SOME PHARMACOLOGICAL AND TOXICOLOGICAL ACTIVITIES OF SOME PLANTS USED IN TRADITIONAL MEDICINE IN MWANZA, TANZANIA

BY

SHAABANI MSHAMU

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ABSTRACT

Three medicinal plants, *Albizia anthelmintica, Salvadora persica* and *Vernonia amygdalina* used in traditional medicine in Magu district, Mwanza were investigated for antimicrobial and anthelmintic activity. Informal interviews carried out in two villages of Nassa ginnery and Mwanangi, revealed several plants used in traditional medicine to treat humans and livestock diseases. These plants were alleged to be effective in treating abdominal pains, worm infestations including schistosomiasis, malaria and eye conditions. Toxicity studies and phytochemical screening of the study plants were also carried out. Antimicrobial activity study showed highest zone of inhibition with *B.subtilis* (23 mm) MIC = 7.8125 mg/ml) for the aqueous extract of *Vernonia amygdalina*. The ethanolic extract of the same plant had highest zone of inhibition of 26 mm, MIC = 62.50 mg/ml with *B.anthracis*. The other two plants tested showed some activity against some of the tested bacteria with decreased zone of inhibition and higher MIC values. In the *in vitro* anthelmintic activity studies, aqueous extract of *Albizia anthelmintica* had highest activity against the egg hatchability and larval survival. The highest percentage hatchability inhibition was 94.67%. The antischistosmal activity of *S. persica* in naturally infected calves showed 77.47% faecal egg count reduction. The brine shrimp lethality assay gave LC_{50} values greater than 20 μgml^{-1}, which is the cut-off point for a substance to be regarded as acutely toxic to biological systems. The phytochemical screening of the study plants, revealed the presence of tannins, flavonoids, terpenoids, cardiac glycosides and saponins. From the findings of this study it can be concluded that, the study plants contains some bioactive principles

which may be responsible for their alleged activities. The study confirms some of the alleged effects of the studied plant preparations. Traditional medicine preparations from the studied plants have been proved efficacious and safe; this justifies their continued use without adverse effects.

DECLARATION

I, Shaabani Mshamu, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been nor concurrently being submitted for a higher degree award in any other university.

…… ……

Shaabani Mshamu Date

(**MVM candidate**)

The above declaration is confirmed

…… ……..

Prof. R.D. Mosha Date

(Supervisor)

…… ……

Prof. AA Kassuku Date

(Supervisor)

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LIST OF ABBREVIATIONS AND SYMBOLS

TMP	-	Traditional Medicine Practitioner
TM	-	Traditional Medicine
WHO	-	World Health Organization
OAU	-	Organization of African Unity; currently African Union
DMSO	-	Dimethyl Sulphoxide
MIC	-	Minimum Inhibitory concentration
MUHAS	-	.Muhimbili University of Health and Allied Sciences
Ml	-	millilitre
µg	-	microgramme
µl	-	microlitre
cfu	-	colon forming units
etc	-	et cetera
INT	-	.Iodo Nitro Tetrazolium
URT	-	United Republic of Tanzania
rpm	-	revolutions per minute
VicRes	-	Lake Victoria Basin Research Initiative
WAAVP	-	World Association for Advancement of Veterinary Parasitology

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Traditional medicine has been in practice for years, and according to the World Health Organization, more than 80% of the world population relies on traditional medicine for their primary health care needs (WHO, 2002). The use of medicinal plants in traditional medicine is well known in rural areas of many developing countries (Veeramuthu *et al*., 2006; Prabuseenivasan *et al*., 2006). In Tanzania, traditional medicine is practiced more today than in the past. This rise is due to several reasons; the most important is poverty especially for the rural resource poor communities. The process which culminated into passing of the Traditional and Alternative Medicine Act in 2002, by the Parliament of the United Republic of Tanzania (URT), which became operational in 2005 (Strangeland *et al*., 2008), replacing the old laws, has also contributed to the development of traditional medical practices.

Ignorance and misconception have also contributed to the popularity of traditional medicine; some people have been made to believe that some ailments cannot be cured in the hospitals where modern allopathic medicine is practiced (Muella *et al*., 2000). These reasons, and the ever-increasing cost of health care for both people and livestock and the fact that increasing number of diseases continues to hit our communities, have forced people to resort to traditional medicine. It is worth mentioning that, the prosperity of traditional medicine stands on the perception that, its use of natural products and methods of treatment, are gentler and less hazardous

than those employed by conventional allopathic medicine (Abere and Agoreyo, 2006). Traditional medicine is therefore, believed to represent an economic and safe alternative to disease treatment for both people and livestock in the resource poor communities (Rojas *et al*., 2006).

From the number of diseases that compounds from plants are capable of treating, it is clear that plants have diverse pharmacological properties, ranging from anthelmintic, antibacterial, antiviral, antifungal, anticancer, insecticidal, antidiabetic to immunomodulatory, etc. It is now known that, increasing numbers of modern commercial medicines are derived from plants, like Quinine (*Cinchona ledgeriana*), Quinidine (*Cinchona ledgeriana*), Vincristine (*Cantharanthus roseus*), Artemisinin (*Artemisia* spp), Azadirachtin (*Azadirachta indica*), Pyrethrins (*Chrysanthemum* spp) and Rotenone (*Lonchocarpus nicou, Derris elliptica*) to mention just a few examples.

