ANTIBACTERIAL ACTIVITY OF THE ROOT EXTRACTS OF *DRACEANA LAXISSIMA* AND *DRACEANA FRAGRANS* ON SELECTED URINARY TRACT PATHOGENS

Wotoyitide Tonny Lukwago, (BSc. Ethnobotany)

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Supervised by

Professor Celestino (Obua MD, MSc, PhD)
Department of Pharmacology and Therapeutics (MakCHS)

Professor Jasper Ogwal-Okeng (MB,ChB, MSc, PhD)
Department of Pharmacology and Therapeutics (MakCHS)

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Most importantly, I express my sincere appreciation to my parents and the entire family for their patience, love, continual encouragement, support, sacrifices and prayers throughout my educational pursuits and daily life.
DEDICATION

My gratitude is expressed to my parents, my wife and my entire family for their continued encouragement, support, and sacrifices throughout my educational pursuits and daily life.
DECLARATION

I Wotoyitide Tonny Lukwago hereby declare that this dissertation is my own original work undertaken in partial fulfillment of the requirement for the award of the Degree of Master of Science in Pharmacology. I have made no use of sources, materials or assistance other than those which have been openly and fully acknowledged in the text. If any part of another person's work has been quoted, this appears in inverted commas. Any direct quotation or source of ideas has been identified in the text by author and the dates immediately after such an item, and full details are provided in a reference list at the end of the text.

I have read and understood the University statement on plagiarism and the repercussions that are involved when breached.

Signed

........................................

Supervisors

1. Professor. Celestine Obua (PhD) .............................................

2. Professor Jasper Ogwal-Okeng (PhD) .............................................
ABSTRACT

Introduction:

Over 150 million people are diagnosed with urinary tract infections (UTI) each year. The prevalence rate for UTIs in the Ugandan population was estimated to be at 1 in 33 or 3.03%. The main causal organisms of UTIs in Uganda are *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*. The emerging resistance of the above organisms to commonly used medicines like quinolones and penicillin’s has forced scientists to look for newer antibacterial agents. Extracts from *Dracaena* species have been used worldwide for many generations in the management of such infections. So these plants could be a possible alternative in the management of urinary tract infections.

Objective

To investigate the antibacterial activity of the root extracts of *Draceana Laxissima* and *Draceana Fragrans* on selected urinary tract pathogens.

Methods:

In this study, Plant materials were collected and cleaned according to the standard plant collection procedures. Extraction was by cold maceration and concentration was either by rotary evaporator or freeze drying. The phytochemical screening was carried out using standard methods. Nathans Agar well diffusion method was used for sensitivity test. The toxicity of the *Dracaena species* was also determined using standard methods.

Results:

The Phytochemical screening of the roots of the selected plants shows that the plants contain alkaloids, tannins and saponins. Both the methanol and water extracts of *Dracaena laxissima and Dracaena fragrans* showed no antibacterial activity against all the three test organisms’ i.e. *S. aureus*, *E. coli* and *P. aeruginosa*. No zones of inhibition were identified. The acute toxicity (LD₅₀) of both plants was estimated to be above 16,000mg/kg suggesting that the extract was very safe since its LD₅₀ value was more than 1000mg/kg.
Conclusions:

The results of this study demonstrated that the methanolic and water extracts of the roots of *Dracaena laxissima* and *Dracaena fragrans* have no antibacterial effect against *E. coli*, *S. aureus* and *P. aeruginosa*. 
DEFINITIONS

1. TRADITIONAL MEDICINE
   Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses.

2. LETHAL DOSE 50 – (LD₅₀)
   The median lethal dose of a substance, or the amount required to kill 50% of a given test population.

3. MINIMUM INHIBITORY CONCENTRATION (MIC)
   The MIC is the lowest concentration of antimicrobial agent which inhibits the growth of the microorganism.

4. URINARY TRACT INFECTION -
   Urinary tract infection is an Infection of the kidney, ureter, bladder, or urethra.

5. PHYTOCHEMICALS
   Phytochemicals are chemical compounds that occur naturally in plants. The term is generally used to refer to those chemicals that may affect health, but are not established as essential nutrients.

6. PATHOGENS
   An agent that causes disease, especially a living microorganism such as a bacterium or fungus.

7. ANTIBIOTICS RESISTANCE
   Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic.
8. **ACUTE TOXICITY**
   Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours.

9. **DOSE**
   Dose is the amount of test substance administered. Dose is expressed as weight of test substance per unit weight of test animal (e.g. mg/kg).

10. **LETHAL DOSE 50**
    LD₅₀ (median lethal oral dose) is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD₅₀ value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

11. **LIMIT DOSE**
    Limit dose refers to a dose at an upper limitation on testing (2000 or 5000 mg/kg).
ABREVIATIONS

ATCC American Type Culture Collection

DMSO Dimethylsulfoxide

LD_{50} Lethal Dose 50

MIC Minimum Inhibitory Concentration

WHO World Health Organization

OECD Organization for economic cooperation and development

FA Forestry Authority

UNESCO United Nations Education and Social Cultural Organization

CNS Central Nervous System

ANS Autonomic Nervous System
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(Enchence; Rukiga)
CHAPTER ONE: INTRODUCTION

1.1. Background

Over 150 million people are diagnosed with urinary tract infections (UTI) each year making it the second most common infectious presentation in community practice Worldwide (Gonzalez and Schaeffer, 1999). According to the US Census Bureau (2004), the prevalence rate for urinary tract infections to the total populations of Ugandans was estimated to be at 1 in 33 or 3.03%. UTI accounts for a significant part of the work load in clinical microbiology laboratories (Ojiegbe and Nworie, 2000).

In Uganda Urinary tract infections are commonly caused by Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Proteus mirabiris (Kyabaggu. et al., 2007). In separate studies carried out in Rubaga hospital (Uganda) by Kolawole et al., 2009 and Kyabaggu et al., 2007, it was established that the most contributing species to the UTIs in that hospital included among others; Escherichia coli (10.9%) Staphylococcus aureus (31.9%), Streptococcus species (9.2%), Klebsiella species (21.0%) and Proteus mirabiris (10.1%).

