

**Evaluation of the Molluscicidal Effect of the Plants
Phytolacca octandra, *Phytolacca dodecandra* and
Balanites aegyptiaca as a Possible Control of
Schistosomiasis (Bilharzia) Transmitting Snails.**

By

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**A thesis submitted to the Graduate School in partial fulfilment for
the requirements of the Master of Science Degree in Chemistry of
Egerton University**

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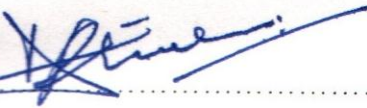
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This thesis has been presented for examination with my approval as the university supervisor.

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PROF. D. R. MUCHIRI, Ph. D.



DEDICATION

This thesis is dedicated to my daughter Lynn Ndimu Mwongela and Musyimi family, you all
inspire my life

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ABSTRACT

Schistosomiasis commonly known as Bilharzia is one of the most widespread parasitic infection only second to malaria. The disease affects more than 250 million people worldwide and requires aquatic snails as intermediate host. One of the methods used for the control of this disease involves the use of synthetic molluscicides. However, owing to their toxicity to the environment and high cost, their use is increasingly becoming unpopular. Plant-derived molluscicides are feasible alternatives that can be used to reduce the incidence of this disease. This study involved the evaluation of the molluscicidal activity of the plants *Phytolacca octandra*, *P. dodecandra* belonging to the Phytolaccaceae family and *Balanites aegyptiaca* belonging to the Balanitaceae family. The whole plant [the berries, leaves, stems and roots] were collected, air dried to a constant weight, macerated to a fine powder and extracted separately using methanol in Soxhlet apparatus. The extracts were screened for activity using Brine shrimp lethality test and thereafter tested for molluscicidal activity. There was no significant [$P > 0.05$] difference observed in the activity of the plant parts studied, that is, the berries, leaves, stem and roots and in the three plants namely *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca* extracts tested for activity using Brine shrimp lethality test. Similarly no significant [$P > 0.05$] difference in molluscicidal activity using genus *Bulinus* snails was recorded for the plant parts and in the three plants studied. Some pure crystalline compounds were isolated from the extracts and their purity confirmed using TLC and melting point ranges. Chemical characterization of these compounds revealed that they were triterpenoid glycosides [saponins]. From this study the Brine shrimp lethality test can be used for preliminary screening of plant extracts for molluscicidal activity since no significant [$P > 0.05$] difference was observed in the activity of the plant parts studied as well as in the three plants studied in both Brine shrimp lethality test as well as molluscicidal test. The three plants studied namely *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca* can be used for the control of Bilharzia transmitting snails and communities should be encouraged to use the plants within their localities. The findings of this research link chemical and pharmacological findings with traditional empirical knowledge by confirming that the plants *Phytolacca octandra*, *Phytolacca dodecandra* and *Balanites aegyptiaca* used as soap substitutes locally contain triterpenoid glycosides [saponins].

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LIST OF ABBREVIATIONS

AR: Analytical reagent

DMSO: Dimethyl sulfoxide

Galp: Galactopyranosyl

Glup: Glucopyranosyl

ppm: parts per million

R_f: Retardation factor

Rhap: Rhamnopyranosyl

TLC: Thin layer chromatography

W.H.O: World Health Organization

Xylp: Xylopyranosyl

CHAPTER ONE

1. INTRODUCTION

Most of the population in the third world is affected by tropical diseases and live in areas where the prevalence of these diseases is high. One approach to control tropical diseases is by elimination of the vectors responsible for their transmission [Maillard *et al.*, 1993]. Schistosomiasis commonly known, as Bilharzia is a wide spread disease which is endemic in many parts of tropical and sub tropical regions of the world and a threat to millions of people in tropical Africa [Amusan *et al.*, 1997]. Schistosomiasis is a human parasitic infection only second to malaria in socio-economic and public health importance in tropical and subtropical areas [Iarotski and Davis, 1981; Doumenge *et al.*, 1987; WHO, 1998]. The current estimate of cases of schistosomiasis infection in over 70 countries is 200 million people residing in rural and agricultural areas and between 500 – 600 million people are endangered mainly due to poverty, poor housing, substandard hygienic practices and the availability of few if any sanitary facilities [WHO, 1984; WHO, 1993; Rodriguez *et al.*, 1996].

Despite its public health importance and being categorised as one of the emerging and re-emerging diseases [Kojima, 1998], schistosomiasis has been ignored and given a low priority in health policies. This is mainly because of the low socio-economic status of developing countries, and the tendency to give economic development priority over health and welfare. Thus, water development projects particularly construction of dams, artificial lakes and irrigation canals, often associated with dynamic population resettlement have become a major factor in the spread and intensification of the disease [Kojima, 1998].

Flat worms of the genus *Schistosoma* whose three main species pathogenic to man are *Schistosoma haematobium*; *S. mansoni* and *S. japonicum* cause the disease. The schistosomes require aquatic snails as intermediate host for their transmission to man and affect the urino-genital, gastro-intestinal and the hepatic systems [Amusan *et al.*, 1997]. Important symptoms of the disease include blood in urine or faeces, stomachache, liver and muscles. There are two forms of schistosomiasis, the urinary form caused by *S. haematobium* and the intestinal form caused by *S. mansoni* and *S. japonicum*.

Community based epidemiological studies of schistosomiasis demonstrate that prevalence [expressed as the percentage of the population found infected at a given point of time] and intensity of infection [expressed in terms of egg output in excreta] are usually higher among young age groups [5-15 years] than in older age groups [Kojima, 1998].

Chemotherapy with orally administered anti-schistosomal drug remains the best method to cure people infected by the parasites [Maillard *et al.*, 1993]. However, this approach is rather expensive and most countries where the disease is prevalent cannot afford this mode of treatment. Besides re-infection occurs when treated people have repeated contact with infected water. The synthetic compounds are expensive and toxic to non-target organisms and thus pose a problem in schistosomiasis control when the deciding factor is cost and environmental safety. Under these conditions plant molluscicides are a possible alternative for control of schistosomiasis [Spengel, 1996].

1.1 Schistosomiasis in Kenya

Schistosomiasis is a major public concern in Kenya [Ouma and Waithaka, 1984] and a highly problematic disease, which affects the school age group [between 5 and 15 years] in Kenya [Cridland, 1955; Webbe and Msangi, 1958; Kinoti, 1971; Southgate and Knowles, 1977; Kristensen, 1987; Noda *et al.*, 1988; Sturock *et al.*, 1990; Brown, 1994]. It leads to a 10 % reduction in activities performed by these children [WHO, 1993]. It is estimated that around one million Kenyans are infected [Highton, 1974]. The rapid growth of population has lead to an increase in the presence of schistosomiasis and it was anticipated that 3.5 million Kenyans would be infected by the year 2000 [Jordan, 1985]. This according to Kojima [1998] being as a result of biological, economic and socio-cultural factors such as

- The distribution, biology and population dynamics of the intermediate host,
- The patterns and extent of environmental contamination with human excreta through insanitary conditions,
- Water development projects including extension of irrigation schemes and constructions of dams and most seriously
- People's knowledge of the disease itself and their attitudes towards, or practices for avoiding infection.

Two species of schistosomes that are prevalent in Kenya are *S. mansoni* and *S. haematobium*. The infection caused by *S. haematobium* occurs at the Kenyan coastal margin, Tana river, Athi river and coastal streams, parts of Kitui and Machakos in Eastern province, warmer parts of Central province especially lower regions of Kiambu and Muranga, wet river courses of Western province and parts of Nyanza province [Highton, 1974]. Fresh water snails of the genus *Bulinus*, sub genus *Physopsis*, group *africanus* and *globosus* are the principle intermediate host for *S. haematobium* [Doumenge et al., 1987; Brown, 1994]. Infection caused by *S. mansoni* on the other hand occurs along the shores of Lake Victoria, Machakos, Kitui, Taveta and Mwea Tebere irrigation schemes. Fresh water snails of the genus *Biomphalaria*, group *pfeifferi* are the principal intermediate host for *S. mansoni* in Kenya [Brown, 1994].

S. mansoni and *S. haematobium* infection rarely occur within the same foci. Focalisation of both *S. mansoni* and *S. haematobium* in Kenya occurs in areas such as Muranga and Kiambu in Central province [Highton, 1974; Ouma and Waithaka, 1984], in Kano plains, swampy habitats and dams in Western province [Kinoti, 1971; Highton, 1974; Masaba, 1980; Brown, 1994], in Taveta areas of Coast province and also in Kitui and Machakos areas of Eastern province [Highton, 1974].

1.2 Statement of the Problem

Schistosomiasis requires aquatic snails as intermediate host. One approach to control it is the elimination of the vectors responsible for the transmission. Plant derived molluscicides are a feasible alternative that can be used to reduce the prevalence of this disease.

1.3 Objectives

The main objective of this study is to evaluate the molluscicidal activity of the plants *Phytolacca octandra*, *Phytolacca dodecandra* and *Balanites aegyptiaca*.

Specific objectives of this study are as follows:

1. To extract and fractionate crude products from the berries, leaves, stems and roots of *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca* using liquid-liquid extraction.
2. To screen for the most active fraction using Brine Shrimp (*Artemia salina*) lethality test and evaluate the molluscicidal activity of the extracts.
3. Chemical characterization of the active fractions.

1.4 Justification

Schistosomiasis, which affects more than 200 million people in over 70 countries, requires aquatic snails as intermediate host. The disease is of high public health importance in many tropical and sub-tropical areas, which is second only to that of malaria and has been categorised as one of the emerging and re-emerging disease in the world [Kojiama, 1998].

Water plays an important role in the transmission of schistosomiasis with the result that communities living near slow moving water or near the banks of dams or rivers being the most affected. Paradoxically, development programs involving dam construction or establishment of irrigation schemes provide ideal breeding sites for transmission and lead to an increase in the incidence of the disease [Maillard *et al.*, 1997]. Urbanization coupled to poor housing and environmental sanitation results in incidences of the disease in urban areas [Mugo, 1974].

At the present time Bayluscide [2, 5-dichloro-4-nitro salicylanilide or niclosamine] is the only synthetic molluscicide recommended by the World Health Organization [WHO] [Maillard *et al.*, 1993]. Synthetic molluscicides are expensive and thus pose a problem in schistosomiasis control programs when the deciding factor is cost. They in addition may result in problems of toxicity to non-target organisms and long-term effects in the environment. The possible development of resistance in schistosomiasis transmitting snails is another important factor. Since cost and toxicity impede the use of synthetic molluscicides there is demand for cheap, non-toxic and safer alternatives.

One possible alternative source of these molluscicides lies in traditional herbs/ natural products used by ethnic groups in Kenya. The main problem facing the use of traditional herbs/ natural products is the proof requirement that the active components contained in medicinal plants are useful, safe, and effective. This is required to assure the medical field and the public regarding the use of medicinal plants as alternatives to synthetic molluscicides. The proofs of pharmacology activity that are available at present are mostly based on empirical experience. The scientific proof then becomes the most important thing, in order to eliminate concern in using traditional herbs/ natural products as alternatives to synthetic molluscicides [Rukangira, 2000].

1.5 Hypothesis

- There is a difference in the molluscicidal activity of the extracts of the berries, leaves, stems and roots of the plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*.
- Brine shrimp lethality test can not be used for screening of plant extracts for molluscicidal activity.
- The active components in the plant extract responsible for molluscicidal activity are mainly saponins.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Human Schistosomes and Their Life Cycle

The human schistosomes or blood flukes are digenic trematodes in the super family schistosomatoidea. They are characterised by having no muscular pharynx and they produce non-operculated eggs [Sturrock, 1993]. The life cycle of all species follow a common pathway from the sexual generation of adult schistosomes within the vascular system of the definitive host to asexual generations in the intermediate host of fresh water snails and a return to the definitive host by skin penetration of infected larval stages [Kojiama, 1998].

The adult male worm normally clasps the female worm in a longitudinal 'schist' or gynaecophoric canal so that the pair assumes a nematoid, worm-like shape ideally suited to life in the minor vessels of the portal or vesicular blood systems of the definitive host [Sturrock, 1993]. The adult worm may live for 20-30 years with a mean life span of 3-8 years. Each worm pair produces 100-300 eggs or more per day [WHO, 1984]. The yellowish eggs are non-operculate and have a terminal spine. The miracidium develops inside the egg over a period of six days. Not all eggs laid are successfully released from the final host via urine or faeces; some are carried back in the venous blood and become lodged in the liver or other organs where they can survive for 11-12 days before forming granulomas [Sturrock, 1993]. The formation of egg granulomas in the various organs especially the liver, is the principle cause of the disease [Sturrock, 1993].

Mature embryonated eggs which are passed with urine, or faeces hatch to liberate the first larval stage the Miracidium when the eggs come into contact with fresh water [Wakelin, 1996] and the process is stimulated by temperature and light [Sturrock, 1993]. The miracidium becomes active and emerges from the eggshell through slits caused by a combination of osmotic effects and movements of the larva. Free living miracidium measure approximately 160-60 μ m. The miracidium has a limited power of survival [Wakelin, 1996], rapidly swims by means of cilia that cover the body surface [Kojiama, 1998], and responds positively towards light but shows negative geotropic response.