Being a tropical country, Tanzania is rich in an array of plant species, which have different importance and uses. While some are used as building materials, others are used as food crops, while others are poisonous and yet others are used as medicines against various ailments inflicting human and animals. Large numbers of plant species are multipurpose in their use (Ruffo *et al*., 2002). The traditional healers and others, who practice traditional medicine, use medicinal preparations derived from various plants species. Most of these plant-derived medicines are administered and used as crude preparations. The fact that, there are a large number of plants used as medicines with different potencies and efficacies, and there are hundreds if not

thousands of traditional healers scattered throughout the country, it is difficult to undertake authentication of the products they administer to their patients and /or clients in a single study. The effective authentication can only be done by taking small number of plants alleged to possess medicinal properties and taking each zone or region at a time as study area.

Among the plants used by the people of Lake Victoria basin are; *Salvadora persica*, *Vernonia amygdalina* and *Albizia anthelmintica*. *Salvadora persica* L. (Salvadoraceae) is widely distributed in Africa, and Asia. In Tanzania, it is found almost in every district and has different uses (Mbuya *et al*., 1994). Various plant parts are edible, in addition, it is believed to possess antimicrobial and other medicinal properties and is important in dental hygiene thus, the plant has been used as toothbrush for centuries (Ruffo *et al*., 2002). *Vernonia amygdalina* (Compositae) is a shrub or small tree of 2 – 5 m. The leaves are green with a characteristic odour and a bitter taste. The roots and the leaves are used in traditional medicine for treatment of fever, kidney problems and stomach discomfort (Ojiako and Nwanjo, 2006; Erasto *et al*., 2007). *Albizia anthelmintica* Brongn (Mimosaceae) is a deciduous shrub or small tree, ranging in height from 3 – 11 m. It has a characteristic grey bark, which can be smooth or deeply reticulated in texture. Various communities in many parts of East Africa have been reported to use infusion of bark decoction against a range of intestinal parasites in both humans and livestock (Galal *et al*., 1991a, b; and Koko *et al*., 2000).

The rapid growing economic importance and use of traditional medicine cannot be overemphasized (Bussmann and Sharon, 2006). In developing countries like Tanzania, traditional medicine has remained as the only accessible and affordable treatment available to the majority resource poor communities. It is known that people use medicines from traditional healers (Prabuseenivasan *et al*., 2006). Most traditional healers administer plant preparations and sometimes, combinations of plant preparations as medicines; therefore, there is a need to screen these plants for their efficacy.

Many plants and their preparations used in traditional medicine have been investigated for their chemical composition and pharmacological activities. Nevertheless, due to the vastness of our country, also the large number of the traditional healers, the medicinal uses of these plants and their preparations differ from one place to another. It is therefore important to study these plants to substantiate the traditional medicinal knowledge; this is only possible by taking a small locality and a number of plants in each study. Therefore, as a part of the on going ethnoveterinary project on medicinal wetland plants in the Lake Victoria basin through the support of Lake Victoria Basin Research Initiative (VicRES), the present study considered three plant species (*Salvadora persica* L.; *Vernonia amygdalina* Del; and *Albizia anthelmintica* Brongn), used in traditional medicine in Magu district, Mwanza. It is expected that, the output from this study will help at providing the scientific based evidence on the alleged activity of these plants.

1.2 Objectives

1.2.1 Main objective

The main objective of this study was to evaluate the extracts from different parts of *Salvadora persica* L., *Vernonia amygdalina* Del., and *Albizia anthelmintica* Brongn for their activities especially anthelmintic, antimicrobial, antischistosomal and brine shrimp lethality test. In the process, the extracts were also being tested for the acute toxicity using brine shrimp lethality assay.

1.2.2 Specific objectives

1.2.2.1. Determination of anthelmintic activity of the commonly used extracts of the study plants.

1.2.2.2. Determination of antimicrobial activity of commonly used extracts of the study plants against selected standard bacterial cultures.

1.2.2.3. Evaluation of Brine Shrimp Lethality of the extracts of the study plants.

1.2.2.4. Identification of the bioactive constituents in the study plants by phytochemical analysis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Traditional Medicine

2.1.1 The Traditional Medicine in the World

Traditional medicine is used globally and has a rapidly growing economic importance. In developing countries, traditional medicine is often the main accessible and affordable treatment available (Bussmann and Sharon, 2006). In Africa up to 80% of the population uses traditional medicine as their primary healthcare system (WHO, 2002). In Latin America, the WHO Regional Office for the Americas (AMRO/PAHO) reports that 71% of the population in Chile and 40% of the population in Colombia uses traditional medicine. In many Asian countries, traditional medicine is widely used, even though western medicine is often readily available. In Japan, 60–70% of allopathic doctors prescribe traditional medicines for their patients, and in China, traditional medicine accounts for about 40% of all health care. The number of visits to providers of traditional medicine now exceeds by far the number of visits to all primary care physicians in the US (Bussmann and Sharon, 2006). Traditional medicine has gained more respect by national governments and health providers (Muella *et al.*, 2000).