The emerging resistance of the above organisms to commonly used drugs like amoxycillin, cotrimoxazole, ciplofloxacin, erythromycin, and to nalidixic acid has forced scientist to look for newer sources of antibacterial agents (Kyabaggu. et al., 2007, Recio, 1989, Cragg et al., 1997). Dracaena species have been used worldwide for many generations in the management of infections (Evans et al., 2002, Cunningham, 1993).

A number of Draceaena species have been reported to have anticytotic, antidiarrhetic, antibacterial antifungal as well as other medicinal properties (Reddy et al., 1984, Milburn, 1984, Miller, 1988). Dracaena loureiri, was reported to posses anti-bacterial activity (Sofowora & Olaniyi 1975). Malcolm
and Sofowora, 1969 reported *Dracaena manii* to possess antimicrobial activity. *Dracaena cinnabar* resin is used in Arabia as an antiseptic (Milner, 1992, Milburn, 1984). According to Burkill (1985) *Dracaena mannii* has been proven to have bacteriostatic and fungi static properties in Senegal and Gambian.

The roots and fruits of *Draceana laxissima* have been used in the treatment of venereal diseases in Nigeria and Tanzania (Burkill 1985). In Rwanda and Congo- Kinshasa *Draceana laxissima* is used in the treatment of Urinary tract infections (UTIs) and sexually transmitted diseases (Cunningham.A.B, 1996). *D. laxissima* locally known as “Enchence” has been used by the people of south western Uganda (Banyankole and Bakiga) in the treatment of UTIs and STIs (Cunningham.A.B, 1996).

*Draceana fragrans* is a native plant species to Africa. It is being used in Angola, Ivory Coast, Mozambique, Sudan, Tanzania, Zambia Kenya and Uganda among others (Dalziel, J. M. 1995). The whole plant is said to act as an insect repellent. It is generally used in treatment of dermatitis and its roots and leaves have inhibitory properties that act against the micro organism *Plasmodium falciparum* (anti malarial) (Agbedahunsi. J.M et al., 2001). A decoction made from its roots is taken by both adults and infants for relief from febrile disease (Chabra, S. C. et al, 1984). *Draceana fragrans* is locally known as “Luwanyi” and “Muramura” by the people in central and south western Uganda respectively.

In an inventory carried out by joint ethnobotanical research and advocacy in 2006, *D. Steudneri, D. Fragrans* and *D. Laxissima* were among the most common species used by the local community in Mpigi district in treatment of UTIs. However there is no sufficient information to support the antibacterial activity of these commonly used dracaena species in Uganda.

### 1.2 Problem statement

Traditionally, Draceana species have been used widely in the management of symptoms of Urinary Tract Infections. In Uganda *D. laxissima and D. fragrans* have been widely used in the management of UTI symptoms. Though several *Draceana* species have been reported to have antimicrobial activity, limited studies have been conducted on *Dracaena laxissima and Dracaena fragrans* to demonstrate their
antibacterial activity despite their wide use locally. More to that evidence of their safety and efficacy still remains unknown.

1.3 Justification

The emergence of resistant microbial strains to the conventional antibiotics has necessitated the use of newer expensive drugs. Herbal medicines provide cheap and readily available alternatives to these newer expensive modern medicines. *Dracaena laxissima* and *Dracaena fragrans* have been used to treat UTI symptoms but without data to show that they have antibacterial activity against the common pathogens that cause the disease. This study has established the activity and safety profile of *Dracaena laxissima* and *Dracaena fragrans* against selected urinary pathogens.

1.4 - Significance

This study has contributed to the existing body of knowledge by generating current information on the effectiveness and safety of *Dracaena laxissima* and *Dracaena fragrans* herbal extracts in Uganda. It has also generated information which can be used by policy makers in the ministry of health as far the use of these plants in primary healthcare; especially treatment of urinary tract infections is concerned.
1.5 Objectives

1.5.1 General objectives

To investigate the antibacterial activity of the root extracts of *Draceana Laxissima* and *Draceana Fragrans* on selected urinary tract pathogens.

1.5.2 Specific objectives

1. To determine the phytochemical profile of the methanolic and water extracts of the root of *D. laxissima* and *D. fragrans*.
2. To determine the *invitro* bioactivity of the root methanol extracts of *D. laxissima* on *E.coli*, *P. aeruginosa* and *Staphylococcus aureus*, common urinary tract pathogens.
3. To determine the *invitro* bioactivity of the root water extracts of *D. fragrans* on *E.coli*, *P. aeruginosa* and *Staphylococcus aureus*, common urinary tract pathogens.
4. To determine the MIC and MBC of *D. fragrans* and *D. laxissima* extracts on *E.coli*, *P. aeruginosa* and *Staphylococcus aureus*
5. To determine acute toxicity of *D. fragrans* and *D. laxissima* extracts on mice.

1.6 Study scope

The study involved only the water and methanolic extracts for purposes of mimicking the local preparations by traditional healers. *Staphylococcus aureus* was selected to represent the gram positive cocci while *Pseudomonas aeruginosa* and *Escherichia coli* were selected to represent the gram negative pathogens.

1.7 Hypothesis

**H₀** There is no antibacterial activity when the root extracts of *D. laxissima* and *D. Fragrans*, are tested against *E.coli*, *P. aeruginosa* and *Staphylococcus aureus*.

**H₁** There is antibacterial activity when the root extracts of *D. laxissima* and *D. fragrans*, are tested against *E. coli*, *P. aeruginosa* and *Staphylococcus aureus*. 
1.8 Limitations

Although this research was carefully prepared, I am still aware of its limitations and shortcomings. First of all, the traditional healers normally boil the plant materials in water to make a decoction. This was not the case because the plant materials were cold macerated. It would be better if it was done the way traditional healers do it.

Second, the current study was unable to analyse the sub chronic and chronic toxicity studies. These two would have helped us find out whether the plants have long term toxicity. And as a result it would have helped the decision maker in the health sector to set the right regulations as far as the use of these plants are concerned. This was not possible because of financial constraints.
CHAPTER TWO: LITERATURE REVIEW

2.1 Urinary Tract Infections

Urinary tract infection (UTI) is a bacterial infection that affects any part of urinary tract system (Stamm and Norrby, 2001). Over 150 million people are diagnosed with urinary tract infections (UTI) each year making it the second most common infectious presentation in community practice worldwide (Gonzalez and Schaeffer, 1999). According to the US Census Bureau (2004), the prevalence rate for urinary tract infections to the total populations of Ugandans was estimated to be at 1 in 33 or 3.03%. UTI accounts for a significant part of the work load in clinical microbiology laboratories (Ojiegbe & Nworie, 2000).