There is evidence that the ecology of the miracidium is related to the ecology of their snail intermediate host [Webbe, 1962; Prentice *et al.*, 1970]. Miracidium remain infective to their intermediate host for 8-12 hours, swimming randomly in long sweeping lines [Sturrock,

[1993]. The continuity of the parasites life cycle depends on the miracidium finding an appropriate intermediate host to which they are probably 'naturally' directed [Chernin, 1974]. The miracidium penetrates the exposed body surface of the snail by secretions from special gland cells [Wajdi, 1963; 1966; 1972]. The ciliated surface of the miracidium is lost during penetration and within few days, the larva develops into a primary sporocyst [Sturrock, 1993; Wakelin, 1996]. Only a portion of the penetrating miracidium develops into mature primary sporocyst [Pan, 1963; 1965]. The primary sporocyst produces secondary sporocyst by asexual reproduction and these then migrate to the digestive gland of the snail and start to produce cercariae by asexual reproduction. Thus a single miracidium results in the development of thousands of cercariae all of the same sex, produced as a result of asexual multiplication within primary sporocyst and by secondary sporocyst [Sturrock, 1993; Wakelin, 1996; Kojima, 1998]. Depending upon environmental temperature, it takes from three to five weeks between miracidial penetration and the emergence of mature cercariae [Anderson and May, 1979]. Mature cercariae escape from the secondary sporocyst and emerge from the snail when stimulated by light and usually at temperatures of 10-30 °C. The peak cercarial shedding occurs between 10.00 and 14.00 hours in natural waters of Tanzania [Webbe, 1966]. Slight variations may occur from one area to another. In Kenya, for example, Prentice and Ouma [1984] have shown that peak cercarial shedding occurred between 13.30 hours and 15.30 hours.

Cercaria is the stage infective to the final host. They are free living, non-feeding and can survive in water for up to 48 hours. Human or animals become infected when they come into contact with cercariae infested water [Sturrock, 1993]. Cercariae penetrate the skin of a definitive host with assistance of lytic substances from the penetrating glands. The cercaria loses its tail during penetration and transforms into a schistosomulum just under the skin where it stays for about two days. It then migrates via the lungs, the liver and the portal veins, where the adult worms pair, before finally entering the vesicle plexus. The cycle is repeated when egg laying by the female begins [Sturrock, 1993].

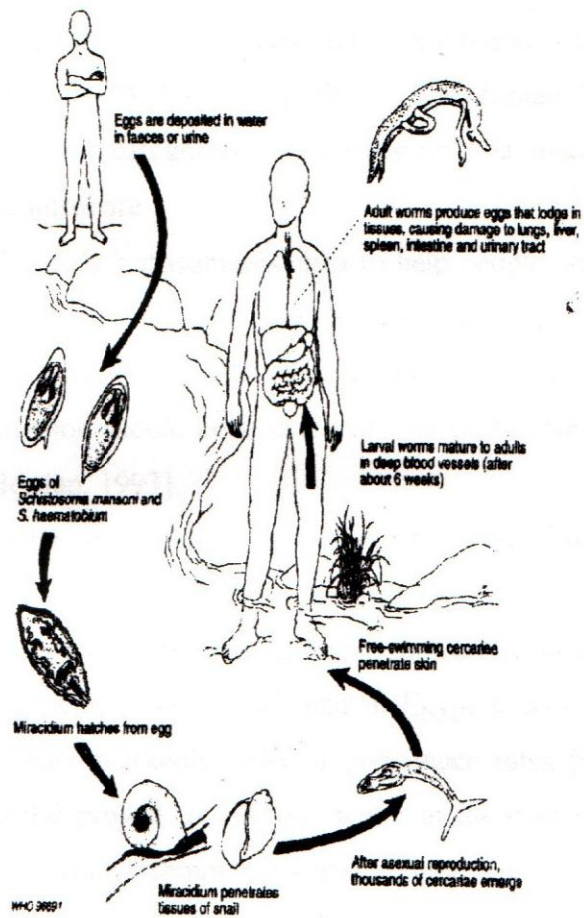


Figure 1: Life Cycle Schistosomiasis [Litwak, 2002]

2.2 Schistosomiasis Control

The primary purpose of schistosomiasis control is to reduce both mortality and transmission of the disease to a level at which it is no longer of public health importance. Although it is not possible currently to completely eradicate the disease, it is possible to control the transmission and morbidity of the disease through integrated control programs run by primary health care institutions [WHO, 1985]. Webbe and Jordan [1993] classified the control programs/ measures into two categories of 'non-specific' and 'disease specific'.

The non-specific methods of control are

- Health education in relation to schistosomiasis aims to help people understand that man is the vector of the infection through indiscriminate urination and defecation and that infection is from water contact. The fact that changing man's behaviour can have profound effect on transmission should be stressed in relation to teaching the life cycle of the parasite [Webbe and Jordan, 1993].
- Safe water supply from wells or boreholes may reduce the contact to infected water and subsequently reduce the risk of infection with the parasite [Greany, 1952; Esrey, 1991]. Lower schistosomiasis prevalence rates among communities having access to well water in Gezira have been reported [Greany, 1952], and in Egypt it was noted that even the partial use of protected water markedly reduced prevalence rates [Farooq and Mallah, 1966]. The first experimental project to evaluate water in the control of schistosomiasis were made in South Africa, where communal water supply was supplemented by simple pools for children and wire fences prevented easy access to infected water [Pitchford, 1970]. Lower prevalence rates were reported.
- Sanitation by having simple toilets reduces the spread of parasitic eggs to waters in rivers and dams, and secures access to safe water bodies for irrigation, bathing and laundry. Lower prevalence rates have been reported in Brazil where a project for the constructions of latrines was done [Barbosa *et al.*, 1971].

The disease specific methods of control include

- Medical treatment with tablets [chemotherapy] is given to infected people to reduce and prevent morbidity of infected individuals [WHO, 1993]. This is an effective and secure treatment, but costly, especially for poor peasant farmers in rural areas in developing

countries. Re-infection occurs more frequently, when treated people have repeated contact with infected water.

- Control of snail intermediate host remains an important measure in an integrated schistosomiasis control approach [Madsen and Christensen, 1992]. A reliable method of achieving significant reductions in snail populations is by use of molluscicides [McCullough, 1986] synthetic or plant derived. This reduces the risk of infection, even with frequent water contact. However synthetic molluscicides may cause environmental disorder, and when people and health authorities have to purchase synthetic molluscicides, it may be beyond the economic means of the poor people. Plant molluscicides present an attractive, cheap alternative to synthetic molluscicides. They can be grown and applied locally.

2.3 Plant Molluscicides

Some saponin containing plants are particularly toxic to schistosome carrying snails and are referred to as plant molluscicides. These are important as vector control agents for schistosomiasis. The recognition of the importance of plant molluscicides has led to evaluation of over 1000 plant species for molluscicidal activity [Maillard *et al.*, 1993]. Some of these plants have been found to contain high concentration of molluscicidal saponins. These molluscicidal saponins are the most powerful natural molluscicides with potencies similar to synthetic molluscicides. Although many plants have been investigated for molluscicidal action only a few are suitable as plant molluscicides for vector control of schistosomiasis in the field. This is because there are several prerequisites for a viable plant molluscicide. Hostettmann and Marston, [1995] gave these prerequisites as

- The molluscicidal activity should be high. The crude extract from which the saponins are obtained should have an activity at concentrations lower than 100mgL^{-1} . The activity of the strongest synthetic molluscicides lies around 1mgL^{-1} for example the LC_{100} for Bayluscide against the schistosomiasis-transmitting snail; *Biomphalaria glabrata* is 0.3mgL^{-1} after an exposure time of 24 hours. Similar values are obtained for *Bulinus* snails. Consequently, to be effective competitors of synthetic molluscicides, plant molluscicides must have LC_{100} values of this order of magnitude. It is advantageous if the molluscicide also kills snail eggs.

- The plant in question should grow abundantly in the natural endemic area. Either the plant should be of high natural abundance or alternatively easily cultivated. In addition regenerating plant parts [fruits, leaves or flowers] should be used and if possible not the roots, since this leads to destruction of the plant.
- Extraction of the active constituents by water is an advantage. The cost of organic solvents and accompanying extraction apparatus could be prohibitive for schistosomiasis control programs in third world countries.
- Application procedures should be simple and safe to the operator. In addition formulations and storage must be straightforward.
- The plant extract or molluscicide should possess low toxicity to non-target organisms [including man]. The discovery of compounds more specifically toxic to snails would be a great advantage. Furthermore, isolation of the active compounds from those molluscicidal plants which are potentially applicable to field trials is important for toxicological and environmental studies.
- Cost should be low.

2.4 The Plants

Previous studies have shown that the plants of *Phytolaccaceae* and *Balanitaceae* family used in traditional medicine locally contain compounds, which may be responsible for molluscicidal action [Parkhurst *et al.*, 1973; Kamel, 1998; Treyvaud *et al* 2000]. This is the basis for the selection of the plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca* for this study.

2.4.1 *Phytolacca dodecandra*

Phytolacca dodecandra plant belongs to the *Phytolaccaceae* family. In Kenya the plants are found widely distributed in areas of high rainfall and altitudes of 1500-2450 m above sea level [Agnew and Agnew, 1994]. The plant is found in bush land, cleared forest and as a perennial climber in riverine forest or sprawling thicket forming shrub [Plate 1]. The plant is woody at the base, arising from a large fleshy tuber and has green some-what succulent stem, alternate, simple leaves and long cylindrical spikes of small white or greenish flowers. The older branches are marked with prominent leaf scars. The leaves are pointed at

the tip and rounded at the base, with smooth translucent margin and stalks up to 2 inches long. The inflorescence arises in the leaf axis with numerous short stalked flowers. The flowers have 4-5 sepals but no petals. Male flowers have about 15 stamens. Female flowers usually have 5 carpel's developing into small orange or red fruits [Ivens, 1971].

Extracts from the roots of this plant have been used as a purgative, taenifuge and cathartic. In some cases the extract from the roots is used to induce vomiting, for treatment of enlarged glands and cure for venereal diseases particularly syphilis [Kokwaro, 1993]. The berries of this plant have a high antimolluscicidal effect [Thiilborg *et al.*, 1993]. Domestically the berries have been used as soap substitute [Eberlee, 1993; Yohannes *et al.*, 1999].

2.4.2 *Phytolacca octandra*

The plant *Phytolacca octandra* belongs to the Phytolaccaceae family. In Kenya the plants are found widely distributed in areas of high rainfall and altitudes of 1500-2450 m above sea level and are found in waste places and disturbed moist forest [Agnew and Agnew, 1994]. The plant is also a perennial climber or sprawling thicket forming shrub, which is woody at the base, arises from a large fleshy tuber and has a pinkish erect and prostrate stem and the entire plant is hairless [Plate 2]. It has alternate existipulate leaves, which are ovate in shape. The flowers occur in condensed racemose inflorescence. Calyx is composed of 5 free sepals, 5 connate cream white petals, 8 stamens and one pistil. The ovary is superior with axile placentation. The fruits are green berries, which turn purple when ripe [Agnew and Agnew, 1994].

Extracts from the leaves of this plant have been used to fortify the stomach, act as appetizer, and alleviate headaches, backaches and stomachache. The extracts from the leaves are also recommended for people debilitated by alcoholism. Extracts from the roots have been used to induce vomiting whereas extracts from the berries are used as a laxative. The berries of this plant have domestically been used as soap substitute.



PLATE 1 *Phytolacca dodecandra* plant

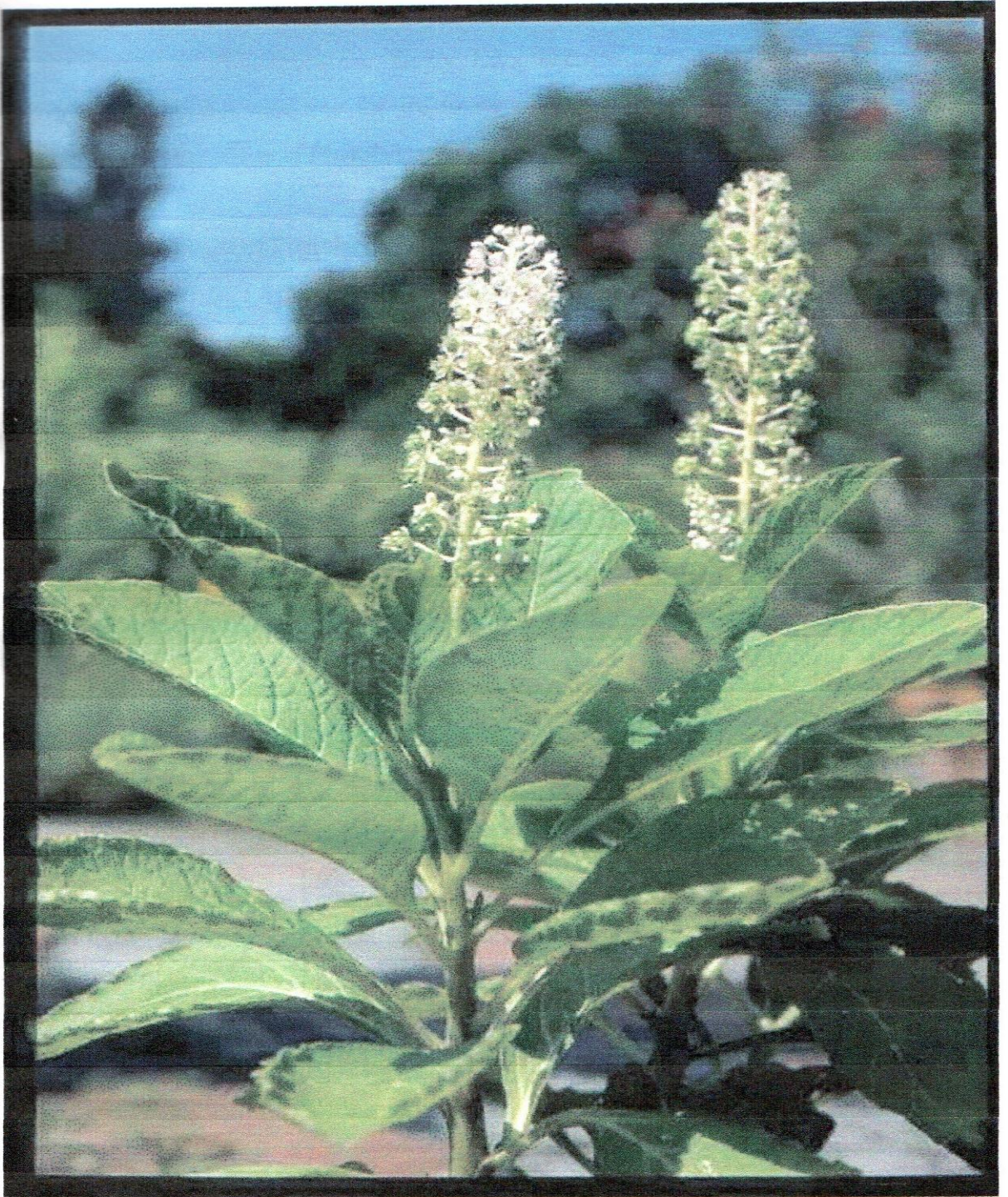


PLATE 2 *Phytolacca octandra* plant

2.4.3 Other Medicinal Plants of Phytolaccaceae Family

Plants of Phytolaccaceae family have been widely used in Africa, oriental and American traditional medicine for treatment of various ailments [Yasuharu *et al.*, 1978]. Examples of other plants of Phytolaccaceae family used are tabulated in table 1 below.