The World Health Organization has defined traditional medicine as the sum total of knowledge or practices whether explicable or inexplicable, used in diagnosing, preventing or eliminating a physical, mental or social disease which may rely exclusively on past experience or observations handed down from generation to generation, verbally or in writing. It also comprises therapeutic practices that have

been in existence often for hundreds of years before the development of modern scientific medicine and are still in use today without any documented evidence of adverse effects (WHO, 1978a, b). According to the World Health Organization, about 80% of the world population relies on traditional medicine for their primary health care needs (WHO, 2002), and the use of medicinal plants in traditional medicine is well known in rural areas of many developing countries (Veeramuthu *et al*., 2006; Prabuseenivasan *et al*., 2006).Traditional healers and the elders are the ones entrusted by the communities to cater for the health of animals and man. They harbour the knowledge and are responsible for diagnosis and prescription against disease conditions inflicting these communities. Traditional medicine is therefore, believed to represent an economic and safe alternative to disease treatment for both people and livestock in these communities (Rojas *et al*., 2006).

2.1.2 Traditional medicine in Tanzania

In Tanzania, like all African countries, traditional medicine(TM) is a well-established system of medicine, parallel to the western or orthodox medicinal system, and it is still in active use especially by rural communities (Muella *et al*., 2000; Strangeland *et al*., 2008). The accessibility to conventional medical doctors is very low (1:33,000) compared to that of traditional medicine practitioners (TMPs) (1:350–450) (IRIN, 2006). Due to lack of proper conventional health care systems, TM is often the first choice for providing primary health care (Strangeland *et al*., 2008).

During colonial era, colonial powers connected TM to the use of supernatural forces or witchcraft, and TM was subject to discredit and legal bans. During the 1990s several conferences and meetings on traditional medicine were held in Tanzania and other African countries, starting with the International Conference of Experts from Developing Countries on Plants, Arusha, in February 1991. In a Meeting of the Inter-African Experts Committee on African Traditional Medicine and Medicinal Plants in the then Organization of African Unity (OAU), the Decade of African Medicine was proclaimed from 2001 to 2010 (Mahunnah, 2002), and the African Traditional Medicine Day was set to be on the 1st of September and Tanzania celebrated the day for the first time in 2003. However, it is during this period the Government of Tanzania put forth legislation to address national health needs, traditional knowledge, and the resource base for TM (e.g., practitioners, biodiversity) (Strangeland *et al.,* 2008). The whole process culminated into the Parliament of the United Republic of Tanzania to pass "The Traditional and Alternative Medicine Act" in 2002, which became fully operational in 2005, thus replacing the old laws. The new legislation aims at integrating traditional medicine into the national health care system, and encourages cooperation between traditional healers and physicians. It also provides protection to the traditional healers' interest. Since then, the traditional medicine is under the Ministry of Health and Social Welfare.

Since 1986, the country has been through a number of Economic Recovery programmes (Strangeland *et al*., 2008). The public health care sector introduced a cost- sharing system in early 1990s, as part of the then ongoing multisectoral

economic reform process in the country. The health sector policy continues to promote community-based, cost sharing health care system and the administration of essential medicines including traditional medicines.

In a typical rural Tanzanian community, a number of factors affect the choice of treatment modality; which are better explained with reference to the social context of illness and its treatment. Diseases are classified into two major groups; the first category is the normal diseases, which includes locally recognized conditions like malaria, schistosomiasis, diarrhoeaic conditions and worms. These are said to belong to the hospital with its specialized personnel and equipments. This view does not hold in case of childhood illnesses and female reproductive conditions where traditional medicine is preferred. The other category is the out of the order conditions, which includes conditions like barrenness, impotence, mental disturbances and chronic debilitating conditions. These can not be attended at the hospital; they can only be treated by traditional medicine practitioners (Muella *et al*., 2000).

Since the mid-1980s Tanzania adopted a different economic policy with regard to animal production, which is characterised by moving away from an economy largely planned and controlled by the government to sharing the tasks of delivering veterinary services with the private sector (Melewas and Rwezaula, 1999). Since then, the role of the government has been to focus on provision of essential functions of public interest in nature such as research, training, monitoring and extension. Due to privatization and commercialisation of veterinary services, there is

a remarkable increase in veterinary pharmacies in towns, drug shops in rural areas, influx of many brands of veterinary drugs and para-veterinarians (Keyyu *et al.*, 2003). But these changes were not all positive for the resource poor rural livestock keepers. Because of the unregulated trend, the prices of the veterinary inputs became high and unaffordable to the livestock keepers; hence forced to look into alternatives means to take care of their livestock. The traditional veterinary medicine, also known as ethnoveterinary medicine is practiced mainly by those ethnic groups who are largely involved in livestock keeping. The Maasai are well known for their richness in ethno-veterinary knowledge (Minja, 1994).

Over centuries survival of ethnoveterinary and other traditional knowledge systems in many developing countries including Tanzania has relied almost exclusively on 'passing on' of information from one generation to the next by word of mouth (Minja, 2004). There have been several initiatives to promote the development of medical and veterinary knowledge and practices, among them are the National policies (Agriculture and Livestock Policy; the Health policy and Forestry policy), development of the Tanzania Indigenous knowledge Database, the FAO-LinKS Project (Minja, 2004).

2.2 The Study Plants

2.2.1 Albizia anthelmintica Brongn

Authority: Brongn

Family: Mimosaceae

Common names: Sukuma (Ngh'otabalashi)

2.2.1.1 Botanic description

Albizia anthelmintica is a deciduous shrub or small tree, ranging in height from 3-11m. It has a characteristic grey bark, which can be smooth or deeply reticulated in texture. Pinnae in 1-2 pairs, leaflets in 1-4 pairs, obliquely obviate or subcircular, apex mucronate, 10-40 by 6-35 mm usually glabrous (Thoithi *et al*., 2002). Flowers usually on leafless twigs, white or yellow, staminal tube not or hardly exserted. Fruit glossy pale brown, 7-18 by 1.5-2.8 cm, usually glabrous. Pod papery, straw-coloured, 3-5 seeded.