Signs and symptoms that indicate UTI infection include new or increased urgency, frequency or dysuria younger patients. However this scenario can also be presented in the elderly patients. These complaints can be common and chronic without bacteriuria. As far as the change in character of urine is concerned, it can be cloudy, bloody, or malodorous in more than 85% symptomatic UTI’s. Other symptoms are less predictive. These may include elevated temperature (vital signs) Elderly patients may require more time to present with fever or may not have any increase in temperature or may even be hypothermic. In the young patients, fever is very common though it might also be absent. According to Palmer, (2004) several studies indicate fever as a marker for serious infection and as the most important clinical indicator for antibiotic treatment.

Pain especially the suprapubic or flank pain can indicate UTI (Palmer, 2004, Osborne, 2004). Incontinence may also be caused by UTI or the altered mental status. Other Possible Signs & Symptoms of UTI are hypotension, tachycardia, tachypnea, rales, respiratory distress, anorexia, nausea, vomiting and abdominal tenderness among others (Palmer, 2004, Swart, Soler & Holman, 2004)
2.2 Management of UTIs

2.2.1 Treatment for uncomplicated UTIs

The standard regimen has traditionally been a 3-day course of trimethoprim-sulfamethoxazole, commonly called cotrimoxazole. A single dose of cotrimoxazole is sometimes prescribed in mild cases, but cure rates are generally lower than with 3-day regimens (Naber KG, 2000). Allergies to sulfa are common and may be serious.

Fluoroquinolone antibiotics, also called quinolones, have usually been a second choice. However, in geographic areas that have a high resistance to cotrimoxazole, quinolones are now the first-line treatment for UTIs. Ciprofloxacin is the quinolone antibiotic most commonly prescribed (Iravani et al, 1999). Quinolones are usually given over a 3-day period. However, pregnant women should not take these drugs.

Nitrofurantoin is a third option. This drug must be given for longer than 3 days (Iravani et al, 1999).

Fosfomycin may also be used during pregnancy but is not as effective as other antibiotics. Resistance rates to this drug are very low (Stein GE, 1999).

Other antibiotics that may also be used, including amoxicillin (with or without clavulanate) and cephalosporins (Susan A.M, 2005). Doxycycline is often effective but cannot be given to children or pregnant women.

2.2.1 Treatment for kidney infections (pyelonephritis)

Patients with uncomplicated kidney infections (pyelonephritis) may be treated at home with oral antibiotics. Patients with moderate-to-severe acute kidney infection and those with severe symptoms or other complications may need to be hospitalized (Drugs. 1990). In such cases, antibiotics are usually given intravenously for several days. Chronic pyelonephritis may require long term antibiotic treatment (Susan A.M, 2005).
2.2.2 Treatments for specific populations

Treating pregnant women.

Pregnant women should be screened for UTIs, since they are at high risk for UTIs and their complications. The antibiotics used during pregnancy include amoxicillin, ampicillin, nitrofurantoin, and cephalosporin (Susan A.M, 2005). Fosfomycin is not as effective as others but may be used during pregnancy (Stein GE, 1999). Pregnant women should not take fluoroquinolones (Iravani et al, 1999).

Treating children with UTIs.

Children with UTIs are generally treated with cotrimoxazole, cephalexin and other cephalosporins, amoxicillin, or amoxicillin/clavulanic acid (Drugs.1990, BNF. 2008). These drugs are usually taken by mouth in either liquid or pill form. Doctors sometimes give them as intravenous.

Vesicoureteral reflux (VUR) is a concern for children with UTIs. VUR can lead to kidney infection (pyelonephritis), which can cause kidney damage (Susan A.M, 2005). The two treatment options for children with VUR are long-term antibiotics to prevent infections or surgery to correct the condition. Children with acute kidney infection are treated with oral cefixime or a short course (2 - 4 days) of an intravenous antibiotic. An oral antibiotic then follows the intravenous.

2.2.3 Non pharmacologic therapies

Physicians commonly recommend non pharmacologic options (e.g., drinking cranberry juice or water) to patients with cystitis. However Jepson, R.G. 2000, found insufficient evidence to recommend the use of cranberry juice to manage UTIs.

2.2.4 Management of catheter-induced urinary tract infections

Catheter-induced urinary tract infections are very common, and preventive measures are extremely important. Catheters should not be used unless absolutely necessary and they should be removed as soon as possible. Intravenous broad spectrum antibiotics may be used to treat these infections (Rosenberg, J.M. 1985, Childs, S.J. 1984).
2.3 Medicinal Plants and Global Trend

Medicinal plants have been used for centuries as remedy components of therapeutic value (Cunningham, 1993). World Health Organization (WHO) documents that medicinal plants are the best source to obtain variety of drugs (Santos, 1995). In Uganda, plants have been a valuable source of natural products and the use of herbal medicine has been practiced since time immemorial. Katende et al., (1995), showed that several plants have been used in the treatment of infections in most regions of Uganda. Anokbonggo (1980) and Adjanohom et al., (1993) demonstrated the use of herbal medicines in the treatment of various illnesses.

According to Davis et al., 1986, Uganda is blessed with a rich diversity of medicinal plants relative to other parts of Africa. Several studies have been carried out in Uganda and they have identified numerous medicinal plants used for treatment of various diseases such as UTIs, malaria and HIV related infections among others (Tabuti et al., 2003, Kokwaro, 1993).

Since microbial resistance to the available antibiotics has been increasing for the past two decades (Martino et al., 2002; Ghandhi and Banker, 1999; Mathur and Tannan, 1999), scientists have been forced to develop new effective but more expensive antimicrobials.

Traditional medicine has also been accepted as an alternative form of health care (UNESCO 1994). This has lead researchers to investigate the antimicrobial activity of medicinal plants. However in Uganda not much work has been done to verify the efficacy of most medicinal plants. It is therefore important to analyze our plant resources for lead compounds (Evans et al., 2002).