Table 1 Other Plants of Phytolaccaceae Family with Medicinal Importance

Plant	Uses	Parts used
<i>P. decandra</i>	Mastitis, varicose ulcers, skin infection, chronic rheumatism of fibrous tissue, obesity, tonsillitis, diphtheria, sore throat, chronic follicular pharyngitis.	Roots, berries
<i>P. americana</i>	Metabolic balancer, anodyne [pain killer], anti fungal, antiviral, anti-inflammatory, antirheumatic, antitumor, cathartic [bowel evacuator], emetic [vomiting inducer], immune stimulant, laxative, lymphatic decongestant, skin infection, disease of mouth and throat.	Leaves, berries
<i>P. bogotensis</i>	Edema, rheumatism, induces vomiting, anti-inflammatory, lymphatic decongestant, antifungal.	Leaves, berries
<i>P. rivinoids</i>	Rheumatism, edema, induce vomiting, laxative, anti-inflammatory, skin infection.	Roots
<i>P. acinosa</i>	Induce vomiting, metabolic balancer, painkiller, skin infections, and edema.	Leaves, roots

2.4.4 *Balanites aegyptiaca*

The plant *Balanites aegyptiaca* belongs to the plant family Balanitaceae. The plant has a wide ecological distribution and is found in most arid, semi arid to sub humid tropical savannah and hot dry areas, along watercourses and in woodlands. The plant is multibranched, spiny shrub or tree up to 10 m tall with spherical crown in one or several distinct masses [Plate 3]. The trunk is short and often branching from near the base. The bark is dark brown to grey, deeply fissured. Branches are armed with stout yellow or green thorns up to 8 cm long. Leaves are with two separate leaflets, which are obovate, asymmetric, 2.5 – 6 cm long, bright green, leathery, with fine hairs when young. Flowers occur in fascicles in the leaf axils and are fragrant and yellowish green in colour. Young fruits are green and tormentose [Plate 4], turning yellow and glabrous when mature [Schmidt and Joker, 2000].

Extracts from the roots of *B. aegyptiaca* are used for treatment of abdominal pains, as a purgative and as an antihelmintic. Gum from the wood is mixed with maize meal porridge to treat chest complains [Kokwaro, 1993]. Fruit extracts are known to kill both fresh water snails that carry bilharzia and the water fleas that carry guinea worm disease [Noad and Birnie, 1989].



PLATE 3 *Balanites aegyptiaca* plant



PLATE 4 *Balanites aegyptiaca* berries

2.4.5 Other Medicinal Plants of Balanitaceae Family

Other plants of Balanitaceae family with medicinal importance are tabulated in table 2 below.

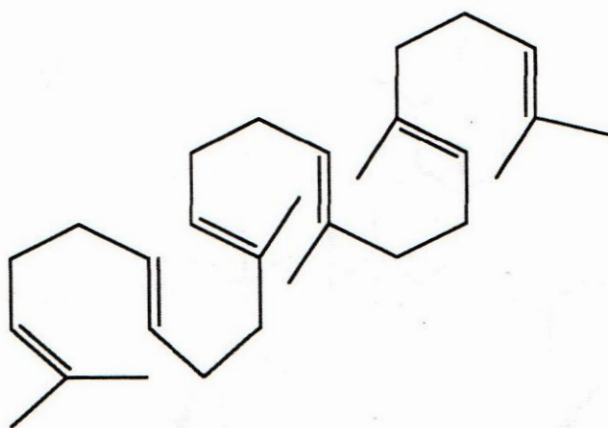
Table 2 Other Plants of Balanitaceae Family with Medicinal Importance

Plant	Uses	Parts used
<i>Balanites wilsonia</i>	Abdominal pains, purgative, antihelmintic, chest complains.	Roots, gum
<i>B. pedicellaris</i>	Abdominal pains, purgative, antihelmintic, chest complains.	Roots, gum
<i>B. orbicularis</i>	Abdominal pains, purgative, antihelmintic, chest complains.	Roots, gum

Although the plants *Phytolacca dodecandra*, *P. octandra* and *Balanites aegyptiaca* are found widely distributed in Kenya, available literature shows that no work has been reported for these plants in Kenya.

2.5 Biologically Active Triterpenoids from Plants

Triterpenoids are compounds with a carbon skeleton based on six isoprene units and which are derived biosynthetically from the acyclic C₃₀ hydrocarbon squalene [Structure 1]. They are relatively complex cyclic structures, most being alcohols, aldehydes, or carboxylic acids. They are colourless, crystalline, often high melting, optically active substances [Harborne, 1984]. Triterpenoids can be divided into at least four groups of compounds: true triterpenes, steroids, saponins and cardiac glycosides with the latter two being triterpenes which occur mainly as glycosides.

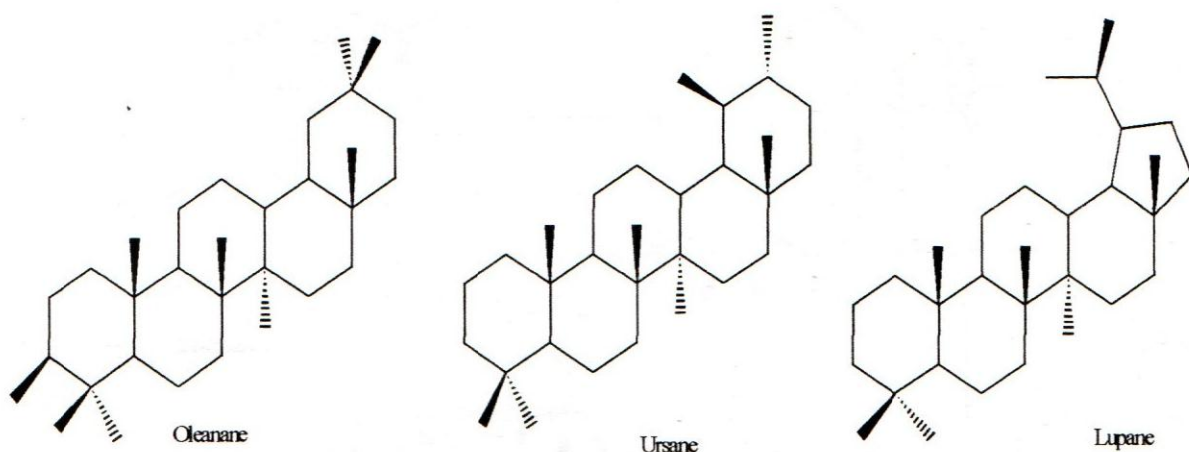


Structure 1: Squalene

Saponins are glycosides of triterpenes [Tschesche and Wulff, 1973]. They are an important group of plant secondary metabolites that are widespread throughout the plant kingdom [Haralampidis *et al.*, 2002]. The name saponin is derived from *sapo* a Latin word for soap since these molecules have surfactant properties and give stable, soap like foams in aqueous solution. Saponins have been variously attributed with a range of properties some of which include both beneficial and detrimental effects on human health [Haralampidis *et al.*, 2002]. Examples of some of the beneficial effects include accessible source of sapogenins, which can be converted to animal sterols of therapeutic importance such as cortisone, contraceptive estrogens and many others [Harborne, 1984]. Other properties of saponins include piscicidal, insecticidal and molluscicidal activity, allelopathic action, antinutritional effect, sweetness and bitterness and as phytoprotectants that defend the plant against attack by microbes and herbivores [Haralampidis *et al.*, 2002]. The glycosidic pattern of the saponins are often complex; many have as many as five sugar units attached and glucuronic acid is a common component.

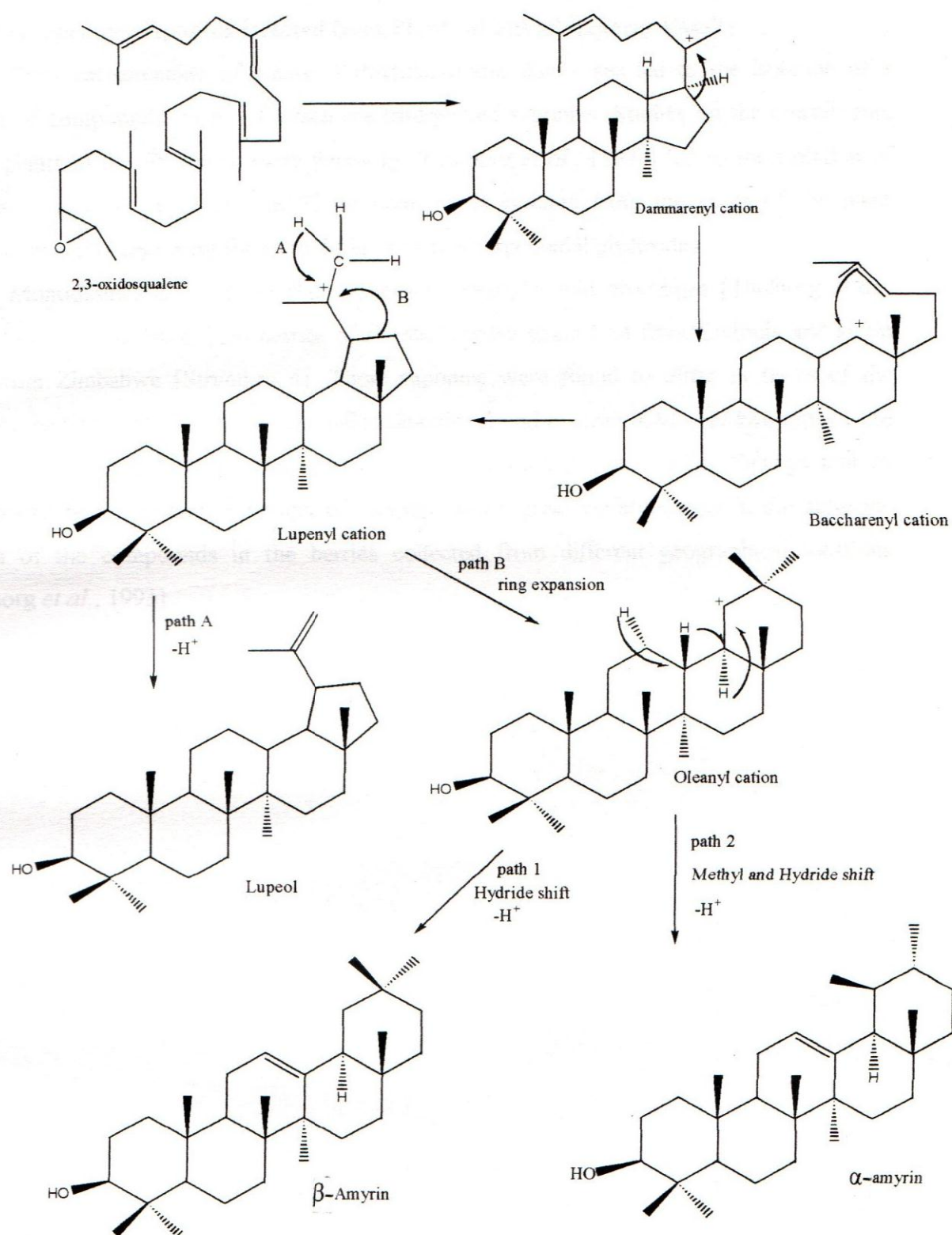
2.5.1 Biosynthesis of Triterpenoid Saponins by Plants

The first committed step in the synthesis of triterpenoid saponins involves the cyclisation of 2-3- oxidosqualene to give one of a number of different potential products. Most triterpenoid saponins are derived from oleanane or dammarane skeleton although lupanes are also common [Hostettmann and Marston, 1995].



Structure 2: Major precursors for triterpenoid saponins

Triterpenoid synthesis involves “chair-chair-chair” conformation of the substrate to give dammarenyl cation. This cation may then be converted to dammarene like triterpenoids or may undergo further rearrangements leading to formation of pentacyclic triterpenoids derived from lupeol, β -amyrin and α -amyrin. From the cyclisation products of 2–3-oxidosqualene a series of additional modifications take place. These may include a variety of oxidation, substitution events and the addition of sugars at different positions of the skeleton [Hostettmann and Marston, 1995]. The presence of a sugar chain attached to the aglycone at carbon-3 hydroxyl position is a common feature shared by all saponins. The sugar chains differ substantially between saponins but are often branched, and may consist of up to five sugar molecules [usually glucose, arabinose, glucuronic acid, xylose or rhamnose] [Hostettmann and Marston, 1995]. The oligosaccharide chain are synthesised by sequential addition of single sugar molecules to the aglycone [Haralampidis, 2002].