2.2.1.2 Natural Habitat

The plant is common on dry bushlands, lava or along seasonal rivers, less often in wooded or bushed grassland or woodland; rarely in evergreen coastal bushland.

2.2.1.3 Phytochemical analysis

Compounds, which have been isolated from the plant, include amino acid derivatives histamine and related imidazole compounds, the saponins musennin and deglucomusennin and echinocystic acid saponins (Kakeru *et al.,* 2008; Galal *et al.*, 1991 a, b)

2.2.1.4 Uses of the plant

A decoction of the stem bark or root is used as an anthelmintic, mainly for tapeworm. The plant is also used as a purgative, sexual stimulant for women, to prevent pregnancy and haemorrhage after birth, for treating malaria, syphilis, gonorrhea, rheumatism, amoebic dysentery and fever (Thoithi *et al*., 2002).Various

communities in many parts of East Africa, have been reported to use infusion of stem or root bark decoction against a range of intestinal parasites in both humans and livestock (Galal *et al.*, 1991a, b; and Koko *et al*., 2000).

2.2.2 Salvadora persica L.

Authority: Linneus (L).

Family: Salvadoraceae

Synonym(s): *Salvadora cyclophylla* Chiov, *Salvadora indica* Wight, and *Salvadora wightiana* Planch

The generic name was given in 1749 in honour of an apothecary of Barcelona, Juan Salvadory Bosca (1598-1681), by Dr Laurent Garcin, botanist, traveller and plant collector. The true specimen of this species came from Persia, as the specific name indicates (Almas *et al*., 2005).

Common names: Arabic: *arrak, siwak*; English: *mustard tree, saltbush, and toothbrush tree*; and Swahili: *mswaki, musuake,* and *msuake*; Sukuma: *mswake.*

2.2.2.1 Botanic description

Salvadora persica is an evergreen shrub or small tree 6-7 m in height; main trunk erect or crawling and profusely branched, wide crown of crooked, straggling and drooping branches; young branches green in colour; bark slightly rough, grayish-brown on main stem, paler elsewhere. Leaves oblong-elliptic to almost circular, light to dark green, rather fleshy, sometimes with wartlike glandular dots and dense, rather loose hairs; apex broadly tapering to round, sharp-tipped; base broadly

tapering; margin entire; petiole up to 10 mm long; leaves in opposite pairs. Flowers are greenish to yellowish, very small in loose, axillary slender-branched or terminal panicles, up to 10 cm long. Fruit spherical, fleshy, 5-10 mm in diameter, pink to scarlet when mature, and single seeded; seeds turn from pink to purple-red and are semi-transparent when mature (Mbuya *et al*., 1994).

2.2.1.2 Natural Habitat

S. persica is widespread, notably in thorn shrubs, desert floodplains, river and stream bank vegetation, and grassy savannahs. The plant prefers areas where groundwater is readily available, by riverbanks, on perimeters of waterholes, in seasonally wet sites, and along drainage lines in arid zones. Also, found in valleys, on dunes and termite mounds (Mbuya *et al*., 1994; Ruffo *et al*., 2002). The tree is able to tolerate a very dry environment with mean annual rainfall of less than 200 mm. Due to the fact that the plant is highly salt tolerant, it can grow on coastal regions and saline soils. Altitude: 0-1800 m. Prefers clays, but is found on loam, black soils and sand soil types. The plant is adapted to alkaline or very saline soils, usually clay-rich, and soils without salt (Mbuya *et al*., 1994).

2.2.2.3 Phytochemical analysis

Several phytochemicals are known to occur in *S. persica*, these includes essential oils, alkaloids, carbohydrates, steroids, terpenoids, saponins, flavonoids, glycosides, amino acids and inorganic constituents (Almas *et al*., 2005).

2.2.2.4 Uses of the plant

The fruits of this plant are edible; leaves are cooked as a sauce and eaten as green vegetable. Tender shoots, seeds and seed oil are also edible. Edible salts are obtained from its ashes. Leaves and young shoots are browsed by all stocks. Toothbrushes made from roots and small branches of about 3-5 mm diameter have been used for over 1000 years, especially by populations in India, Arabia and Africa (Mbuya *et al*., 1994). Several agents occurring in the bark and wood have been suggested as aids in dental care, relieving toothache and gum disease, suppressing bacterial growth and the formation of plaque. A decoction of the root is used to treat gonorrhoea, spleen trouble and general stomachache. Roots are also used for chest diseases or pounded and used as a poultice to heal boils. The bark is scratched and the latex used for treating sores. Seeds are used as a tonic, and seed oil is used on the skin for rheumatism (Hines and Eckman, 1993; Mbuya *et al*., 1994).

2.2.3 Vernonia amygdalina Del.

Authority: Del

Family: Asteraceae

Common names: Sukuma: *Bhukoka, Mbilizi*; Haya: *Mubilizi*.