2.4 Draceana Species and Traditional Medicine

Dracaenas are monocotyledons in the Dracaenaceae family. According to Kubitzki (1998), the genus comprises about 100 species worldwide. Although variable, the genus as a whole is a homogenous group of considerable ancestry. Most Dracaenas produce resins. The resins differ widely in both appearance and purity even if they were from the same locality (Kubitzki, 1998).
In Uganda, *Dracaena steudneri* is among the plants used by traditional medical practitioners in the treatment of various AIDS conditions and opportunistic infections such as carbuncles, sores, venereal diseases and dysuria among others (Lamorde *et al.*, 2010).

Today *Dracaena cinnabar* resin is used in Socotran folk medicine and throughout Arabia for treating a wide range of ailments both internally and externally and it also has antiseptic properties (Milner, 1992, Milburn, 1984). According to Burkill (1985) *Dracaena mannii* has been used in Senegal and Gambian tradition medicine practice for treating various infections including UTIs and phytochemically it has been proven to have bacteriostatic and fungi static properties.

*Dracaena angustifolia* has several traditional uses, including use for the treatment of various physical ailments. The Khasi and Garo tribes of India use decoctions of the leaves and roots to treat various ailments that include kidney troubles among others (Maikhuri and Gangwar, 1993). An indigenous tribe in the Andaman Islands in the Bay of Bengal uses the bark and twigs of *D. angustifolia* to clean their genitals after menstruation (Mukhopadhyay *et al.*, 2002).

The roots and fruits of *Draceana laxissima* have been used in the treatment of venereal diseases in Nigeria and Tanzania (Burkill 1985). In Rwanda and Congo- Kinshasa *Draceana laxissima* is used in the treatment of Urinary tract infections (UTIs) and sexually transmitted diseases (Cunningham.A.B, 1996). *D. laxissima* locally known as “Enchence” has been used by the people of south western Uganda (Banyankole and Bakiga) in the treatment of UTIs and STIs (Cunningham.A.B, 1996).

Dracea fragrans is a native plant species to Africa. It is being used in Angola, Ivory Coast, Mozambique, Sudan, Tanzania, Zambia Kenya and Uganda among others (Dalziel, J. M. 1995). The whole plant is said to act as an insect repellent. It is generally used in treatment of dermatitis and its roots and leaves have inhibitory properties that act against the micro organism *plasmodium falciparium* (anti malarial) (Agbedahunsi. J.M et al., 2001). A decoction made from its roots is taken by both adults and infants for relief from febrile disease (Chabra, S. C. *et al* 1984). The plant is locally known as “Luwanyi” and “Muramura” by the people in central and south western Uganda respectively.
2.5 Phytochemical Profile and Antimicrobial Activity of the Dracaena Species

Previous chemical studies with species of genus *Draceana* were related to the isolation of spiranol saponins, spirostanol sapogenins, furostano saponin and steroidal saponins (Tran *et al.*, 2001; Banskota, *et al.*, 2003). *Dracaena loureiri*, a Thai medicinal plant possesses anti-bacterial activity and *Draceana manii* has got antimicrobial activity (Sofowora & Olaniyi, 1975, Malcolm and Sofowora, 1969). *D. afromontana* has been phytochemically investigated and it was found to posses’ afromontoside, a cytotoxic steroidal saponin (Reddy *et al.*, 1984). *Dracaena draco* has been found to have an antimicrobial steroidal dracogenin and several flavonoids.(Gonzalez *et al.*,1983; Camarda *et al.*,1983)

Palmitic acid and n-heptacosane have also been isolated from *D. manii* (Sofowora & Olaniyi, 1975). *D. loureiri* has been found to possess antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* (Wasuwat, 1967). In a phytochemical study carried out on 13 genera of family *Agavaceae*, *Dracaena* was reported to have flavanoids (Carmarda *et al.*, 1983). Though a number of studies have been carried out on family *Agavaceae*, limited phytochemical studies have been performed on *D. laxissima* and *D. fragrans*. 
CHAPTER THREE: MATERIALS AND METHODS

3.1 FIGURE 1: Flow chart for the Research work

- Literature review
- Plant collection
- Herbarium/Plant identification
- Plant processing e.g. drying, grinding and storage
- Preparation of plant extracts (soaking, filtering, concentrating)

**Draceana laxissima** extract (DST)
- Water methanol
- **Antibacterial assay of DST** (Susceptibility tests)
- Minimum inhibitory concentration (MIC)
- Phytochemical analysis of DST
- Toxicity studies

**Draceana fragrans** extract (DFR)
- Water methanol
- **Antibacterial assay of DFR** (Susceptibility tests)
- Minimum inhibitory concentration (MIC)
- Phytochemical analysis of DFR
- Toxicity studies
3.2 Study settings

Plant materials were collected from the Zika forest in Wakiso district, Uganda after seeking permission from the Forestry Authority and Uganda wildlife society. Zika forest is located 40 km from Kampala and a few kilometers from Entebbe town on the main road to Kampala. The plants were collected from Zika forest because the environment around the forest is still natural and also a number of traditional healers normally collect from the same place. The plant material was identified and verified at Makerere University Herbarium with the help of a taxonomist Mr Kamoga Denis. A voucher specimen was deposited in the herbarium. The water and methanolic extracts were prepared from the Department of Pharmacology and Therapeutics Makerere University. Bioassays were carried out at the College of Veterinary Medicine, Animal resources and Bio security Makerere University (COVAB).

Phytochemical studies were carried out at the Department of Pharmacology and Therapeutics Makerere University. Freeze drying was done at the Dept of nuclear medicine Mulago Hospital.

3.2 Study design

This was a laboratory experimental study to establish the biological activity of the extracts of *D. fragrans* and *D. laxissima* on purchased strains of *Pseudomonas aeruginosa* (ATCC 27853) a gram negative bacillus, *Staphylococcus aureus* (ATCC 25923) which represent gram positive cocci and *Escherichia coli* (ATCC 25922) an enteric gram, negative bacillus. *Draceana laxissima* and *Draceana fragrans* were selected because they were among the plants being used by traditional healers in managing symptoms of UTIs in Uganda (JERA. 2008). However, there was no evidence to verify these claims.

3.3 Plant material collection

The roots of *D. laxissima* and *D. fragrans* were obtained from the Ziika forest in Wakiso district with the help of a local herbalists Ssalongo Musisi, a forest warden Mr Okot Ivan and a taxonomist Mr Kamoga Denis, after seeking permission from the forestry authority (FA) and Uganda wildlife society. The plant materials were taken to Makerere University Herbarium for identification and voucher specimens were prepared and kept at the herbarium.
3.4 Processing of roots for extraction

The roots of *Draceana laxissima* and *Draceana fragrans* were open air dried in the shade. The dried roots were pounded into a moderately coarse powder. The powder was stored in sealed plastic bags in a cool dry place, ready for extraction.