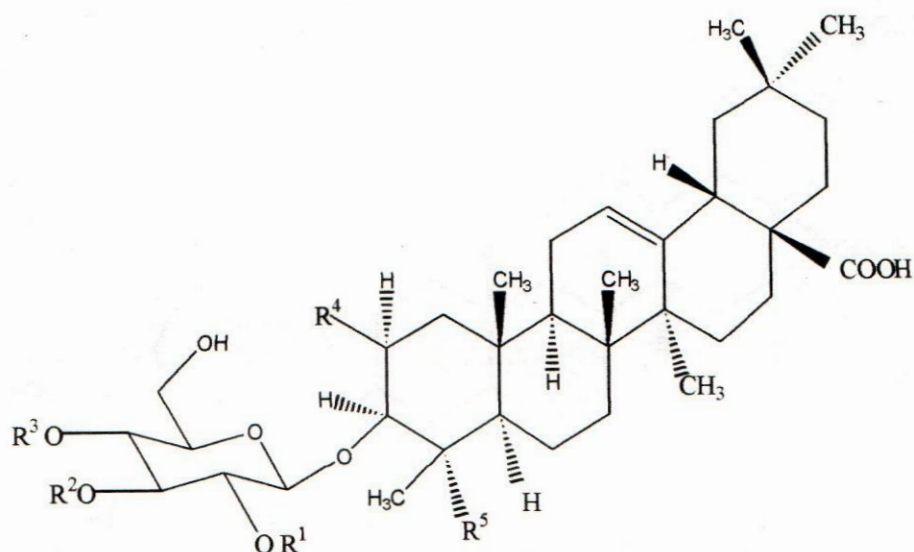


Structure 3 Biosynthesis of triterpenoids [Haralampidis *et al.*, 2002]

2.5.2 Triterpenoid Saponins Isolated from Plants of Phytolaccaceae Family

Chemical screening of plants of Phytolaccaceae family has led to the isolation of a number of compounds, many of which are triterpenoid saponins. Studies on the constituents of the plants in the *Phytolaccaceae* family by Yasuharu *et al.*, [1978] led to the isolation of Phytolacca saponin B, E and G. These compounds isolated from the roots of the plant *Phytolacca americana* were found to differ in their triterpenoidal glycosides.

Monodesmosidic saponins that are mainly oleanolic acid glycosides [Thiilborg *et al.*, 1993] have been isolated from berries of *P. dodecandra* strain E44 from Ethiopia and strain MS1 from Zimbabwe [Structure 4]. These saponins were found to differ in terms of the aglycone pattern such that out of the major saponins found in strain E44 only two types were also found in strain MS1 [Thiilborg *et al.*, 1994]. This reaffirmed earlier findings that *P. dodecandra* berries contain a variety of saponins and a great variation exist in the aglycone pattern of the compounds in the berries collected from different geographical locations [Thiilborg *et al.*, 1993].



Ethiopian plant E44

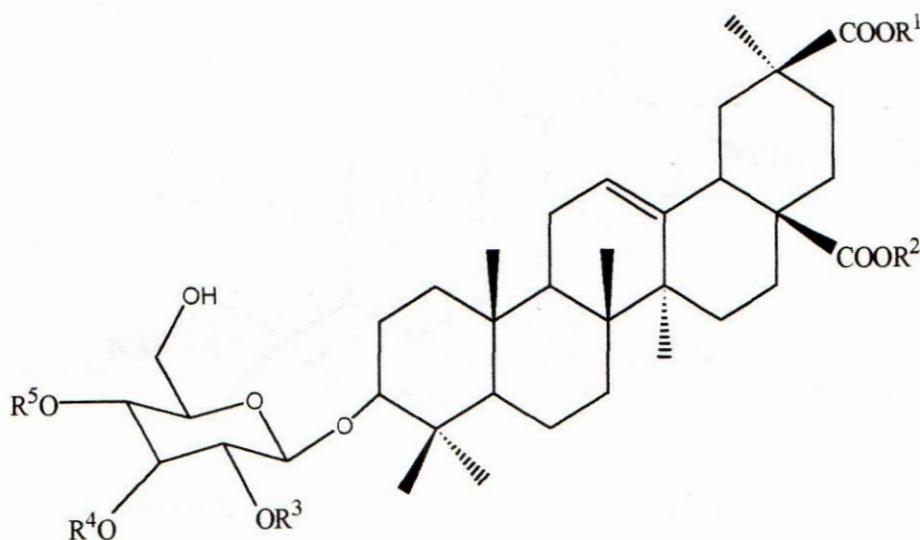
1	$R^1 = \alpha\text{-L-Rhap- (1}\rightarrow\text{2)-}\beta\text{-D-Glup}$	$R^2 = R^4 = \text{H}$	$R^3 = \beta\text{-D-Glup}$
2	$R^1 = R^3 = \beta\text{-D-Glup}$	$R^2 = R^4 = \text{H}$	
3	$R^1 = R^4 = \text{H}$	$R^2 = \beta\text{-D-Gal}$	$R^3 = \beta\text{-D-Glup}$
4	$R^1 = R^3 = R^4 = \text{H}$	$R^2 = \alpha\text{-L-Rhap- (1}\rightarrow\text{2)-}\beta\text{-D-Galp}$	
5	$R^1 = R^3 = R^4 = \text{H}$	$R^2 = \beta\text{-D-Xylp- (1}\rightarrow\text{6)-}\beta\text{-D-Glup}$	

Zimbabwean plant MS1

1	$R^1 = \text{H}$	$R^2 = \beta\text{-D-Gal}$	$R^3 = \beta\text{-D-Glup}$	$R^4 = \text{OH}$	$R^5 = \text{CH}_2\text{OH}$
2	$R^1 = R^3 = \beta\text{-D-Glup}$	$R^2 = R^4 = \text{H}$			$R^5 = \text{CH}_2\text{OH}$
3	$R^1 = R^3 = \beta\text{-D-Glup}$	$R^2 = \text{H}$		$R^4 = \text{OH}$	$R^5 = \text{CH}_3$
4	$R^1 = R^3 = \beta\text{-D-Glup}$	$R^2 = R^4 = \text{H}$			$R^5 = \text{CH}_3$
5	$R^1 = R^3 = \text{H}$	$R^2 = \alpha\text{-L-Rhap- (1}\rightarrow\text{2)-}\beta\text{-D-Galp}$		$R^4 = \text{OH}$	$R^5 = \text{CH}_3$
6	$R^1 = R^3 = \text{H}$	$R^2 = \beta\text{-D-Galp}$		$R^4 = \text{OH}$	$R^5 = \text{CH}_3$
7	$R^1 = R^3 = R^4 = \text{H}$	$R^2 = \beta\text{-D-Xylp- (1}\rightarrow\text{6)-}\beta\text{-D-Glup}$			$R^5 = \text{CH}_3$

Structure 4 Major monodesmosidic saponins from *P. dodecandra* berries of Ethiopian strain E44 and Zimbabwean strain MS1 strain cultivars [Thiilborg *et al.*, 1993; 1994; Ndamba *et al.*, 1993]. MS1/4 and MS1/7 are identical to E44/2 and E44/3 respectively

Seven triterpenoid glycosides of serajic acid [structure 5] have been isolated from *P. rivinoids* and *P. bogotensis* plants [Nielsen *et al.*, 1995].



From *P. bogotensis*

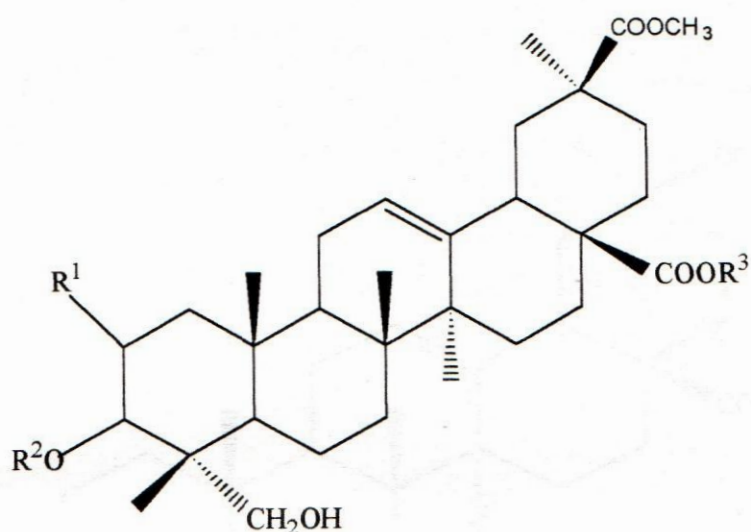
1 $R^1 = CH_3$	$R^2 = \beta\text{-D-Glup}$	$R^3 = R^5 = H$	$R^4 = \beta\text{-D-Galp}$	
2 $R^1 = CH_3$	$R^2 = R^4 = \beta\text{-D-Glup}$	$R^3 = H$		$R^5 = \beta\text{-D-Galp}$
3 $R^1 = R^2 = R^3 = H$			$R^4 = \beta\text{-D-Glup}$	$R^5 = \beta\text{-D-Galp}$
4 $R^1 = CH_3$	$R^2 = \beta\text{-D-Glup}$	$R^3 = \beta\text{-D-Glup-}(2\rightarrow1)\text{-}\alpha\text{-D-Rhap}$		$R^4 = R^5 = H$
5 $R^1 = R^2 = R^4 = R^5 = H$		$R^3 = \beta\text{-D-Glup-}(2\rightarrow1)\text{-}\alpha\text{-D-Rhap}$		$R^4 = R^5 = H$

From *P. rivinoids*

1 $R^1 = CH_3$	$R^2 = R^4 = \beta\text{-D-Glup}$	$R^3 = H$		$R^5 = \beta\text{-D-Galp}$
2 $R^1 = CH_3$	$R^2 = \beta\text{-D-Glup}$	$R^3 = R^5 = H$	$R^4 = \beta\text{-D-Galp-}(3\rightarrow1)\text{-}\beta\text{-D-Glup}$	
3 $R^1 = CH_3$	$R^2 = R^3 = H$		$R^4 = \beta\text{-D-Glup}$	$R^5 = \beta\text{-D-Galp}$

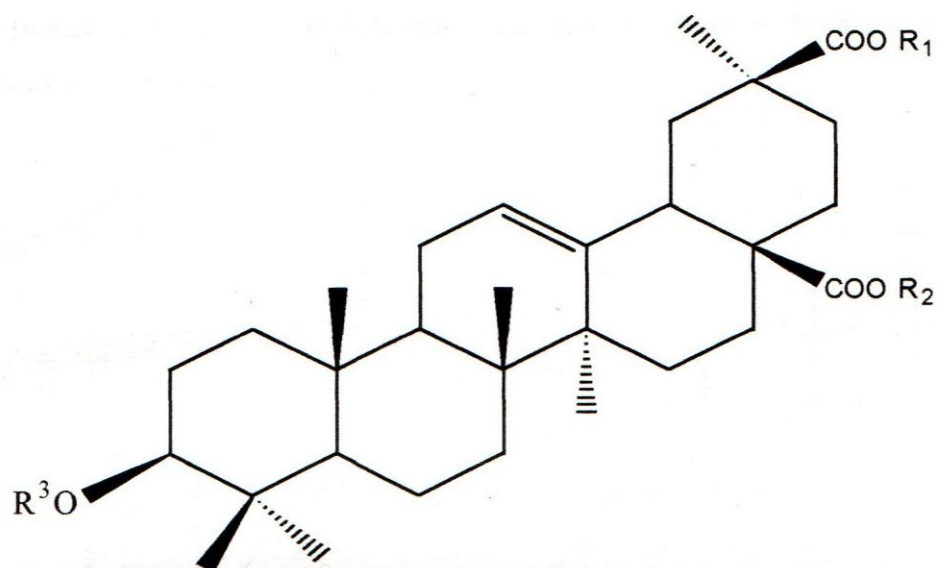
Structure 5 Triterpenoid saponins isolated from *P. rivinoids* and *P. bogotensis* [Nielsen *et al.*, 1995]

Strauss *et al.*, 1995 isolated twelve triterpene saponins from root cultures of *P. acinosa*. Among these esculentoside B, S, A, H and Phytolaccaside F [structure 6] have also been detected in roots and leaves of *P. acinosa* and *P. americana*. Treyvaud *et al.*, 2000 isolated five monodesmosidic serajic acid saponins, a monodesmosidic spergulagenic acid saponin from berries of *P. icosandra* in Indonesia [structure 7].