2.2.3.1 Botanic description

Vernonia amygdalina is a small tree or shrub, growing up to 10 m tall. It has somewhat coarse and rough leaves, which are alternate, simple, green above and pale below, ovolanceolate, up to 20 cm long with regularly toothed margins. The bark colour ranges from rusty to dark-brown, slightly fissured and sometimes much

branched, the young branches have numerous white breathing pores (lenticels). The flowers white, tinged purple or pink and sweetly scented particularly in the evening, arranged in numerous heads at the end of the branches (Erasto *et al*., 2006).

2.2.3.2 Natural Habitat

Vernonia amygdalina is found in a wide range of bushlands, often associated with termite mounds, woodland, river fringes, and forest habitats at altitudes from sea level up to 2800 m, it is also often found around houses where it is used as fencing. Its rainfall range is from 750 to 2000 mm per year. It is widely distributed in Africa and it is found in Angola, Burundi, Cameroon, Central African Republic, Congo, Cote d'Ivoire, Eritrea, Ghana, Guinea, Kenya, Liberia, Malawi, Mali, Nigeria, Rwanda, Sierra Leone, Sudan, Tanzania, Uganda, Yemen, Zaire, Zambia and Zimbabwe (Erasto *et al.*, 2006).

2.2.3.3 Phytochemical analysis

Phytochemical analysis of the plant material have revealed the presence of anthracene glycosides, sesquiterpene lactones, steroids, flavonoids, proteins, carbohydrates, reducing sugars, saponins and tannins (Igile *et al.*, 1995; Babalola *et al.*, 2001; Ojiako and Nwanjo, 2006; Erasto *et al*., 2007). Members of the genus *Vernonia* are good sources of sesquiterpene lactones. This class of compounds has been reported to be insect antifeedant, antifungal, cytotoxic and antitumour (Krishna *et al*., 2003; Erasto *et al*., 2007). Several sesquiterpene lactones have been identified from *Vernonia amygdalina*; these include vernolide, vernolepin, vernodalin,

hydroxyvernolide, vernoamygdalin and steroid glycosides like vernonioside B1 and vernoniol B. (Koshimizu *et al*., 1994; Erasto *et al*., 2007).

2.2.3.4 Uses of the plant

All parts of the plant are pharmacologically active. The stem and root (after removing the bark) are used as chew-sticks (Ojiako and Nwanjo, 2006). *Vernonia amygdalina* is probably the most used medicinal plant in the genus Vernonia. The most documented medicinal uses of this plant include the treatment of helmithiasis, amoebic dysentery and gastrointestinal problems (Huffman *et al.*, 1996). It is also used in the treatment of malaria, venereal diseases, wounds, hepatitis and diabetes (Hamill *et al*., 2000; Kambizi and Afolayan, 2001). The roots and the leaves are used in ethnomedicine to treat fever, hiccups, kidney problems, menstruation pain and stomach discomfort (Nwanjo, 2005). Both aqueous and alcoholic extracts of the stem bark, roots and leaves are reported to be extensively used as a purgative, antimalarial and in the treatment of eczema for maintaining healthy blood glucose levels. Having been proved in human medicine to possess potent antimalarial and anthelminthic properties (Abosi and Raseroka, 2003) as well as antitumorigenic properties (Izevbigie *et al*., 2004), the plant has gained great popularity. As it is easily recognized and used for self-medication by parasitized chimpanzees (Huffman, 2003), the plant is believed to posses an amazing antiparasitic activity in zoopharmacognosy. Pharmacological studies in experimental animals have shown that the leaf extract has both hypoglycaemic and hypolipidaemic properties and so could be used in managing diabetes mellitus (Akah and Okafor, 1992).

2.3 Helminthosis

Parasitic helminthes affect animals and man, causing considerable loss due to stunted growth in animals and the cost of controlling the infections for both animals and man (Iqbal *et al*., 2003). In tropical and subtropical regions like Tanzania, where the parasites are more abundant due to favourable environmental conditions, helminthosis is even more devastating (Waller, 1997; Keyyu *et al*., 2003).

Most diseases caused by helminths are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. In animals, the prevalence of helminth diseases is very high, especially during the wet season (Makundi *et al*., 1998; Iqbal *et al*., 2003; Keyyu *et al*., 2003). Trematode and gastrointestinal nematode infections are known to be highly prevalent in most livestock keeping areas of Tanzania (Makundi *et al*., 1998; Keyyu *et al*., 2003). The most commonly encountered nematodes in Tanzania includes; *Haemonchus contortus, Trichostrongylus colubriformis*, *Oesophagostomum columbianum*, *Bunostomum trigonocephalum, Strongyloides papillosus, Trichuris ovis* and *Moniezia expansa*. While the most commonly encountered trematodes are *Fasciolla* spp, *Amphistome spp,* and *Schistosoma* spp. (Makundi *et al*., 1998; Keyyu *et al*., 2005; Swai *et al*., 2006). Such high infection rates prevent them from attaining optimum productivity, especially under the traditional husbandry system (Iqbal *et al*., 2003).

The conventional method for control of helminthiasis is mainly by chemotherapy, with best results being obtained when integrated with proper grazing management

and resistant animals. The most commonly used anthelmintics in the livestock industry in Tanzania, including both traditional and commercial sectors are; Benzimidazoles (Albendazole Bolus®, Farbenda®, Tramazole®, Benzol®), Combination of Levamisole and Oxyclozanide (Milsan®, Milverm®, Wormicid Bolus® , Wormicid Drench), Levamisole alone (Levoxy®), Nitroxynil (Trodax®) and Endectocides (Ivermectin), Ivermectin and Clorsulon(Ivomec Super®) (Ngomuo *et al*., 1990; Keyyu *et al*., 2003; Swai *et al*., 2006). However, misuse of this approach in the last few decades due to over-dependency and indiscriminate use have led to consequent evolution of anthelmintic resistance especially among major nematode species (Ngomuo *et al*., 1990; Prichard, 1994). Together with the problem of anthelmintic resistance, in the livestock sector also there is a problem of poor availability and affordability of anthelmintics to small-scale farmers in developing countries which adds on the problem (Hammond *et al*., 1997).

