotic properties. The lower doses 200 and 400mg/kg produced response comparable to loperamide at the dose tested (3mg/kg). This indicates that increase in the dose of the extract does not cause an increase in activity (non-dose dependent effect). The non-dose dependent effect may possibly be that with increasing concentration of the extract, the constituent(s) responsible for its activity against magnesium sulphate-induced diarrhoea may act differently with increasing dose.

The methanol rhizome extract of *Nymphaea lotus*produced a dose dependent decrease in mean frequency of castor oil-induced diarrhoea and a non-dose dependent decrease in the mean frequency of magnesium sulphate-induced diarrhoea.

The test of the methanol rhizome extract of *N. lotus* on gastric transit time is a method used to study the effect of drugs on the motility of intestine (peristaltic movement) (Shamkuwar *et al.,* 2012). Activated charcoal is a non-absorbable agent that prevents absorption of chemicals and drugs due to its adsorption properties.

Atropineis a parasympatholytic drug which acts by blocking the actions of acetylcholine at muscarinic receptors. Atropine, as an antidiarrhoeal, possesses anti-motility effect and acts by increasing the intestinal transit time probably due to its anti-cholinergic effect (Brown and Taylor, 2001).In this study, atropine and different doses of the extract decreased the propulsive movement in the gastric transit time study with the former being more potent than the rhizome extract at the doses used. The methanol rhizome extract of *N. lotus*significantly reduced the intestinal propulsive movement in the charcoal meal treated model at doses of 200, 400 and 800mg/kg of body weight.The effect was comparable to atropine sulphate (5 mg/kg).The results show that the extract suppressed the propulsion of charcoal meal (probably in the same way as atropine sulphate) and increased the time for absorption of water and electrolytes. The reduction in percentage distance travelled can be a proof of gastrointestinal smooth muscle relaxation and antisecretory effect. This results in increase in gastric transit time allowing more time for water and electrolyte absorption into the system. The smooth muscle relaxation may be responsible for the use of the plant ethnomedically for the treatment of diarrhoea. Several antidiarrhoeal medications are well known for reduction of intestinal contractions and thereby reducing the intestinal transit (Mathad *et al.,*2005).

In the peripheral nervous system, acetylcholine activates gastrointestinal smooth muscles, and is a major neurotransmitter in the autonomic nervous system.Acetylcholine, with increasing concentration, causes a dose dependent stimulation of rabbit jejunum contraction. This effect attributed to the muscarinic effect of the drug. In the spontaneously contracting rabbit jejunum preparation, the methanol rhizome extract of *Nymphaea lotus* exhibit spasmogenic effect at lower concentration (4×10^{-4} - 2×10^{-2}mg/ml), followed by concentration dependent spasmolytic effect at higher concentration (4×10^{-2} – 512×10^{-2}mg/ml)in contrast to concentration-dependent contractility caused by acetylcholine which indicates the presence of a combination of gut stimulant and inhibitory constituents in the methanol rhizome extract of *N. lotus***.** The extract blocked the effect of acetylcholine on the spontaneous contraction of the rabbit jejunum. The effect produced by the interaction is similar to that of atropine (a cholinergic blocker) when interacted with acetylcholine. This shows that the extract may have anticholinergic activity.

The co-existence of spasmogenic and spasmolytic activity is common in herbal remedies (Khan *et al.,* 2012) such as *Calendula officinalis* (Bashir *et al.,* 2006), Ginger (Ghayur and Gilani, 2005), *Lavandula stoechas* (Jabeen et al., 2007),*Forsskalea tenacissima* (Shah *et al.,* 2010),*Plantago ovata* (psyllium husk) (Mehmoud *et al.,*2011), *Amaranthus spinosus* (Chaudhary*et al.,*2012)etc. These herbs have spasmolytic and spasmogenic effect depending on the solvent used for extraction, the concentration of the herbal extract or the type of isolated tissue used as well as the plant part used. This effect may be mediated through muscarinic receptor blockade and muscarinic receptor activation respectively. This is probably meant by nature not to allow the gut stimulant effect to go beyond a certain limit beyond which it could have been harmful, causing abdominal cramp. This is in line with the general perception that natural products

possess “side effects neutralizing” combination (Gilani and Atta-Ur-Rahman, 2005). There is growing evidence supporting this concept (Kumar *et al.*, 2015). The presence of this effect in medicinal plants is a long established concept put forth by the Hippocrates and strengthened by Ibn Sina and others. However, until now, this concept remained dormant and lacks sufficient scientific evidence mainly due to scarcity of ethnopharmacologists with wider background (Gilani *et al.*, 2000; 2005). Food plants are considered relatively safe as they are likely to contain synergistic and/or side effect neutralizing combinations of activities (Gilani and Atta-ur-Rahman, 2005). The rhizome of *Nymphaea lotus* may be a good example of such plants.