3.6 Extraction of plant material

3.6.1 Methanol extraction and calculation of yields

Twenty grams of the powdered roots of *D. laxissima* and *D. fragrans* were transferred to sterile wide-mouthed screw-capped bottles of 200 ml volume. One hundred milliliters (100 ml) of methanol 95% conc. was added to the powder samples and was allowed to soak for 24 h at room temperature. The extractions were repeated three times to ensure that all the active components have been extracted. The filtrates were concentrated on a rotary evaporator model Rotavapor® R-210/R to remove the solvent. The resulting methanolic extracts was put in bottles which were first autoclaved to ensure sterility and then kept under refrigerated conditions until use. The yield was determined as a proportion of the dry weight of the extracts with that of the powder. The percentage (%) yield of the extract was calculated using the following equation below where, W = weight.

\[
% \text{ yield} = \frac{W_{\text{crude extract}}}{W_{\text{dried plant}}} \times 100
\]

Where: \(W_{\text{crude extract}}\) is the weight of the crude extract

\(W_{\text{dried plant}}\) is the weight of the dried plant powder

3.6.2 Aqueous extraction and calculation of yields

Twenty grams of the powdered roots of *D. laxissima* and *D. fragrans* were transferred to sterile wide-mouthed screw-capped bottles of 200 ml volume. One hundred milliliters (100 ml) of sterile de ionized distilled water was added to the powder samples which will then be allowed to soak for 24 h at 4°C. The mixtures were then be centrifuged at 2000 rpm for 10 min. at 4°C. The extractions were repeated three times to ensure that all the active components have been extracted. The supernatants were filtered through a Whatman No. 4 filter paper and distributed into dark bottles. The filtrates were then freeze-
dried. The yields of root extracts were calculated as the amount of freeze dried extract expressed as weight percentage of the plant material used for extraction (w/w%). The percentage (%) yield of the extract was calculated using the following equation below where, \( W \) = weight.

\[
\text{% yield} = \frac{W_{\text{freeze dried extract}}}{W_{\text{dried plant}}} \times 100
\]

Where: \( W_{\text{freeze dried extract}} \) is the weight of the freeze dried extract

\( W_{\text{dried plant}} \) is the weight of the dried plant powder

3.7 Preliminary phytochemical screening:

The phytochemical analysis was carried out at the College of Health Sciences, The department of pharmacology and therapeutics Makerere University. The stock solution consisting of 100mg of crude extract \( \times 10\% \) of dimethyl sulfoxide (DMSO) was prepared using distilled water as an eight fold serial dilution to cover ranges from 100mg\text{\textgreek{l}}m to 0.78125mg\text{\textgreek{l}}m and stored in the dark at 4\degree C (Appendix 1). The extracts thus obtained was subjected to preliminary phytochemical screening following the methods described by Harborne (1998), Trease and Evans (1989). This was conducted for determination of the presence of saponins, tannins, alkaloids, flavonoids, cardiac glycosides and cyanogenic glycosides (Appendix IV).

3.8 Test Bacterial Organisms

Standard bacterial organisms from the American Type Culture Collection were used. These included, \textit{Pseudomonas aeruginosa} (ATCC 27853) a gram negative bacillus, \textit{Staphylococcus aureus} (ATCC 25923) which represent gram positive cocci and \textit{Escherichia coli} (ATCC 25922) an enteric gram, negative bacillus. They were collected from the microbiology department of the College of Health Sciences Makerere University. The bacterial cultures were aseptically sub-cultured onto nutrient agar and incubated aerobically at 37\degree C for 24 hours to obtain discrete bacterial colonies.
3.8.1 Preparation of the test organisms

Before the preparation of test organisms a 0.5 Mc farland standard was prepared by dissolving 9.95mls of 1% H₂SO₄ with 0.05mls of BaCl₂ (Becton and Dickson., 2010) against which the test inoculum was compared. To 5 mls of sterile 0.9 % normal saline 24 hour old test organism was carefully added colony by colony while comparing the forming turbidity with that of the prepared Mc farland standard until the exact matching was obtained (Case, 1998), this was approximately equivalent to 1.5 *10⁸ CFU/ml.

3.9 Test for antibacterial activity

3.9.1 Preparation of the extract for study

One gram of the extract was carefully weighed using a top load weighing balance ( model: Sartorius Werke GMBH 2204) and there after dissolved in 10% dimethylsulfoxide (DMSO), Distilled water was added up to 10 mls to obtain a concentration of 100mg/ml (Stock Solution). One mill was obtained from the stock solution and serially diluted using distilled water in a two-fold pattern to obtain concentrations of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml ( Arwa et al., 2008 with modification).

3.9.2 Preparation of media for agar well diffusion assay.

Mueller hinton agar (Corda laboratories ltd, South Africa) was used because it is suitable for all organisms in the study. Thirty eight grams of agar powder was dissolved in one liter of distilled water, boiled to dissolve and there after the solution was autoclaved using Baujahrs 1995 model at 121ºc for 15 minutes and then cooled to 50 ºc in a water bath. It was then transferred into sterile petri dishes where it cooled and solidified under sterile conditions. The petri dishes with the media were incubated for 24 hours at 37 ºc to ensure that there is no contamination by bacterial organisms.
3.9.3 Agar well diffusion assay

Sterile plates of solidified agar were inoculated with a loopful of test inoculum and streaked to form a lawn of a test microorganism. Five wells of 6mm diameter were made on each Petri plate using sterile borer (Carter and Cole, 1990). The wells were clearly labelled.

Exactly 0.05mls of each of the selected concentrations (100mg/ml, 25mg/ml and 6.25mg/ml) was carefully dispensed in the extract well. An equal volume was used for the controls. DMSO and water mixture were used as negative control to nullify the effect of the solvent on the test organisms, while10mg/ml gentamycin was used as a positive control to produce a standard bacterial effect (Waako., 1996, Olila et al., 2001, Bbosa., 2004). The inoculated plates were incubated aerobically at 37°C for 24 hours (Waako., 1996, Olila et al., 2001, Bbosa., 2004). Triplicate tests were made for each experiment. Plates were finally examined for presence of zones of inhibition. The zones of inhibition were measured using vernier calipers and expressed in millimeters. In this case smaller zone of inhibition meant little activity and vice versa for a bigger zone of inhibition.