The increased growth of organic agriculture, which strongly restricts the use of synthetic products, adds on the need for alternatives for helminth control. Therefore, a search for alternative and more sustainable anthelmintics is the best approach to the control of helminthiasis. The search on prophylactic strategies such as grazing management, biological control with nematophagous fungi or food supplementation with leguminous plants accumulating high amounts of condensed tannins so far has been promising. Condensed tannins (CT) have been found to improve general performance of parasitised sheep (Max *et al*., 2002).

Plant anthelmintics have long been used and known in many countries worldwide including Tanzania, but little has been done to validate their use, especially in veterinary medicine. Some reports on plants with anthelmintic properties come mainly from Asia and Africa (Alawa *et al*., 2003). The tested plants include the seeds of *Azadirachta indica* A. Juss (Meliaceae), *Caesalpinia crista* L. (Caesalpiniaceae) and *Vernonia anthelmintica* (L.) Willd. (Asteraceae), the whole plants of *Fumaria parviflora* Lam. (Fumariaceae) and the fruits of *Embelia ribes* Burm (Myrsinaceae); *Nigella sativa* (essential oil); *Areca catechu* (The Areca nut); *Curcubita pepo* (seeds); *Zanthoxylum species*; *Acacia auriculiformis*; *Carica papaya* (seeds); *Chenopodium ambrosioides*; *Fructus mume*; *Picraena excelsa*; *Artemisia absinthium* (wormwood) *and Juglans nigra* (black walnut), these plants are well known to herbalists with particular application to treating nematode infestation.

2.4 Schistosomiasis

Schistosomiasis tops all the endemic parasitic diseases worldwide.

2.4.1 Aetiology

Schistosomiasis is caused by digenic trematodes of the superfamily schistosomatoidea. Adult worms inhabit the mesenteric veins (*S. mansoni*, *S. japonicum*, *S. mekongi* and *S. intercalatum*) or the veins of the vesical and pelvic plexuses (*S. haematobium*). Adult schistosomes live in pairs in the portal system and in the mesenteric venules; males are shorter (7-12 mm in length and 2 mm wide) and have a ventral infolding from the ventral sucker to the posterior end, forming the gynaecophoric canal (WHO, 1993). Females are slender (1 mm in diameter) and

longer (9-17 mm in length), and are held in the gynaecophoric canal during copulation. *Schistosoma bovis* is one of the *Schistosoma* species that usually occurs in the portal and mesenteric vessels of cattle, sheep, goats, sitatunga and the more rarely equines in S. Europe, S. Asia and Africa. *S. bovis* has been isolated from baboons in Zimbabwe. It is of little significance as zoonosis.

2.4.2 Epidemiology

Schistosoma bovis is endemic in 43 countries in Africa and occurs in the Americas in Brazil, Suriname, Venezuela and in the Caribbean (WHO, 1993).

2.4.3 Life cycle

The life cycle is common to all species with a sexual generation in vascular system of the definitive host and an asexual generation in the intermediate hosts (snails) (WHO, 1993). Embryonated eggs are discharged in faeces and urine; in water miracidia hatch from the eggs and penetrate the intermediate host: Different genera of snails: intermediate host of *S. bovis* are snails of the genus *Bulinus*. After penetration in the snail, the miracidium develops into sporocysts and, in about 5 weeks (35 days), thousands of cercariae are produced (asexual multiplication). Cercariae are the infective forms, the infection of the definitive host occurs by penetration of the skin, although experimental *per os* infection has been documented. After encountering the skin, the cercariae penetrate and lose the tail transforming into schistosomulae. After penetration schistosomula migrate to the lungs and elongate (in 3-4 days), after which they penetrate into the pulmonary capillaries where they are carried into the systemic circulation and into the portal

system. In the hepatic circulation, schistosomes mature to adult, and in pairs, they migrate to the mesenteric veins. After 42 - 56 days, embryonated eggs are excreted in faeces and/or urine and the cycle repeats.

2.4.4 Cultural characteristics

Under the laboratory conditions, schistosomes can be maintained from one generation to the next in the intermediate host snails and the laboratory animals i.e. guinea pigs and mice. The eggs in a faecal sample are incubated under illumination and heat to hatch into miracidia. Once obtained, the miracidia are used to infect the intermediate hosts i.e. snail species (*Bulinus* sp), after 30 -35 days incubation period, the snails start to shed cercariae which are used to infect the laboratory animals in which they will develop into adult schistosomes and the cycle can be repeated.

2.4.5 Diagnosis

The clinical signs taken alone will not suffice to arrive at a definite diagnosis, but they should indicate the necessity of faecal examination, which will reveal the eggs of the parasite (WHO, 1993).