Methanol rhizome extract of *Nymphaea lotus* at higher concentrations(4×10^{-2}–512×10^{-2} mg/ml)exerted a dose dependent relaxant effect on the guinea pig ileum but has no effect at lower concentrations (4×10^{-4} - 2×10^{-2} mg/ml). The methanol rhizome extract of*N. lotus* blockedthe contractile effect of histamine upon interaction but this effect was not similar to when mepyramine was interacted with histamine. The extract produced significant relaxation while mepyramine did not because it is a histamine H_1 receptor blocker. The blockade of the effect of histamine by the extract may be as a result of physiologic antagonism. This activity may be as a result of a mechanism that does not involve binding to the same histamine receptor.

Histamine occurs throughout the gastrointestinal tract, in enterochromaffin-like (ECL) cells, restricted to the fundic mucosa of the stomach, mast cells and nerves. Histamine is actively produced and released in ECL cells, rich in the synthesis enzyme, histidine decarboxylase (HDC), while it is mainly stored in mast cells.Contraction of intestinal smooth muscle is described as one of the best characterized responses mediated by H_1 receptors (Coruzzi *et al.,* 2000). Receptors mediating the histamine induced contraction on the isolated guinea pig ileum are of the H_1 type (Bertaccini *et al.,* 1979). Throughout the gastrointestinal tract, contractile effects are also exerted on vascular smooth muscle and on endothelial cells, this latter resulting in an increase in vascular permeability (Coruzzi *et al.,* 2000).

Methanol rhizome extract of *N. lotus* contains chemical(s) with gut stimulatory effect mediated partly through cholinergic activation and also gut inhibitory constituent(s) which not only is/are likely to offset the side-effects associated with cholinergic components but also provide scientific explanation for the traditional use in diarrhoea.

The result of the phytochemical screening is in agreement with other studies such as analysis of the ethanol leaf extract of *N. lotus* by Akinjogunla *et al.*,(2010) and Afolayan *et al.*,(2013).Analysis of ethanolic extract of *N. lotus* by Dadi-Mamud and Musa (2013)in all the different parts of the plant analysed is in agreement with the phytochemical analysis except for tannins that were absent in the flowers extract.

The spasmolytic activity may be attributed to the phytochemicals found in the extract, such as saponins, flavonoids, sterols, tannins and triterpenes as studies on medicinal plants have been reported to have relaxant activities (Naiz and Wadood, 2011; Cortes *et*

al., 2006).The presence of phytochemicals like terpenoids, sterols, flavonoids and phenolic compounds have also being found to have spasmogenic or laxative activity in animals (Longanga-Otshudi *et al.,* 2000). Presence of cholinomimetic constituents may be responsible for the spasmogenic activity of methanol rhizome extract of *N. lotus*.

Alkaloids, glycosides, quinone, terpenoids and essential oils inhibit release of autocoids and prostaglandins (Tiwari *et al.,* 2011; Yasmeen *et al.,* 2010). Quinone (anthraquinones)also inhibits contractions caused by spasmogens and stimulates normalization of the deranged water transport across the mucosal cells. Flavonoids inhibit gastrointestinal release of acetylcholine (Tiwari *et al.,* 2011). Tanninsstimulatenormalization of deranged water transport across the mucosal cells and reduction of the intestinal transit (Tiwari *et al.,* 2011). Tannins denature proteins by forming a complex (protein tannate) and this complex coats the intestinal mucosa and makes it more resistant while simultaneously diminishing gastric secretions (Westendarp, 2006). Saponins inhibit histamine release *in vitro* (Rao and Gurfinkel, 2000; Tiwari *et al.,* 2011). Steroids enhance intestinal absorption of sodium and water (Kumar *et al.,* 2010).Therefore, the phytochemical constituents present in the methanol rhizome extract of *Nymphaea lotus* are thought to be responsible for anti-diarrhoeal activity by increasing colonic water and electrolyte reabsorption and inhibiting intestinal motility.