1 $R^1 = \text{OH}$	$R^2 = \beta\text{-D-Xylp}$	$R^3 = \text{H}$	Esculentoside B, Phytolaccaside B, Phytolaccasaponin G
2 $R^1 = \text{OH}$	$R^2 = \beta\text{-D-Xylp}$	$R^3 = \beta\text{-D-Glup}$	Esculentoside B
3 $R^1 = \text{OH}$	$R^2 = \beta\text{-D-Xylp (3}\rightarrow\text{1)-}\beta\text{-D-Glup}$	$R^3 = \text{H}$	Esculentoside A, Phytolaccaside A,
			Phytolaccasaponin E
4 $R^1 = R^3 = \text{H}$	$R^2 = \beta\text{-D-Xylp (2}\rightarrow\text{1)-}\beta\text{-D-Glup (2}\rightarrow\text{1)}\alpha\text{-D-Rhap}$		Phytolaccaside F
5 $R^1 = \text{H}$	$R^2 = \beta\text{-D-Xylp (2}\rightarrow\text{1)-}\beta\text{-D-Glup}$	$R^3 = \beta\text{-D-Glup}$	Esculentoside L
6 $R^1 = \text{OH}$	$R^2 = \beta\text{-D-Xylp (4}\rightarrow\text{1)-}\beta\text{-D-Glup}$	$R^3 = \beta\text{-D-Glup}$	Esculentoside H
7 $R^1 = \text{H}$	$R^2 = \beta\text{-D-Xylp (2}\rightarrow\text{1)-}\beta\text{-D-Glup (2}\rightarrow\text{1)}\alpha\text{-D-Rhap}$	$R^3 = \beta\text{-D-Glup}$	Esculentoside R
8 $R^1 = \text{H}$	$R^2 = R^3 = \text{H}$		Phytolaccagenin
9 $R^1 = R^2 = R^3 = \text{H}$			Phytolaccagenic acid

Structure 6: Triterpenoid saponins isolated from root cultures of *P. acinosa* [Strauss *et al.*, 1995]

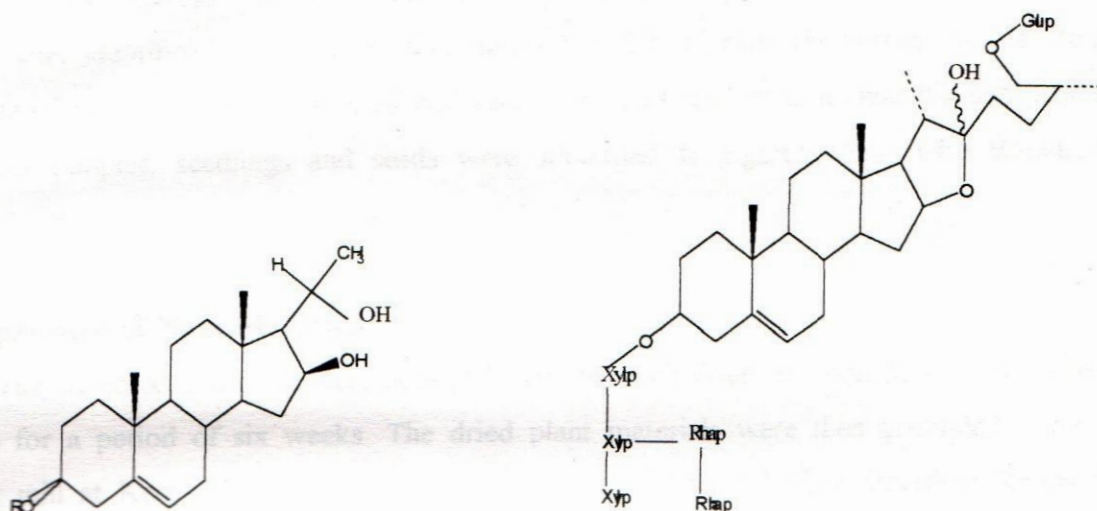


1 $R^1 = CH_3$	$R^2 = H$	$R^3 = \beta\text{-D-Glup}$
2 $R^1 = CH_3$	$R^2 = H$	$R^3 = \beta\text{-D-Glup-(3}\rightarrow\text{1)} - \beta\text{-D-Galp}$
3 $R^1 = CH_3$	$R^2 = H$	$R^3 = \beta\text{-D-Glup-(3}\rightarrow\text{1)} - \beta\text{-D-Glup}$
4 $R^1 = CH_3$	$R^2 = H$	$R^3 = \beta\text{-D-Glup-(3}\rightarrow\text{1)} - \beta\text{-D-Glup-(3}\rightarrow\text{1)} - \beta\text{-D-Glup}$
5 $R^1 = CH_3$	$R^2 = H$	$R^3 = \beta\text{-D-Glup-(2}\rightarrow\text{1)} - \beta\text{-D-Glup-(2}\rightarrow\text{1)} - \alpha\text{-D-Rhap}$
6 $R^1 = H$	$R^2 = H$	$R^3 = \beta\text{-D-Glup-(2}\rightarrow\text{1)} - \beta\text{-D-Glup-(2}\rightarrow\text{1)} - \alpha\text{-D-Rhap}$
7 $R^1 = CH_3$	$R^2 = \beta\text{-D-Glup}$	$R^3 = \beta\text{-D-Glup-(3}\rightarrow\text{1)} - \beta\text{-D-Galp}$
8 $R^1 = CH_3$	$R^2 = \beta\text{-D-Glup}$	$R^3 = \beta\text{-D-Glup-(3}\rightarrow\text{1)} - \beta\text{-D-Glup}$
9 $R^1 = CH_3$	$R^2 = \beta\text{-D-Glup}$	$R^3 = \beta\text{-D-Glup-(2}\rightarrow\text{1)} - \beta\text{-D-Glup-(2}\rightarrow\text{1)} - \alpha\text{-D-Rhap}$

Structure 7: Triterpenoid saponins isolated from the plant *P. icosandra* [Treyvaud *et al.*, 2000]

2.5.3 Triterpenoid Saponins Isolated from Plants of Balanitaceae Family

Kamel and Koskinen, [1995]; Kamel [1997] were able to isolate two pregnane glycosides [structure 8] as well as Furostanol saponins [structure 9] from the mesocarp of fruits of *Balanites aegyptiaca*.



1 R= α -D-Rhap (2 $\rightarrow$ 1) - β -D-Glup (2 $\rightarrow$ 1)- α -D-Rhap

1a R=H

2 R= β -D-Glup

Structure 8: Pregnane glycosides from *B. aegyptiaca*. **Structure 9:** Furostanol saponin from *B. aegyptiaca*

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Collection and Identification of the Plants

The plants *Phytolacca octandra* and *P. dodecandra* were collected in Njoro area in Nakuru district whereas the plant *Balanites aegyptiaca* samples were collected in Kitui district. After identification by a plant taxonomist Mr. S.T. Kariuki the berries, leaves, stem and roots of the plants were collected and each plant part packed in a clear-labelled plastic bag. Plant samples, seedlings and seeds were submitted to Egerton University Botanical Garden.

3.2 Preparation of Plant Materials

The collected berries, leaves, stem and roots were air dried to a constant weight under a shade for a period of six weeks. The dried plant materials were then grounded using a hammer mill at Kenya Agricultural Research Institute – National Plant Breeding Research Centre [KARI-NPBRC] Njoro. The powdered samples were then packed in clear-labelled plastic bags ready for extraction.

3.3 Extraction of Plant Material

A total of 250 g of each grounded sample, that is, the berries, leaves, stem and roots of each of the three plants was extracted using methanol in a Soxhlet apparatus for a period of eight hours. The solvent was removed in *vacuo* to give the concentrated methanol extract, which was used for bioactivity test, and the rest dried in an evacuated dessicator.

3.4 EXPERIMENT ONE:

3.4.1 The Use of Brine Shrimp Lethality Test to Screen *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca* Extracts.

3.4.1.1 Preparation of Test Extracts Concentrations

Each extract was tested at 1000 ppm, 100 ppm and 10 ppm. Five test tubes and one control were prepared at each concentration for a total of 18 test tubes per extract. Fifty mg of the dried extract were weighed and dissolved in 5 mL of methanol. From this solution 500 μ L, 50 μ L and 5 μ L were transferred into test tubes corresponding to 1000 ppm, 100 ppm and 10

ppm respectively using a micropipette syringe. The solvent was evaporated using a blow drier. Alternatively extracts that were insoluble in methanol were dissolved in dimethylsulfoxide [DMSO]-AR and up to 50 μ L per 5 mL of brine were used to avoid DMSO toxicity affecting the results. The prepared extracts were stored in a dry place.

3.4.1.2 Hatching Brine Shrimps

Thirty-three grams of brine salt were weighed and dissolved in 1 litre of distilled water. Ten milliliter of this saline solution was transferred into a petri dish. Two milligram of brine shrimp eggs were sprinkled into the petri dish containing the saline solution. The petri dish was then covered half way and placed in a wooden cupboard that served as an incubator. A 40 w bulb was hung above the uncovered part of the petri dish to attract the hatched larvae as well as maintain a constant temperature in the cupboard. The shrimps were allowed to hatch for 24 hours.

3.4.1.3 Bioassay Test

After 24 hours [when the shrimp larvae were ready] ten shrimps were transferred into each prepared extract test tube [50 shrimps per dilution + 10 shrimps control] using a Pasteur Pipette. Five milliliters of the prepared saline solution was then added to each test tube containing the shrimps and the extracts. Twenty-four hours later the number of surviving shrimps were counted and recorded.

3.4.1.4 Calculation of % Mortality

The percent larvae mortality was determined after correction for control using Abbott's formula as shown below, and the means separated using Duncan's Multiple Range Test [DMRT]

$$\% \text{ Mortality} = \frac{[\text{sample mortality} - \text{control \% mortality}]}{100 - \text{Control \% mortality}} \times 100$$

3.4.1.5 Statistical Analysis

The collected data was analysed using Probit procedure to determine the LD₅₀. These values were subjected to analysis of variance using the General Linear Model [GLM] procedure and the means separated using Duncan's Multiple Range Test [DMRT]. These analyses were carried out using the SAS proprietary software release 8.1[1999-2000].

3.5 EXPERIMENT TWO:

3.5.1 Evaluation of the Molluscicidal Effect of the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca* Extracts.

3.5.1.1 Collection of Snails

The sampled area for snail collection was Timboni dam in Kilifi District near Mazeras Township. The site was chosen as a result of having sandy beach, established vegetation such as water lilies and other floating vegetation [Plate 5], which offer an ideal environment for the *Bulinus* snails. The infection caused by *S. haematobium* or urinary schistosomiasis is very common in this area especially among school going children who swim and play in the infected waters of Timboni dam.

Sieves approximately 20 cm in diameter and 17 cm deep, with holes 2 or 3 mm wide drilled through a metal bottom together with a snail scoop made of steel screen soldered to a wire frame were used for snail collection. Both the sieve and snail scoop were attached to handles of about 4 – 6 m long. With the sieve a sweeping motion was used to collect the snails whereas a shovelling motion was employed with a scoop. The action with either one [the scoop or the sieve] had to be vigorous since some snails adhered firmly to the vegetation. Snails found sticking to star grass, grass sedges, and on the sandy substrates were collected using forceps or hand picked. The collected snails were put into a container, which contained some wet cotton wool to prevent the snails from dehydration. The snails were transported in this container to the Institute of Primate Research Malacology laboratory at Fort Jesus, Mombasa [Plate 6] where an indoor culture was established.

3.5.1.2 Establishment of an Indoor Culture

Medium size aquaria made of polycarbonate material were used. The inside surface of the aquaria was rough in order to provide a gripping surface for the snails. Each aquaria measured 45 cm length by 32 cm width by 16 cm depth. Approximately 5 litres of dechlorinated water was needed for each aquarium. The aquarium was lined at the bottom with about 2 cm deep substrate of sand. To maintain a high level of oxygen, the aquaria were continually aerated using a whisper number 100 air pump, which was operated at low pressure to create minimal turbulence in the aquaria. The room temperature was maintained between 26 °C and 27 °C. The collected snails were fed with lettuce and kept in these aquaria for twenty-four hours for acclimatization process before they could be used for the bioassay procedure.

3.5.1.3 Bioassay

The World Health Organization guidelines were followed. Each extract was tested at 200 mg, 100 mg and 50 mg per litre of dechlorinated water. Three plastic containers plus one control were prepared for each extract concentration. Five acclimatised snails were placed into each of these containers with a total volume of 200 mL of the test extract solution. The snails were exposed to the test extract solution for 24 hours at room temperature and normal diurnal lighting. The extract was decanted after 24 hours and the snails rinsed twice with a stream of dechlorinated water. The snails were then fed with lettuce and left in clean water for a further 24 hours in order to recover. Snails were considered dead if they either remained motionless, not feeding, no heartbeat when observed under a microscope or shell looked discoloured at the end of the period. Control snails were not exposed to the test extract solution.

3.5.1.4 Statistical Analysis

The collected data was analysed using the Probit procedure to determine the LD₅₀ values. The LD₅₀ values obtained were subjected to analysis of variance using the General Linear Model [GLM] procedure and the means separated using the Duncan's Multiple Range Test [DMRT]. These analyses were carried out using the SAS proprietary software release 8.1 [1999-2000].