2.4.7 Treatment

Currently, praziquantel chemotherapy is the mainstay of control of schistosomiasis. This dependence of a single drug to control disease raises concerns about the development of tolerance and/or resistance (Adam *et al*., 2006). Other alternatives which include the use of plant extracts have been investigated (Botros *et al*., 2004). The role of plants extract in the fight against schistosomiasis has been shown by the

discovery of Myrrh, an oleo-gum resin from the stem of *Commiphora molmol* plant. Treatment of schistosome infected mice with Myrrh, resulted in death of living *Schistosoma* ova in biopsy and proved very effective and safe in the treatment of *Schistosoma haematobium* infections (Botros *et al*., 2004). Extracts from plants like *Abrus precatorius* (Leguminosae), *Pterocarpus angolensis* (Leguminosae) and *Ozoroa insignis* (Anacardiaceae) have been shown to be lethal to adult schistosomes.

2.4.6 Control of infection

The priorities are: health education, the supply of safe and clean drinking water and the planning of adequate healthcare facilities, diagnosis and treatment, management of the environment, and control of the intermediate hosts (freshwater snails). Schistosomiasis control is far more effective when placed in the context of a general health system. The integration process is slow, but this "horizontal" approach is now becoming an integral part of health care at village level. Schistosomiasis prevention and control measures should be implemented before dam construction work begins. A control approach for each form of schistosomiasis varies and must be adapted to the epidemiological situation, available financial resources, and the particular local culture.

Health education on schistosomiasis has greater importance than ever before. The introduction into schools of diagnosis and treatment has made children and parents much more aware of the problem connected with disease. School teachers and local health workers are effective in explaining the role played by people in the

transmission of schistosomiasis. Campaigns in the mass media have proved particularly successful in increasing awareness of the need for diagnosis and treatment. The supply of safe drinking water is fundamental to schistosomiasis control. The beneficial results of chemotherapy - normally quite spectacular - are even more marked in communities with adequate water supplies. The high prevalence of schistosomiasis is clearly a reliable criterion to select communities for installing a clean water supply.

2.5 Brine shrimp, Artemia salina Leach

Brine shrimp of the genus *Artemia* are members of the fairly primitive crustacean sub-class Branchipoda, order Anostraca. They are found world-wide in saline lakes and ponds, and have truly remarkable mechanisms for surviving under harsh and variable environmental conditions. Survival depends in part on the formation of extremely resistant “eggs” (cysts). These “eggs” are, in fact, embryos arrested at the gastrula stage, and encased in chitinous shells. The eggs will not hatch unless they have first dried out to extremely low water content. In the dehydrated state, metabolism is completely arrested; the embryos are in a state of cryptobiosis. After this dehydration, exposure to water activates the *Artemia* eggs. In a few hours or days, larval *nauplii* emerge and swim actively by rhythmic movements of the head appendages. For a few days, the nauplius subsists on yolk; then begins to feed and develop through a series of molts and successive instars to the reproductive stage.

The most striking physiological capacity of *Artemia* is its ability to tolerate extremes of environmental salinity. *Artemia* can survive and even thrive in media ranging

from 1/2 the tonicity of sea water (about 1.5% NaCl) to concentrated brines in which salts actually crystallize out; this would be approximately at a 15% NaCl concentration.

2.6 Brine shrimp Lethality Assay

The brine shrimp, because of its characteristics and its short life span, has been useful to researchers in genetics, histology, toxicology, biochemistry, molecular biology, and ecology. Brine shrimp, *Artemia salina* has also been used in screening pharmacological activity of chemical compounds and plant extracts (Carballo *et al*., 2002). The organism has been used in laboratory bioassay of toxicity and other biological actions through estimation of medium lethal concentration (LC_{50} values).

The test for toxicity of chemicals against the brine shrimp *Artemia salina* was developed by Michael *et al*., 1956 and was adapted and improved by others (Meyer *et al*., 1982; McLaughlin *et al.,* 1991; Solis *et al*., 1993). Since the brine shrimp is highly sensitive to a variety of chemical substances, it is a convenient preliminary toxicity test. The brine shrimp lethality (BSL) bioassay has been shown to be a useful and quick *in vitro* test for predicting toxicity of plant extracts and guiding their phytochemical fractionation (Meyer *et al*., 1982).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study area

The field work (gathering the information on the preparation, uses of the study plants and plant materials collection) was conducted at Nassa ginnery and Mwanangi, both in Magu district, Mwanza. The laboratory work on preparation of plant materials and extracts, antimicrobial, anthelmintic activity studies and phytochemical screening was undertaken at the Faculty of Veterinary Medicine of the Sokoine University of Agriculture, Morogoro, Tanzania. The determination of minimum inhibitory concentration and the brine shrimp lethality assay were conducted at the Institute of Traditional Medicine (ITM), Muhimbili University of Health and Allied Sciences (MUHAS), Dar-es-Salaam, Tanzania. The field work on antischistosomal activity study of *S.persica* was done at Ipogolo, Tagamenda and Kitayawa villages of Iringa district; and the laboratory work conducted at the Veterinary Investigation Centre, Southern Highlands Zone, Iringa, Tanzania.