A number of plants exhibit their antidiarrhoeal properties through their antimicrobial activities (DiCarlo *et al.,* 1993; Ilyas *et al.,* 1995). Ethanolic extract of *Nymphaea lotus* leaves has been shown in previous studies to possess activity against some Gram negative and Gram positive bacteria such as *Staphylococcus aureus*and *Escherichia coli*(Akinjogunla *et al.,* 2009; Dadi-Mamud and Musa, 2013)which are among the microorganisms that are implicated in diarrhoea. The methanol rhizome extract of *Nymphaea lotus* may also contain similar constituents responsible for the antimicrobial activity. This may, therefore, contribute to its antidiarrhoeal activity.

A high rate of intestinal absorption might lead to a decrease in intestinal accumulation and together with reduced intestinal motility may result in increased transit time (Jarbur and Sjovall, 2000). This in turn might give chance for further absorption.The obtained anti-diarrhoeal activities of *Nymphaea lotus* in this study might be due to possession of chemicals that facilitate the aforementioned actions.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

The methanol rhizome extract of *Nymphaea lotus* was found to be relatively safe with the median lethal dose (LD_{50}) above 5000mg/kg *p.o.* in mice.

The extract was capable of inhibiting the castor oil induced diarrhoea in a dose dependent manner with its effect at 800 mg/kg being comparable to loperamide(3mg/kg).It is likely that the plant extract mediate its effect through similar mechanisms as that of loperamide.

The extract was capable of inhibiting magnesium sulphate-induced diarrhoea in mice. But the lower doses of 200 and 400mg/kg produced better response comparableto loperamide (3mg/kg) unlike that of the higher dose, 800 mg/kg (non-dose dependent effect).

The methanol rhizome extract of *N. lotus* suppressed the propulsion of charcoal meal in the test for gastric transit time in mice. The extractsignificantly reduced the intestinal propulsive movement at doses of 200, 400 and 800mg/kg of body weight probably in a manner similar to atropine sulphate (5mg/kg). Theincrease in gastric transit time will thereby cause an increase in the time for absorption of water and electrolytes into the system.

The methanol rhizome extract of *Nymphaea lotus* exhibits spasmogenic activity at lower concentrations (4×10^{-4} - 2×10^{-2} mg/ml) and concentration dependent spasmolytic activity at higher concentrations (4×10^{-2} – 512×10^{-2} mg/ml)on isolated rabbit jejunum preparation indicating the presence of a combination of gut stimulant and inhibitory constituents. The extract, at concentration that causes relaxation of the rabbit jejunum, blocked the effect of acetylcholine on the spontaneous contraction of the rabbit jejunum. The interaction is similar to when atropine (a cholinergic blocker) when interacted with acetylcholine.

On isolated guinea pig ileum,methanol rhizome extract of *Nymphaea lotus* at higher concentrations (4×10^{-2}–512×10^{-2} mg/ml)exerted a dose dependent relaxant effect but has no effect at concentrations below 4×10^{-2} mg/ml. The extract blocked the contractile effect of histamine upon interactionas a result of physiological antagonism. The interaction is not similar to when mepyramine (a H_1histamine receptorblocker) is interacted with histamine.

Presence of secondary metabolites such as: flavonoids, tannins, terpenoids, alkaloids, glycosides, quinone, steroids as well as saponins in the extract have been found to be responsible for its antidiarrhoeal activity.

6.2 Conclusion

The methanol rhizome extract of *N. lotus* may be considered potentially safe for oral use.The extract was found to have antidiarrhoeal activity against secretory diarrhoea, osmotic diarrhoea and motility-related diarrhoea. In addition, the extract was found to have spasmogenic and spasmolytic effects at low and high concentrations respectively on the rabbit jejunum and only spasmolytic effect on guinea pig ileum. Alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, tannins and triterpenes were found to be present in the methanol rhizome extract of *Nymphaea lotus*.

This study has shown that the methanol rhizome extract of *Nymphaea lotus* contains pharmacologically active substances with antidiarrhoeal propertiesthus justifying its use by the local population for this purpose.

6.3 Recommendation

Based on the findings and limitations in this work, the following recommendations are made:

- Further research is to be carried out to fractionate and purify the extract, in order to find out the molecule responsible for the antidiarrhoeal activity observed.
- Further study should be done to evaluate the exact mechanism of action
- Sub chronic and chronic toxicological studies on the methanol rhizome extract of *Nymphaea lotus* should be carried out to ascertain its safety.

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