3.10 Acute toxicity tests

The toxicity of *D. fragrans* and *D. laxissima* extracts were determined and expressed in terms of LD50 which was calculated according to Ghosh (1984). The LD50 of the *Draceana species* was determined in mice of both sexes. Seventy (70) Swiss albino mice of both sexes were kept in standard laboratory cages to acclimatize for 7 days then starved overnight (16 hours) to empty the Gastric Intestinal Tract (GIT). This was to minimize the interaction of the plant extract with food and to increase absorption over the large surface area. A preliminary study was done to provide dose ranges for the subsequent study. In this study two groups of five mice each were treated using widely separated doses by oral route administration.

The animals were divided randomly into 10 groups of 6 mice in each. Group 9 and 10 served as control, by receiving 1ml of distilled water; group 1, 2, 3 and 4 received the extract at the dose levels of 8000mg/kg, 12,000mg/kg, 16,000mg/kg and 20,000mg/kg per body weight of *Draceana laxissima* preparation while group 5, 6, 7 and 8 received the same dose rates of *Draceana fragrans* by oral route of administration with the help of a gavage tube size 4. The dose level selection was based on the earlier run preliminary study. The observations lasted for 24 hours, including drug administration and observation period with signs of toxicity and any mortality recorded.
3.11 Data analysis

The data were recorded in a table format and there after transferred into Excel® spreadsheets for coding and preliminary analysis. The datasets of the different pathogens were summarized in means and presented in tables. Analysis of the data was reported in the form of diameter of inhibition zone during susceptibility testing of all bacterial isolates by agar well test against different classes of antimicrobial agents.

3.12 Ethical Considerations

The research proposal was presented to the department of Pharmacology and Therapeutics, the ethics and research Committee of the College of Health Sciences of Makerere University before the research was carried out. The animals were also handled according to the animal ethics guidelines and use of live animals for experimental purposes.
CHAPTER FOUR: RESULTS

4.1 Extracts of *Dracaena fragrans* and *Dracaena Laxissima* and their phytochemical composition

Phytochemical screening of the roots of the plants shows that the plants contain alkaloids, tannins and saponins. The root extracts of both *D. fragrans* and *D. laxissima* lacked Flavonoids and glycosides. Unlike the root extract of *D. fragrans* which had moderate concentrations of both tannins and alkaloids, the root extracts of *D. laxissima* had very low concentration of both tannins and alkaloids. Alkaloids and tannins were completely absent in the water extract of *D. laxissima* root. Although saponins were present in all extracts, only *D. fragrans* methanol extract had moderate concentrations of the Saponins as shown in table 1 below.

**Table 1;** Classes of compounds identified in the methanolic, and water root extracts of *Draceana fragrans* and *Draceana laxissima.*

<table>
<thead>
<tr>
<th>Phytochemical groupings</th>
<th><em>Dracaena fragrans</em> root extract</th>
<th><em>Dracaena Laxissima</em> root extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>Methanol</td>
</tr>
<tr>
<td>Flavinoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Key:  - : Absent, + : Low concentration, ++ : Moderate Concentration,
4.2 Antibacterial activity of root extracts of Draceana fragrans and Draceana laxissima.

Using the agar-well diffusion method the both the methanol extract and water extracts showed no antibacterial activity on all the three test organisms i.e. *S. aureus*, *E. coli* and *P. aeruginosa*. No zones of inhibition were identified. Gentamycin which was used as the positive control showed sensitivity to all the three organisms as shown in table 2 below.

**Table 2: Antibacterial activity of the methanolic and water extracts of D. fragrans and D. laxissima against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa using the agar well diffusion method**

Gentamycin, a standard drug, is included for purposes of comparison.

<table>
<thead>
<tr>
<th>Medicinal plant extract</th>
<th>Solvent</th>
<th>S. a</th>
<th>E. c</th>
<th>P. a</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. fragrans</em></td>
<td>Methanol</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gentamycin</td>
<td></td>
<td>21</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td><em>D. laxissima</em></td>
<td>Methanol</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gentamycin</td>
<td></td>
<td>20</td>
<td>20</td>
<td>15</td>
</tr>
</tbody>
</table>

Key: S.a- Staphylococcus aureus, E.c- Escherichia coli, P.a- Pseudomonas aeruginosa
### 4.3 Acute Toxicity Study Results For the Water Draceana laxissima and Dracaena fragrans.

Table 3 below shows the different groups of mice, their body weight and the dosage of dracaena flaxissima extract which was used to dose the mice. It also shows the percentage number of dead mice and probits.

**Table 3: Results of the lethal doses of Draceana laxissima for determination of LD_{50} after oral dosing in mice (n=6).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean body wgt (gm)</th>
<th>Dose (mg/kg)</th>
<th>Log dose</th>
<th>Number dead</th>
<th>% dead</th>
<th>Probits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.84</td>
<td>8,000</td>
<td>3.90</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>14.23</td>
<td>12,000</td>
<td>4.08</td>
<td>1/6</td>
<td>16.67</td>
<td>4.01</td>
</tr>
<tr>
<td>3</td>
<td>12.73</td>
<td>16,000</td>
<td>4.20</td>
<td>2/6</td>
<td>33.33</td>
<td>4.64</td>
</tr>
<tr>
<td>4</td>
<td>14.14</td>
<td>20,000</td>
<td>4.30</td>
<td>4/6</td>
<td>66.67</td>
<td>5.71</td>
</tr>
</tbody>
</table>

Figure 2 below shows a plot of probits against log dose for dracaena laxissima plant extract. The slope of the graph which was drawn from the figures obtained from table 3 is $y=13.9x-53.87$. Since probit 5 represents the 50% death, let’s assume that $y=5$.

By using the above formula, we can derive the value of $x$.