PLATE 5 Timboni dam snail collection site

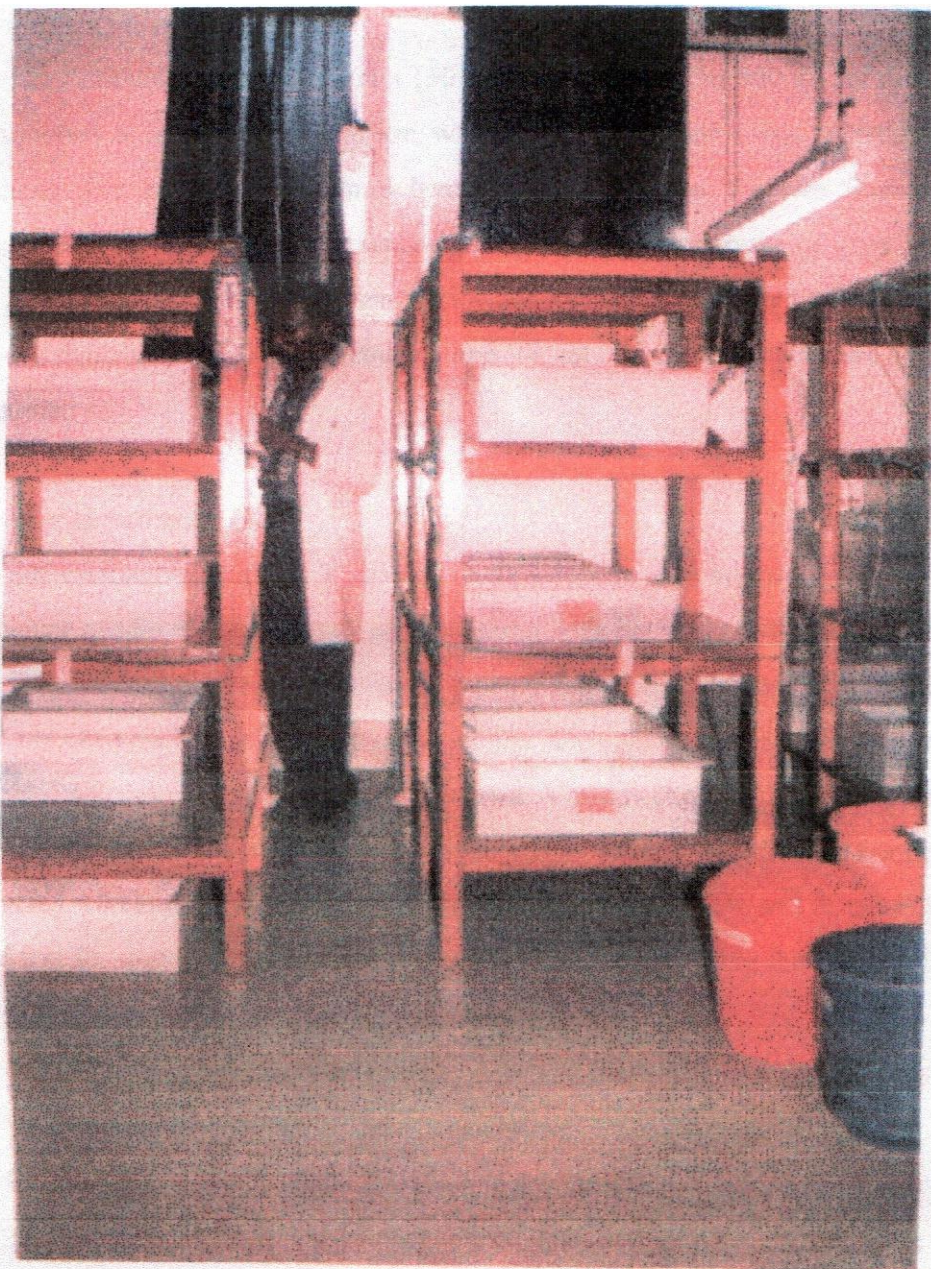


PLATE 6 Indoor breeding and culture facility in Fort Jesus Malacology Laboratory, Mombasa, Kenya.

3.6 Isolation and Purification of the Active Compounds.

3.6.1 *Phytolacca octandra* Berries

Dried *P. octandra* berries extract [30 g] were dissolved in 300 mL of distilled water to give an aqueous solution. This solution was then fractionated using *n*-butanol to give an aqueous layer and an organic butanol layer. After separating the two layers, the organic butanol extract was concentrated *in vacuo* using a rotary evaporator to give 14.8 g of solid material. A test for saponins was then performed on the butanol extract where 2 g of the extract were dissolved in 20 mL methanol and 10 mL of diethyl ether was added slowly in small amounts.

3.6.2 *Phytolacca octandra* Stem

Crude methanol extract [8.37 g] was re-dissolved in cold methanol and filtered to give 0.81 g of white needle shaped crystals after washing with pet-ether, ethyl acetate and finally methanol. The melting point was determined using a Sanyo melting point apparatus. TLC analysis of the crystals was done with methanol and silica gel. Antimony chloride in concentrated HCl was used as the spraying reagent. Various chemical tests done included a test for triterpenoids using Liebermann-Buchard reagent [acetic anhydride in concentrated sulphuric acid], alkaloids using Dragendorff reagent [Bismuth nitrate and Potassium iodide in water] and test for saponins. Elemental analysis was carried out using the CE 440 Elemental Analyser Exeter Analytical instrument.

3.6.3 *Phytolacca octandra* Roots.

Concentration of the methanol extract *in vacuo* using a rotary evaporator led to the formation of a brown precipitate. Glass sintered filtration of this extract gave a brownish powder residue and a filtrate which was put in a sample bottle and dried in a dessicator where some crystalline compound was formed. The residue from the filtrate was re-dissolved in methanol and filtered to obtain 2.21 g of white sugar like crystals after washing with pet-ether, ethyl acetate and finally methanol. The melting point was determined using a Sanyo melting point apparatus. TLC analysis of the crystals was done using methanol and silica gel, and antimony chloride in concentrated HCl used as the spraying reagent. Tests for

triterpenoids, alkaloids and for saponins were carried out on the crystals obtained. Elemental analysis was carried out using the CE 440 Elemental Analyser Exeter Analytical instrument. The brownish powder obtained after glass sintered filtration [3.0 g] was triturated with chloroform then recrystallised from methanol: water [4:1] to give some colourless crystals whose melting point was determined. TLC analysis of the crystals was done with methanol/ silica gel and antimony chloride in concentrated HCl used as the spraying reagent. Tests for triterpenoids, alkaloids and saponins were carried out on the crystals obtained.

3.6.4 *Phytolacca dodecandra* Berries

Methanol extract residue [8.37 g] obtained was redissolved in cold methanol and filtered to give 1.22 g of sugar like crystals after repeatedly washing with methanol. The melting point was determined and TLC analysis was done with methanol/ silica gel and antimony chloride in concentrated HCl used as the spraying reagent. Tests for triterpenoids, alkaloids and saponins were carried out. Elemental analysis was also carried out.

The filtrate methanol solution used to redissolve the extract was removed *in vacuo* to give 4.3 g of residue, which was dissolved in 300 mL of distilled water and fractionated using n-butanol to give an aqueous layer and an organic butanol layer. The layers were separated and the organic butanol layer concentrated *in vacuo* using a rotary evaporator to give 0.58 g of the organic butanol extract. A test for saponins was then performed on the butanol extract where 0.1 g of the extract were dissolved in 2 mL methanol; to this solution diethyl ether was added slowly in small amounts.

3.6.5 *Phytolacca dodecandra* Stem

After concentration of the methanol extract *in vacuo* some needle shaped crystals started forming in the round bottom flask. Cold methanol was added and the solution filtered. The crystals were cleaned repeatedly using cold methanol to give 0.38 g of clean needle shaped crystals whose melting point was determined using a Sanyo melting point apparatus. TLC analysis of the crystals was done with methanol/ silica gel and antimony chloride in concentrated HCl used as the spraying reagent. Test for triterpenoids, alkaloids, saponins and elemental analysis were carried out on the crystals obtained.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Extraction of the Plants Using Soxhlet Apparatus

Table 3: Percent Yield

Plant	Part	% Yield	Mean % Yield
<i>Phytolacca octandra</i>	Berries	30.95	15.27 ^a
	Leaves	18.56	
	Stem	8.56	
	Roots	8.24	
<i>Phytolacca dodecandra</i>	Berries	30.89	14.04 ^a
	Leaves	7.92	
	Stem	11.53	
	Roots	5.82	
<i>Balanites aegyptiaca</i>	Berries	2.75	9.76 ^a
	Leaves	14.76	
	Stem	11.08	
	Roots	10.45	

^aMeans with the same superscript are not significantly different at 5 % level

From the percent yield obtained after extraction Soxhlet extraction method seems to have given the highest yield for the berries of *Phytolacca octandra* [30.95 %] and *P. dodecandra* [30.89 %], and the lowest for *Balanites aegyptiaca* [2.75 %] berries [Table 3]. However analysis of variance showed no significant [$P > 0.05$] difference in mean percent yields of the three plants.

4.2 EXPERIMENT ONE: Brine Shrimp Assay

4.2.1 Mean Percent Mortality for the Berries, Leaves, Stem and Roots of the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*.

Calculation for the mean percent mortality after correction for control for the different plants parts showed that the leaves of the *P. octandra* had the highest percent [37.67 %] mortality when compared to other plants [*P. dodecandra* and *B. aegyptiaca*] parts, whereas the lowest mean percent mortality was recoded in the stem of *B. aegyptiaca* [11.23 %] [Table 4]. The berries with the highest percent mortality were those of *P. octandra* [27.00 %]. The stem of *P. octandra* [32.50 %] was found to have the highest mean percent mortality whereas the roots of *B. aegyptiaca* were found to have the highest mean percent mortality [36.02] [Fig 2].

Table 4 Mean Percent Mortality for the Berries, Leaves, Stem and Roots of the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*.

Mean ^a			
	<i>P. octandra</i>	<i>P. dodecandra</i>	<i>B. aegyptiaca</i>
Berries	27.00 ± 5.42	22.00 ± 9.37	24.89 ± 7.35
Leaves	37.67 ± 2.83	18.00 ± 2.34	35.11 ± 8.57
Stem	32.5 ± 6.21	21.44 ± 6.59	11.23 ± 2.89
Roots	32.19 ± 4.49	32.89 ± 3.02	36.02 ± 2.20

Mean^a percent mortality ± standard error of the mean

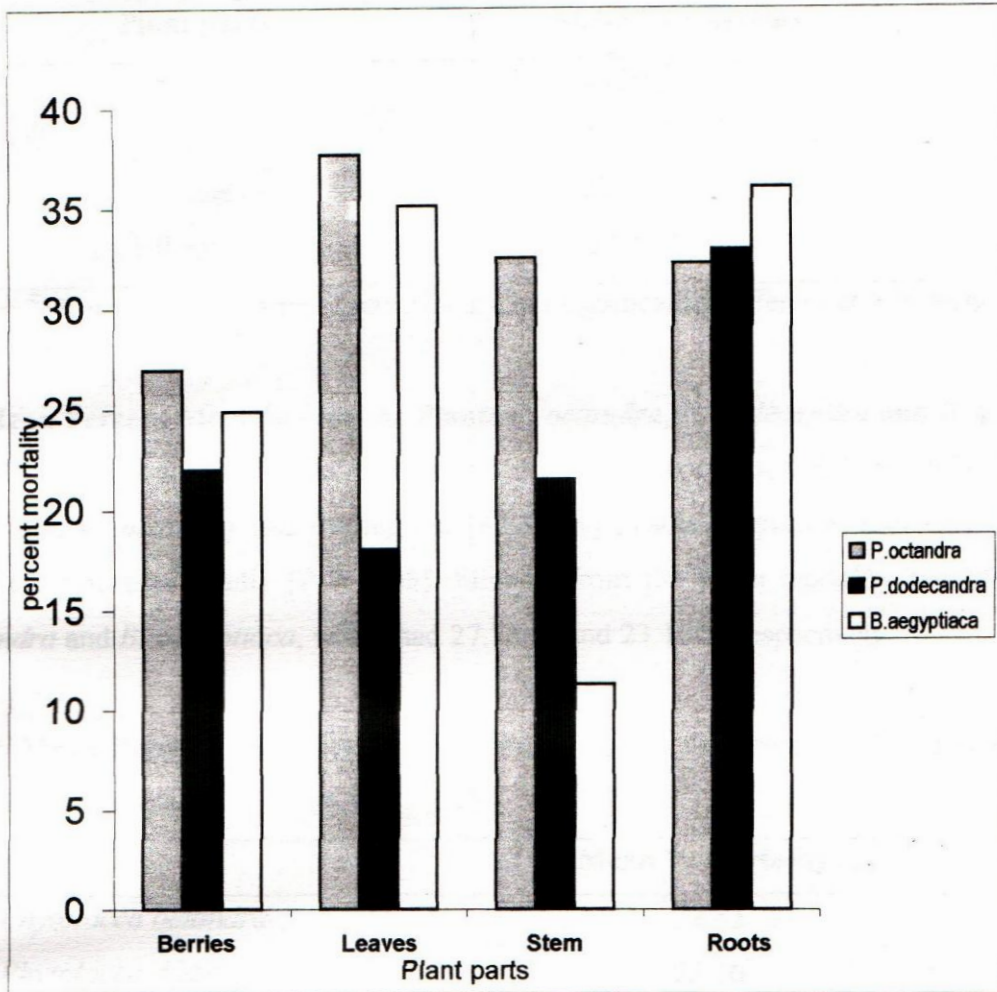


Figure 2 Graph of Mean Percent Mortality for the Berries, Leaves, Stem and Roots for the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*.

4.2.2 Mean Percent Mortality for the Berries, Leaves, Stem and Roots

Calculation for the mean percent mortality after correction for controls for the different plant parts showed that the roots had the highest percent mortality [33.17 %] [Table 5]. However these were not significantly [$P > 0.05$] different from the mean mortality values recorded by the regenerative parts such as the leaves [29.33 %], berries [24.25 %] and the stems [23.43 %], which recorded the lowest mean percent mortality.

Table 5 Mean Percent Mortality for the Berries, Leaves, Stems and Roots

Plant parts	Mean % mortality
Berries	24.25 ^a
Leaves	29.33 ^a
Stems	23.43 ^a
Roots	33.17 ^a

^aMeans with the same superscript are not significantly different at 5 % level

4.2.3 Mean Percent Mortality for the Plants *P. octandra*, *P. dodecandra* and *B. aegyptiaca*

Calculation for the mean percent mortality after correction for the different plants showed that *P. octandra* had the highest [32.33 %] [Table 6] percent mortality. However these were not significantly [$P > 0.05$] different from the mean mortality recorded for *P. dodecandra* and *B. aegyptiaca*, which had 27.73 % and 23.16 % respectively

Table 6 Mean Percent Mortality for the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*

Plant	Mean % mortality
<i>Phytolacca octandra</i>	32.33 ^a
<i>Phytolacca dodecandra</i>	23.16 ^a
<i>Balanites aegyptiaca</i>	27.73 ^a

^aMeans with the same superscript are not significantly different at 5 % level

4.2.4 LD₅₀ for the Berries, Leaves, Stem and Roots for the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*

LD₅₀ is defined as the dose, which kills 50 % of organisms exposed to the test substance within 24 hours of exposure. Based on this definition, substances that are highly potent are usually expected to give lower LD₅₀ values. In this study therefore, plant extracts that give lower LD₅₀ values will be perceived as better than those with higher LD₅₀ values. Analysis of variance showed no significant [$P > 0.05$] difference in the mean LD₅₀ values for the different plant parts that is the berries, leaves, stem and roots of the three plants [Table 7].