Therefore;

$$y = 13.94x - 53.87$$

$$5 = 13.94x - 53.87$$

$$x = 4.223$$
Since x represents the log dose value at 50% death on the x axis, the antilog of x will represent the LD50. Therefore;

\[
\text{Antilog of } x = \text{LD50}
\]

\[
\text{Antilog of } x = 16714
\]

\[
\text{LD50} = 16714 \text{ mg/kg}
\]

**Figure 2: A graph of Probits vs Log dose for Draceana laxissima extract**

![Graph of Probits vs Log dose for Draceana laxissima extract](image)

Table 4 below shows the different groups of mice, their body weight and the dosage of dracaena fragrans extract which was used to dose the mice. It also shows the percentage number of dead mice and probits

![Graph of Probits vs Log dose for Draceana laxissima extract](image)
Table 4: Results of the lethal doses of Draceana fragrans for determination of LD$_{50}$ after oral dosing in mice (n=6).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean body wgt (gm)</th>
<th>Dose (mg/kg)</th>
<th>Log dose</th>
<th>Number dead</th>
<th>% dead</th>
<th>Probits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.93</td>
<td>8,000</td>
<td>3.90</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>20.83</td>
<td>12,000</td>
<td>4.08</td>
<td>1/6</td>
<td>16.67</td>
<td>4.01</td>
</tr>
<tr>
<td>3</td>
<td>17.42</td>
<td>16,000</td>
<td>4.20</td>
<td>2/6</td>
<td>33.33</td>
<td>4.64</td>
</tr>
<tr>
<td>4</td>
<td>15.67</td>
<td>20,000</td>
<td>4.30</td>
<td>3/6</td>
<td>50</td>
<td>5.15</td>
</tr>
</tbody>
</table>

Figure 3 below shows a plot of probits against log dose for dracaena fragrans plant extract. The slope of the graph which was drown from the figures obtained from table 4 is Y=12.81x- 49.34. Since probit 5 represents the 50% death, let’s assume that y= 5.

By using the above formula, we can derive the value of x.

Therefore;

Y=12.81x- 49.34

Y= 5

5= 12.81x- 49.34

X= 4.5283

Since x represents the log dose value at 50% death on the x axis, the antilog of x will represent the LD50. Therefore;

Antilog of x = LD50

Antilog X = 17458

LD$_{50}$ = 17458mg\kg
The acute toxicity (LD$_{50}$) of Draceana laxissima and Draceana Fragrans is 16714 mg/kg and 17458mg/kg respectively.

The general toxic signs and symptoms which were observed in mice 24 hours after the animals were dosed are, hypo activity, abdominal twitches, hyper urination, drowsiness, shivering, convulsions, hard breathing, vocalization, grinding of teeth and diarrhea.

Although the following toxicity signs were observed in the higher doses these signs might have been associated with CNS, ANS and behavioral profile.
 CHAPTER FIVE: DISCUSSION

5.1 Discussion

This study reports that the roots of Draceana laxissima and Draceana fragrans contain alkaloids, tannins and saponins. These phytochemicals are responsible for the extract colours observed. Flavonoids and glycosides were absent in all extracts. Other studies have reported presence of flavonoids in D. draco, D. manii, and D. afrormontana and all these had antibacterial activity (Gonzalez et al., 1983; Carmarda et al., 1983). Flavonoids are believed to have antibacterial activity and their absence in these root extracts may imply that the extract could have limited antibacterial activity.

Though some alkaloids are pharmacologically associated with inhibition of nucleic acid proteins and membrane phospholipids biosynthesis (Shelton 1991), some alkaloids are unstable when removed from their natural environment. This could have affected the activity of these plant extracts. In the study the methanol extract of D. fragrans had the highest concentration of the phytochemicals.

The method used to detect antibiotic susceptibility was agar well diffusion methods. In the present study, the antibacterial susceptibility tests were performed to Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa isolates associated with urinary tract infections. The results showed that Gentamycin which was used as a control showed great antibacterial activity against all pathogens.

In the present study, the methanolic extracts of Dracaena laxissima and Dracaena fragrans showed no activity against E. coli. These results agree with those reported by Voravuthikunchai et al., 2004 who found that E. coli showed high rates of resistance to Dracaena loureiri extract which is close relative of D. laxissima and D. fragrans. The obtained data also showed that, Staphylococcus aureus and Pseudomonas aeruginosa showed high rates of resistance to the water extracts of D. laxissima and D. fragrans. This result is in agreement with the study carried out by Prashanth et al., 2006 which showed that the Dracaena cinnabari a close relative of D. laxissima and D. fragrans was resistant to both Staphylococcus aureus and Pseudomonas aeruginosa.

However, these organisms are known to cause urinary tract infections. This further suggests that maybe these plants are being used to treat other symptoms e.g. urethral inflammation associated with UTI. Saponins are known to have quite a number of biological activities among which are analgesic (Lei et al., 1984; Oshima et al., 1984) and antipyretic activities (Gan and Chen, 1982). This agrees with the
traditional healers’ claims that the extracts relieve painful urination. Also agrees with Zhou et al. (2001) and Liu et al. (2006) who isolated several compounds showing analgesic and anti-inflammatory activity from *Dracaena cochininchinensis* which is also a close relative of *D. laxissima* and *D. fragrans*.

Acute toxicity test is the most common type of toxicity tests. This establishes the concentration required to kill a proportion of test organisms within a relatively short period of time, usually seven days or less (24 hours). The most common test of acute toxicity is the LD$_{50}$ test (Medium Lethal dose). The LD$_{50}$ is also known as the "lethal dose test" or the fifty percent test. This test examines the toxicity of a chemical by assessing the dosage needed to kill half of the animal test subjects (Richards, 2008).

The acute toxicity studies were conducted as per the OECD guidelines 2001a, where the limit test dose of 2000 mg/kg was used. No test substance-related mortality was observed at 2000 mg/kg. So, the two plants extracts were considered to be practically non-toxic (Gosh, 1984). These results were also in agreement with the classification of toxicity based on LD 50 dose ranges which stipulates that a drug is considered to be completely non toxic if the LD50 is above 1500mg/kg (Loomis and Hayes 1996, Pascoe, 1983).