The best mean LD₅₀ value for the berries [2.2627], leaves [2.4181] and stem [2.4197] were recorded for the plant *P. octandra* extracts [Fig 3]. However these were not significantly [$P > 0.05$] different from the LD₅₀ values recorded for berries [2.9865], leaves [2.5397], stem [2.8193] of *P. dodecandra* and berries [2.6839], leaves [2.6770] and stem [2.8523] of *B. aegyptiaca*. The plant *B. aegyptiaca* recorded the best mean LD₅₀ value for the roots [2.0931] extract although it is not significantly [$P > 0.05$] different from the LD₅₀ value recorded for the *P. octandra* roots extract [2.5672] and *P. dodecandra* [2.5332] extract.

Table 7 LD₅₀ for the Berries, Leaves, Stem and Roots for the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*.

	MEAN ^a		
	<i>P. octandra</i>	<i>P. dodecandra</i>	<i>B. aegyptiaca</i>
Berries	2.2627 ± 0.7096	2.9865 ± 0.0243	2.6839 ± 0.1837
Leaves	2.4181 ± 0.1923	2.5397 ± 0.1466	2.6770 ± 0.4604
Stem	2.4197 ± 0.3446	2.8193 ± 0.2517	2.8523 ± 0.0954
Roots	2.5672 ± 0.1815	2.5332 ± 0.1089	2.0931 ± 0.00

^aMean LD₅₀ value ± standard error of the mean

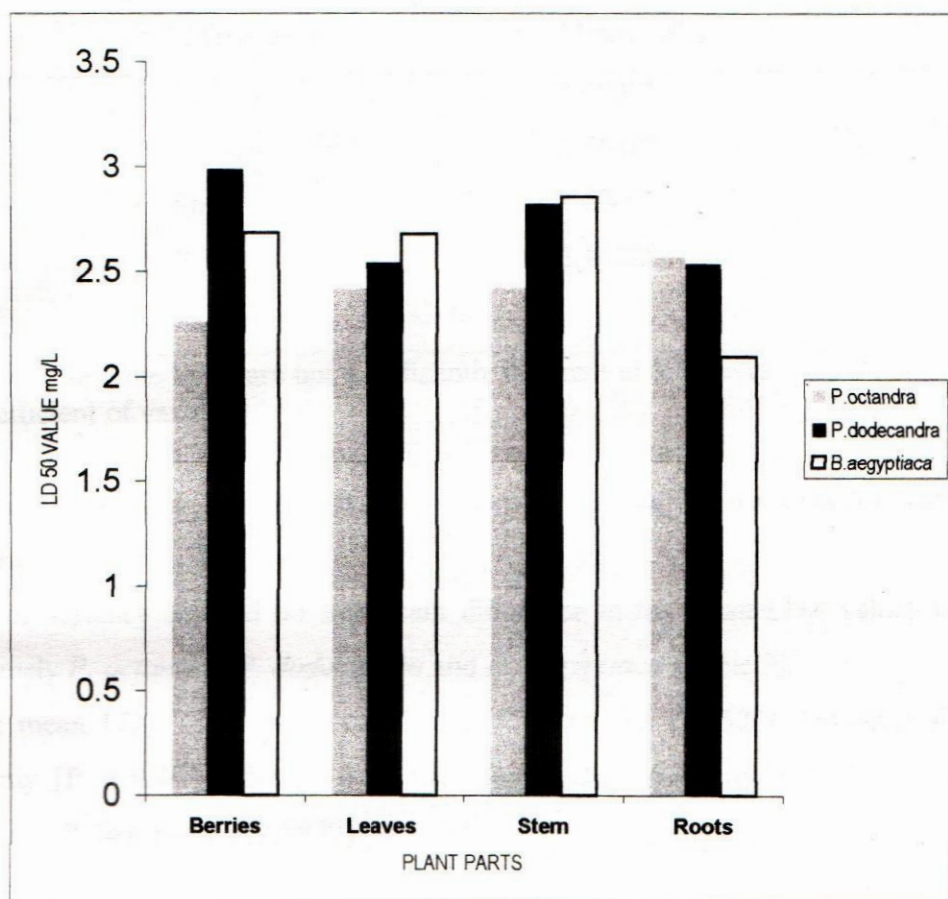


Figure 3 Graph of LD₅₀ for the Berries, Leaves, Stem and Roots of *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*.

4.2.5 Mean LD₅₀ for the Berries, Leaves, Stem and Roots

Analysis of variance showed no significant [$P > 0.05$] difference in the mean LD₅₀ value for the berries, leaves, stem and roots [Table 8]. The best mean LD₅₀ value was recorded in the roots [2.4622], although it was not significantly [$P > 0.05$] different from the mean LD₅₀ values recorded for the berries [2.6932], leaves [2.5638] and stem [2.6800].

Table 8 Mean LD₅₀ for the Berries, Leaves, Stems and Roots.

Plant part		Mean LD ₅₀
Berries		2.6932 ^a
Leaves		2.5638 ^a
Stem		2.6800 ^a
Roots		2.4622 ^a
C.V	18.82 %	

^aMeans with the same letter are not significantly different at 5 % level

CV = coefficient of variance

4.2.6 Mean LD₅₀ for the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*

Analysis of variance showed no significant difference in the mean LD₅₀ values for the three plants namely *P. octandra*, *P. dodecandra* and *B. aegyptiaca* [Table 9].

The best mean LD₅₀ value was recorded in *P. octandra* [2.4520]. However this was not significantly [$P > 0.05$] different from the mean LD₅₀ values recorded for *P. dodecandra* [2.7488] and *B. aegyptiaca* [2.5877].

Table 9 Mean LD₅₀ for the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*

Plant	Mean LD ₅₀
<i>Phytolacca octandra</i>	2.4520 ^a
<i>Phytolacca dodecandra</i>	2.7488 ^a
<i>Balanites aegyptiaca</i>	2.5877 ^a
C.V	18.82 %

^aMeans with the same letter are not significantly different at 5 % level

CV: coefficient of variance

4.2.7 Comparison of Mean LD₅₀ and Mean Percent Mortality.

Correlation analysis performed on the mean LD₅₀ and the percent mortality for the plants *P. octandra*, *P. dodecandra* and *B. aegyptiaca* and their parts that is the berries, leaves, stem and roots showed some significant [$P < 0.005$] negative correlation [$P = 0.0345$ and correlation coefficient $r = -0.61178$]. This means that an increase in the LD₅₀ value may be associated to a greater extent with a decrease in the percent mortality for the plants and plant parts studied [Fig 4].

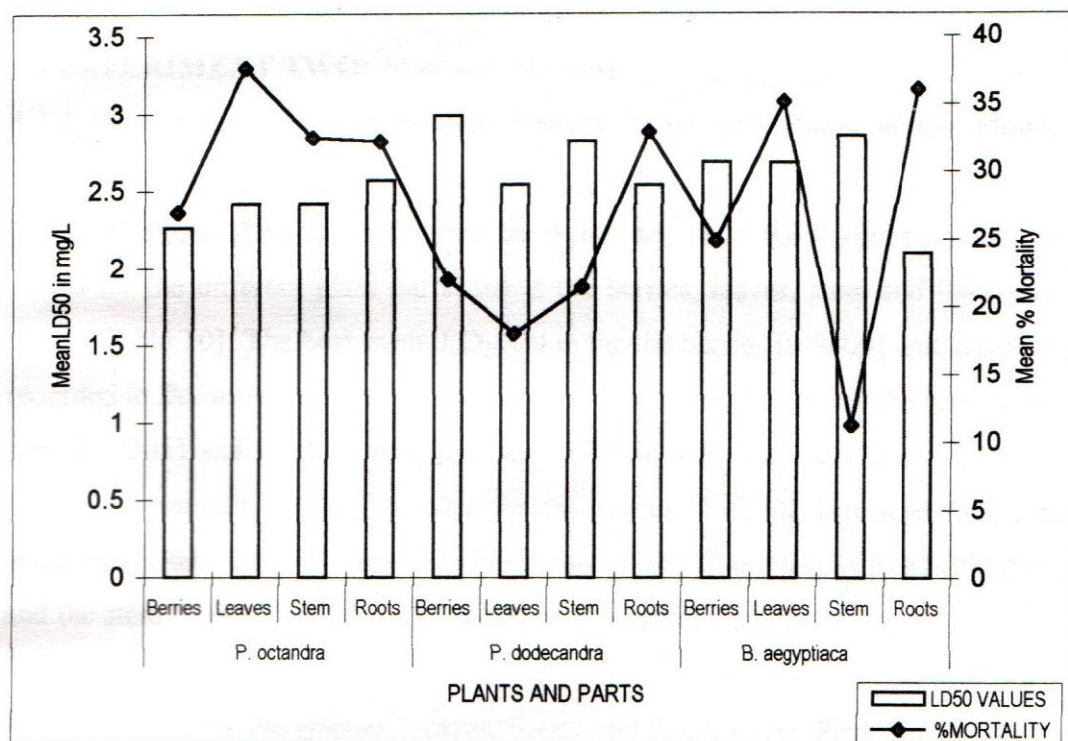


Figure 4 Graph of Comparison of Mean LD₅₀ and Mean Percent Mortality for the Berries, Leaves, Stems and Roots of the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*

From the experimental data, the regenerative parts of the plants appear to be equally active as any other part of the plant since no significant [$P > 0.05$] difference exists. This makes them suitable candidates for development of a new molluscicide since their exploitation would not endanger the plants. The three plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca* grow abundantly in Kenya and therefore from the pre-

screening results they have a potential use in controlling snail host for Schistosomiasis. Since from the results all plants and plant parts have been found to exhibit almost same activity [no significant ($P > 0.05$) difference in LD_{50} values]. These results are supported by earlier findings of the World Health Organisation that the effect of a potential molluscicide on fish and shrimp can be used to determine the effectiveness of a compound [WHO, 1961]. The results also serve as a guide to further research on the biological activity [molluscicidal activity] of the plant extracts.

4.3 EXPERIMENT TWO: Molluscicide Test

4.3.1 LD_{50} Values for the Berries, Leaves, Stem and Roots of the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*

Analysis of variance showed no significant [$P > 0.05$] difference in the mean LD_{50} values for the different plant parts that is the berries, leaves, stem and the roots of the three plants [Table 10]. The best mean LD_{50} value for the berries [0.9609] and leaves [0.7975] were recorded in *Balanites aegyptiaca*, whereas *P. dodecandra* recorded the best mean LD_{50} for the stem [0.7305] and roots [0.8885]. However these were not significantly [$P > 0.05$] different from the mean LD_{50} values recorded for the berries [1.4520], leaves [0.8846], stem [1.0438] and roots [1.0310] of *P. octandra*, the berries [1.1427] and leaves [0.8749] of *P. dodecandra* and the stem [0.9415] and roots [2.2482] of *B. aegyptiaca* [Fig 5].

Table 10 LD_{50} for the Berries, Leaves, Stems and Roots of the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*

	MEAN ^a		
	<i>P. octandra</i>	<i>P. dodecandra</i>	<i>B. aegyptiaca</i>
Berries	1.4520 ± 0.1011	1.1427 ± 0.1356	0.9609 ± 0.0020
Leaves	0.8846 ± 0.0742	0.8749 ± 0.0356	0.7975 ± 0.0719
Stem	1.0438 ± 0.0093	0.7305 ± 0.1349	0.9415 ± 0.0062
Roots	1.0310 ± 0.1205	0.8885 ± 0.0742	2.2482 ± 0.1551

Mean ^a LD_{50} value ± standard error of the mean

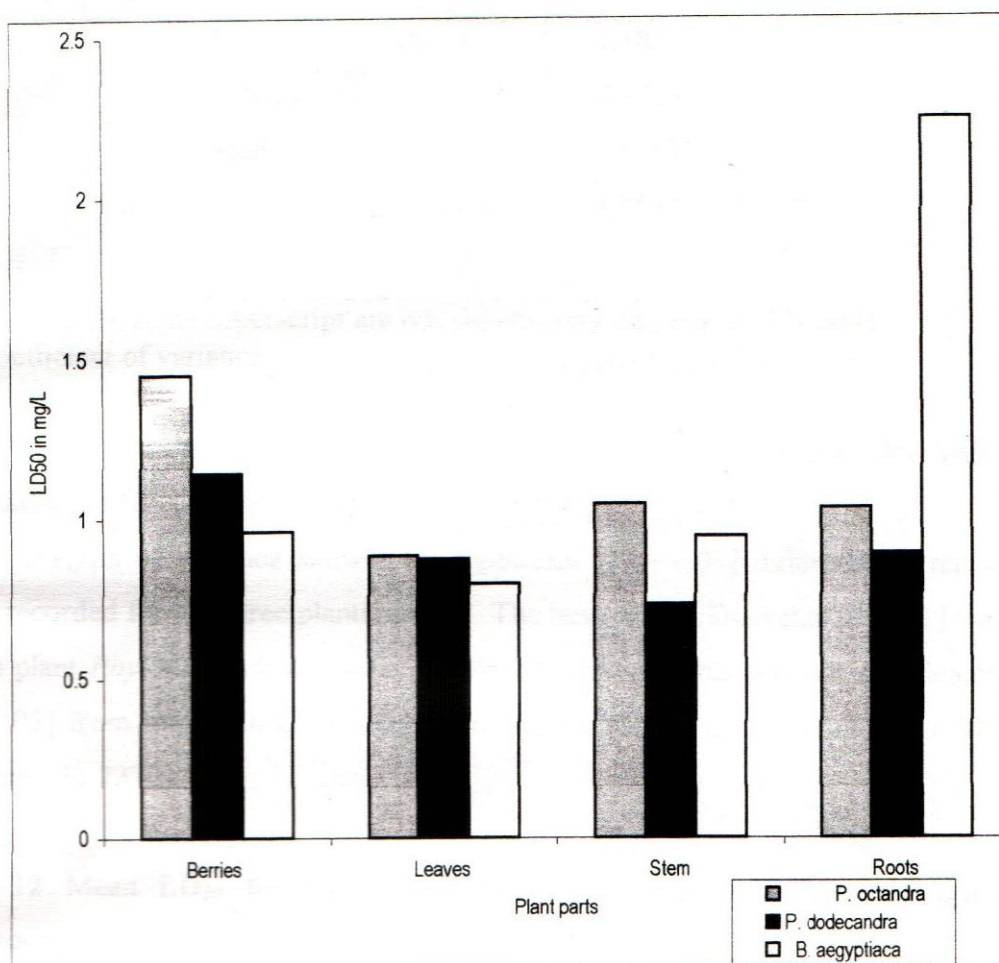


Figure 5 Graph of LD₅₀ for the Berries, Leaves, Stems and Roots of the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*