These results are in agreement with the fact that traditional healers have been using the plant as a placebo without encountering any major adverse effects. Much as the extracts where rendered non toxic, many saponins exhibit toxic effects at high doses over long periods of time causing problems such as excessive salivation, vomiting, diarrhea, loss of appetite and manifestations of paralysis (Spinks and Fenwick, 1990). This is also in agreement with the result of this study where some of these toxic effects manifested in the animals 24 hours after the animals were dosed.
CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

In conclusion, the results of this study demonstrated that water extracts of the roots of *Draceana laxissima* and *Draceana fragrans* have no antibacterial effect against *E. coli*, *S. aureus* and *P. aeruginosa*. It is also true that *D. fragrans* and *D. laxissima* water and methanolic extracts contain tannins, alkaloids, and saponins, but lack flavonoids. Results of the acute toxicity study demonstrate that *D. laxissima* and *D. fragrans* water extract are safe when used within 24 hours. So herbal medicines should be validated scientifically before we encourage the public to take it as an alternative form of medicine.

6.2. Recommendations

Further studies should be conducted to find out whether *D. laxissima* and *D. fragrans* have got antipyretic, anti-inflammatory, or analgesic effects. Since traditional healers normally use a cocktail of plant extracts, a study needs to be carried out to find out whether the other plant extracts which are being used by the traditional healers could be having any synergistic effect. Sub-acute and chronic toxicity studies should also be carried out.
REFERENCE


Dose, Short-Course Ciprofloxacin And Standard 7 Day Therapy With Co-Trimoxazole Or Nitrofurantoin In The Treatment Of Uncomplicated Urinary Tract Infection. *J Antimicrob Chemother* ; 43(suppl a): 67–75.


Appendix I: How serial dilutions were made

1000mg (1000000µg) of each extract of water and methanol was dissolved in a few drops of DMSO and then topped up to 10ml to form a100mg/ml or 100000 ug/ml solution. Dilution of the extract was made per ml starting with 100mg, 50mg, 25mg, 12.5mg, 6.5mg, 3.125mg, 1.562mg, 0.78125mg and 0 (DMSO and water mixture). Those of gentamycin will also be made per ml starting with 10ug, 5ug, 2.5ug, 1.25ug, 0.65ug, 0.3125, 0.15625ug and 0 (DMSO and water mixture).

Appendix II: Properties of some common solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Formula</th>
<th>Bp °C</th>
<th>Density (g/mL)</th>
<th>Hazzard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diethyl ether</td>
<td>C₄H₁₀O</td>
<td>35</td>
<td>0.7</td>
<td>highly flammable</td>
</tr>
<tr>
<td>Methanol</td>
<td>CH₃OH</td>
<td>65</td>
<td>0.8</td>
<td>flammable, toxic</td>
</tr>
<tr>
<td>Water</td>
<td>H₂O</td>
<td>100</td>
<td>1.0</td>
<td>–</td>
</tr>
</tbody>
</table>
**Appendix 111: Classification of toxicity based on LD50 dose ranges**

The LD50 has been used by some authors to devise categories of the chemicals necessary to produce harm. Such categorization along with respective lethal doses is given in the following table.

**Table 4**

<table>
<thead>
<tr>
<th>Category</th>
<th>LD50 mg/kg</th>
<th>LD50mg/kg</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extremely toxic</td>
<td>1 or less</td>
<td>Less than 5</td>
<td>Super toxic</td>
</tr>
<tr>
<td>Highly toxic</td>
<td>1 to 50</td>
<td>5 to 50</td>
<td>Extremely toxic</td>
</tr>
<tr>
<td>Moderately toxic</td>
<td>50 to 500</td>
<td>50 to 500</td>
<td>Very toxic</td>
</tr>
<tr>
<td>Slightly toxic</td>
<td>500 to 5000</td>
<td>500 to 5000</td>
<td>Moderately toxic</td>
</tr>
<tr>
<td>Practically non toxic</td>
<td>5000 to 15000</td>
<td>5000 to 15000</td>
<td>Slightly toxic</td>
</tr>
<tr>
<td>Relatively harmless</td>
<td>More than 15000</td>
<td>More than 15000</td>
<td>Practically non toxic</td>
</tr>
</tbody>
</table>

(Loomis and Hayes 1996) (Pascoe, 1983)
Appendix IV: Screening procedure

Test for alkaloids: Five ml of the extract will be added to 2 ml of HCl. To this acidic medium, 1 ml of Dragendorff’s reagent will be added. An orange or red precipitate produced will indicate the presence of alkaloids.

Test for amino acids: One ml of the extract will be treated with few drops of Ninhydrin reagent. Appearance of purple colour will show the presence of amino acids.

Test for anthraquinones: Five ml of the extract solution will be hydrolyzed with diluted Conc. H₂SO₄ extracted with benzene. 1 ml of dilute ammonia will be added to it. If a rose pink coloration appears it will indicate a positive response for anthraquinones.

Test for flavonoids: To one ml of the extract, a few drops of dilute sodium hydroxide will be added. If an intense yellow colour is produced in the plant extract, which becomes colourless on addition of a few drops of a dilute acid, it will indicate the presence of flavonoids.

Test for glycosides: The extract will be hydrolyzed with HCl for few hours on a water bath. To the hydrolysate, 1ml of pyridine will be added followed by a few drops of sodium nitroprusside solution. It will then be made alkaline with sodium hydroxide solution. Appearance of pink to red colour will indicate presence of glycosides.

Test for phytosterol: The extract will be refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture will be diluted and extracted with ether. The ether layer will be evaporated and the residue will be tested for the presence of phytosterol. The residue will be dissolved in few drops of diluted acetic acid; 3 ml of acetic anhydride will be added followed by few drops of Conc. H₂SO₄. Appearance of bluish green colour will show the presence of phytosterol.
Test for saponins: The extract will be diluted with 20 ml of distilled water and it will be agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam will show the presence of saponins.

Test for steroids: One ml of the extracts will be dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid will be added by sides of the test tube. If the upper layer turns red and sulphuric acid layer shows yellow with green fluorescence, this will indicate the presence of steroids.

Test for tannins: Five ml of the extract and a few drops of 1% lead acetate will be added. If a yellow precipitate is formed, it will indicate the presence of tannins.

Test for triterpenoids: Ten mg of the extract will be dissolved in 1 ml of chloroform; 1 ml of acetic anhydride will be added following the addition of 2 ml of Conc. H₂SO₄. Formation of reddish violet colour will indicate the presence of triterpenoids.

Dimethyl sulfoxide (DMSO) dissolves a variety of organic substances. This highly pure reaction solvent provides a simpler impurity profile. This can reduce costs associated with impurity identification and make product purification easier.