4.3.2 Mean LD₅₀ for the Berries, Leaves, Stems and Roots

Analysis of variance showed no significant [$P > 0.05$] difference in the mean LD₅₀ values for the berries, leaves, stems and roots of the three plants [Table 11]. The best LD₅₀ value was recorded in the leaves [0.8523], although this was not significantly [$P > 0.05$] different from the mean LD₅₀ value recorded for the berries [1.1852], stems [0.8757], and roots [1.3381] of the three plants studied.

Table 11 Mean LD₅₀ for the Berries, Leaves, Stems and Roots.

Plant part		Mean LD ₅₀
Berries		1.1852 ^a
Leaves		0.8523 ^a
Stem		0.8757 ^a
Roots		1.3381 ^a
C.V	34.47 %	

^aMeans with the same superscript are not significantly different at 5 % level

CV: coefficient of variance

4.3.3 Mean LD₅₀ for the Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*

Analysis of variance showed no significant [$P > 0.05$] difference in the mean LD₅₀ values recorded for the three plants studied. The best mean LD₅₀ value [0.9091] was recorded for the plant *Phytolacca dodecandra*, [Table 12] although this was not significantly different [$P > 0.05$] from the mean LD₅₀ value recorded for the plants *P. octandra* [1.1001] and *B. aegyptiaca* [1.2370].

Table 12 Mean LD₅₀ for Plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*

Plant	Mean LD ₅₀
<i>Phytolacca octandra</i>	1.1001 ^a
<i>Phytolacca dodecandra</i>	0.9091 ^a
<i>Balanites aegyptiaca</i>	1.2370 ^a
C.V.	34.47 %

^aMeans with the same superscript are not significantly different at 5 % level

CV: coefficient of variance

In the experiment the extracts were tested within the range of between 200 mg/L to 50 mg/L instead of the recommended 100 mg/L to 10 mg/L [WHO 1961]. This is due to the low activity recorded in the recommended range. This low activity within the recommended range could probably be as a result of very low concentration of saponins in the extracts as reported by Treyvaud *et al*, [2000]. The mode of action of the extracts is probably haemolysis since the colourless extract solution turned reddish in colour six hours after exposure of the snails to the extract. These findings are consistent with those of Harborne, [1984].

From the results of molluscicidal activity no significant [$P > 0.05$] difference exist between the plant parts that is the berries, leaves, stem and roots and the three plants studied. These results are consistent with those obtained in the Brine shrimp lethality in that no significant difference in activity was recorded between the plant parts and between the plants studied. Therefore brine shrimp lethality test can be used to screen plant extracts for activity and specifically for molluscicidal activity.

Extracts from the regenerative parts of the plants are equally good as any other part of the plants [no significant ($P > 0.05$) difference in the mean LD_{50}] and therefore satisfy the requirement for a viable source of plant molluscicide. Moreover the three plants grow abundantly in Kenya and based on the findings of this research, the plants have potential for use in controlling the snail intermediate host for schistosomiasis.

4.4 Chemical Characterization of the Isolated Compounds

From the melting point analysis the crystals exhibited very sharp melting points with a range of 3 °C from the onset of melting to the point where the crystals had melted completely [Table13]. TLC analysis of the crystals revealed the presence of single spots [R_f given in table 13], which appeared as pink spots upon spraying with antimony chloride in concentrated HCl acid and heating at 90 °C for 10 minutes. The appearance of a single spot for each compound confirms that the crystals were pure. Antimony chloride in concentrated HCl acid used as a spraying reagent is also used to confirm the presence of saponins, which normally appear as either pink or purple spots [Harborne, 1984]. Therefore the pure compounds could probably be saponins since they gave pink spots whose R_f 's are given in table 13.

Table 13 Table of Results Obtained in Chemical Characterization

Sample	M.P ^a	Test Performed				
		TLC ^b	Alkaloids	Triterpenoids	Glycosides	Saponins
MWS	347-349	3.5/7	- VE	+ VE	+ VE	+ VE
MWR	344-346	4.3/7	- VE	+ VE	+ VE	+ VE
GSC	370.3-370.8	4.5/7	- VE	+ VE	+ VE	+ VE
BBC	245-248	3.8/7	- VE	+ VE	+ VE	+ VE
BSC	334-336	3.0/7	- VE	+ VE	+ VE	+ VE

^a melting point in °C , ^b TLC R_f[retardation factor]

Test for alkaloids was performed using Dragendorff's reagent. However no precipitate was formed implying that the crystals were not alkaloids [Table 13]. The crystals were tested for triterpenoids using the Liebermann-Buchard reagent. This colourless solution when added to the crystals turned blue – green in colour indicating the presence of triterpenoids. Since triterpenoids can be divided into four groups: true triterpenes, steroids, saponins and cardiac glycosides [Harborne, 1984] either of these could be present but earlier TLC analysis suggested the presence of saponins therefore the triterpenoid saponins are the most probable compounds that are responsible for the blue-green coloration hence positive test.

By use of concentrated H₂SO₄ the crystals were tested for the presence of glycosides. A positive test indicated by development of an intense red coloration was exhibited by the sample MWR. The others produced specks of red coloration. These specks of red coloration could probably be as a result of glycosides linked to the aglycone chain of the saponin. A test for saponins was carried out by adding diethyl ether to a solution of the crystals in methanol. A white precipitate was formed indicating the presence of saponins. Fractionated extracts of *P. octandra* and *P. dodecandra* tested positive for the presence of saponins. These findings are in line with those by Treyvaud *et al.*, [2000] and Thilborg *et al.*, [1994] that the berries of *P. octandra* and *P. dodecandra* are rich in saponins and therefore one of the best sources of plant molluscicide.

4.5 Elemental Analysis

Elemental analysis of the crystalline compounds showed that the MWR, BBC and GSC are basically made of carbon, hydrogen and oxygen varying in percent composition. Correlation analysis was done to compare the observed similarities between the isolated crystalline compounds and known isolated compounds from plants of the Phytolaccacea family [Thiilborg *et al.*, 1993; Treyvaud *et al.*, 2000]. From the correlation analysis [Table 14], there is a high likelihood that the isolated compounds are saponins since a high positive correlation was obtained [$r > 0.93$] for all tested samples.

Table 14: Percent Composition and Correlation Coefficients

Sample	% composition	Correlation coefficients [r]					
		a	b	c	d	e	f
GSC	C = 69.16 H = 6.53 O = 24.31	0.9969	0.9879	0.9969	0.9998	0.9938	0.9775
BBC	C = 76.66 H = 7.29 O = 16.05	0.9701	0.9497	0.9710	0.9899	0.9626	0.9304
MWR	C = 74.69 H = 7.10 O = 18.22	0.9786	0.9609	0.9794	0.9946	0.9722	0.9436

a: 3-O- {o- α -L-Rhap-(1 $\rightarrow$ 2)-o- β -D-Glup-(1 $\rightarrow$ 2)-O-[β -D-Glup-(1 $\rightarrow$ 4)]- β -D-Glup} oleanolic acid
b: 3-O-[2',4'-Di-O-(β -D-Glup)- β -D-Glup] oleanolic acid
c: 3-O-{ α -L-Rhap-(1 $\rightarrow$ 2)-O-[β -D-Galp-(1 $\rightarrow$ 3)]- β -D-Glup } oleanolic acid
d: 3-O- β -D-Glup-serjanic acid
e: 3-O-[β -D-Galp-(1 $\rightarrow$ 3)]- β -D-Glup-serjanic acid
f: 3-O-{ α -L-Rhap-(1 $\rightarrow$ 4)]- β -D-Glup-(1 $\rightarrow$ 2)-O- β -D-Glup} serjanic acid

The greatest correlation was registered between the isolated compounds and known compounds [c: 3-O- $\{\alpha$ -L-Rhap-(1 $\rightarrow$ 2)-O- $\{\beta$ -D-Galp-(1 $\rightarrow$ 3)]- β -D-Glup }]oleanolic acid and [d: 3-O- β -D-Glup-serjanic acid]. This therefore suggests that the isolated compounds GSC, BBC and MWR could either be Oleanolic acid saponins or Serjanic acid saponins. These findings concur with the findings of Thiilborg *et al.*, [1993] and Treyvaud *et al.*, [2000] who have isolated Oleanolic acid and Serjanic acid saponins from plants of the Phytolaccaceae family.

Elemental analysis of the sample MS revealed that the compound contained carbon [74.51 %], hydrogen [6.98 %], oxygen [7.646 %] and nitrogen [10.86 %]. The presence of nitrogen was interesting to note since the sample had given a negative Dragendorff test for alkaloids. This compound could therefore be a steroidal alkaloid glycoside. Wanyonyi *et al.*, [2002] isolated steroidal alkaloids glycosides with molluscicidal activity.

CHAPTER FIVE

5.CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

1) From the results obtained in this study no significant [$P > 0.05$] difference exists in the molluscicidal activity between the plant parts that is the berries, leaves, stem and roots studied. This therefore encourages the use of the regenerative parts such as the berries since they are equally active as any other part of the plants. Also no significant [$P > 0.05$] difference exists in the molluscicidal activity of the three plants studied. Therefore communities should be encouraged to utilize either of these plants and especially those found within their locality for controlling schistosomiasis-transmitting snails.

2) Similar results were observed in the Brine shrimp lethality test where no significant [$P > 0.05$] difference exist in the activity of the plant parts that is berries, leaves, stem and roots studied. Also no significant differences exist in the activity of the three plants studied. Thus it is possible to monitor active extracts for molluscicidal activity using Brine shrimp lethality test rather than the more expensive *in-vivo* and *in vitro* assays. The *in-vivo* lethality test in simple zoologic organisms has the advantages of being rapid [24 hours following introduction of the shrimps], inexpensive, and simple [for example no aseptic technique are required]. It easily utilizes a large number of organisms for statistical consideration and requires no special equipment and a relatively small amount of sample [20 mg for screening initial extracts at 1000 ppm, lesser amounts for active fractions]. It has the added advantage, of not requiring higher animal serum and animal rights activist have not yet objected to the use of these invertebrates in experimental work. Self-reliance and rapid results are important advantages in this bioassay test.

3) Chemical characterisation of the isolated crystalline compounds indicates that they are saponins. Melting point and TLC analysis were used as a purity probe with observation of a single TLC spot and a sharp melting point being used as indicators for the purity of the compounds. Since these crystalline pure saponin compounds were extracted using Soxhlet apparatus further research could be carried out to optimise the use of Soxhlet apparatus as a method for extracting pure crystalline saponin compounds as demonstrated by the results of this study.

4) The pharmacological evidence and chemical characterization results from this study serve as a link between scientific findings and traditional empirical knowledge. The results also confirm existing traditional empirical knowledge that the studied plants, which are used as soap substitutes locally, contain saponins and can be used to control the schistosomiasis.

5) From the results obtained in this study no significant [$P > 0.05$] difference exists in the molluscicidal activity between the plant parts that is the berries, leaves, stem and roots studied. Also no significant [$P > 0.05$] difference exists in the molluscicidal activity of the three plants studied. Therefore we reject hypothesis that “there is a difference in the molluscicidal activity of the extracts from the berries, leaves, stems and roots of the plants *Phytolacca octandra*, *P. dodecandra* and *Balanites aegyptiaca*”.

Similarly from the Brine shrimp lethality test no significant [$P > 0.05$] difference exist in the activity of the plant parts that is berries, leaves, stem and roots studied. Also no significant differences exist in the activity of the three plants studied. Therefore we reject hypothesis that “Brine shrimp lethality test cannot be used for screening of plant extracts for molluscicidal activity”.

Chemical characterisation of the isolated compounds indicates that they are saponins. Therefore we accept hypothesis that “the active component responsible for molluscicidal activity are mainly saponins”.

5.2 Recommendations

- A further study should be carried out to test the extracts and compounds against genus *Biomphalaria* snails which cause *schistosoma mansoni* or fecal schistosomiasis.
- A study should be carried out to evaluate the miracidiacidal and cercariacidal properties of the extracts and compounds.
- Spectroscopic [x-ray crystallography, NMR, Mass] analysis should be carried out on the isolated compounds to determine their structure.

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