3.2 Plant sample collection

Informal interviews were conducted with traditional healers and elders in two villages in Nassa ward, Magu district during several visits to these villages. Questions on the plant type, parts used, method of collection and preparation, diseases treated by the preparations and dose were asked during these interviews. The villages were Mwanangi and Nassa ginnery. Many plants used in traditional medicine in these villages were mentioned during the interviews and from the list (Table 1) three plants were selected for this study. Preliminary identification of the

plants was done in the field by the traditional healers and/ or elders. Herbarium specimens were prepared and photographs taken for confirmation of the identification of the plants to species level. Different parts of the plants (roots for *A. anthelmintica* and *S. persica*; leaves for *V. amygdalina*) used in disease treatments were collected, properly packed and taken to Sokoine University of Agriculture where the activity studies were conducted. A botanist was consulted for the final identification of the plants.

3.3 Preparation and Extraction of plant material

3.3.1 Preparation of the root barks

The roots of *A. anthelmintica* and *S. persica* were separately cleaned of debris and the barks removed, chopped into small pieces when necessary and then air dried for 14 days under shade on a laboratory bench. The dried root barks (450 g of *A. anthelmintica* and 326 g of *S. persica*), were separately ground into fine powder using a laboratory mill (Christy Hunt Engineering Ltd, England) at the Department of Animal Science and Production (DASP), Sokoine University of Agriculture.

3.3.2 Preparation of the leaves

The leaves of *V. amygdalina* were cleaned of any debris, chopped into small pieces and then air dried for 14 days under shade on a laboratory bench. The dried leaves (513 g) were ground into fine powder using a laboratory mill (Christy Hunt Engineering Ltd, England) at the Department of Animal Science and Production (DASP), Sokoine University of Agriculture.

3.3.3 Preparation of plant extracts

3.3.3.1 Preparation of ethanolic extracts

The ethanolic extracts of the root barks of *A. anthelmintica* and *S. persica* and leaves of *V. amygdalina* were prepared by soaking 96 g of *A. anthelmintica* root bark powder, 45 g of *S. persica* root bark powder and 138 g of *V. amygdalina* leaves powder each in 250 ml of 80% ethanol each overnight at room temperature. Then, the mixtures were separately filtered using filter funnels fitted with Whatman® filter paper No. 1 (Whatman International Ltd, England). The filtrates were concentrated on water bath at 50°C using a rotavapor (BUCHI Labortechnik AG, Switzerland). The extracts were then freeze dried to obtain dry extracts using a freeze drier (Edwards High Vacuum International, England), which were stored in the refrigerator at 4 °C until use.

3.3.3.2 Preparation of aqueous extracts

The aqueous extracts of the root barks of *A. anthelmintica* and *S. persica* and leaves of *V. amygdalina* were prepared by boiling 96 g of *A. anthelmintica* root bark powder, 45 g of *S. persica* root bark powder and 138 g of *V. amygdalina* leaves powder each in 250 ml of distilled water and thereafter leaving the mixture to soak overnight at room temperature. Then, the mixtures were separately filtered using filter funnels fitted with Whatman® filter paper No. 1 (Whatman International Ltd, England). The filtrates were concentrated on water bath at 50°C using a rotavapor (BUCHI Labortechnik AG, Switzerland). The extracts were then freeze dried to obtain dry extracts using a freeze drier (Edwards High Vacuum International, England), which were stored in the refrigerator until use.

3.3.3.3 Preparation of the stock solutions

The stock solutions were prepared basing on the concentration of extracts which was able to inhibit microbial growth during the pilot studies. The stock solution 50% w/v for the ethanolic extract was prepared by dissolving 2 gram of the dry extract in 4 ml of Dimethyl Sulphoxide (DMSO), and that for aqueous extract (40% w/v) was prepared by dissolving 2 gram of the dry extract in 5 ml of DMSO. For the minimum inhibitory concentrations determination, 2 gram of dry extracts was dissolved in 4 ml of DMSO. The stock solution for the egg hatchability inhibition studies was prepared by dissolving 2 gram of dry extract in 5 ml of DMSO for the ethanolic extracts (40% w/v) and 0.5 gram of dry extract in 5 ml of DMSO for the aqueous extracts (10% w/v).

3.4 Laboratory materials

3.4.1 The solvents

The solvents used for extraction process were ethanol and distilled water. The ethanol (99.9%, Harris reagent, Philip Harris limited, Sheristone, England) was bought from a local dealer in Morogoro. The distilled water was obtained from the Protozoology Laboratory, Department of Veterinary Microbiology and Parasitology. Dimethyl Sulphoxide (DMSO) (Sigma Poole, Dorset, UK), that was used in preparing the working solutions of the extracts was also obtained from a local dealer in Morogoro Municipality.

3.4.2 The growth media for the microorganisms

Nutrient broth and nutrient agar (Oxoid Ltd., Basingstoke, Hants., England), and Saboraud's Dextrose Agar (Laboratorios CONDA., La Forja, 9-28850 Torrejon de Ardoz- Madrid (Spain), both were obtained from a local dealer in Morogoro Municipality.

3.4.3 The test organisms

Artemia salina Leach (*Artemia salina*, Sanders^{TM} Great Salt Lake, Brine Shrimp Company L.C., U.S.A.) was used for brine shrimp lethality bioassay. The eggs of *Artemia salina* were donated by Dr. M.J. Moshi of the Institute of Traditional Medicine, MUHAS. Standard strains of *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, were obtained from the Bacteriology and Mycology Laboratory, Department of Veterinary Microbiology and Parasitology, Sokoine University of Agriculture.

3.4.4 Helminth Eggs

Faecal samples, from which the nematode eggs used in the *in vitro* anthelmintic activity studies, were obtained from goats, from a farmer at Kihonda, a suburb of Morogoro municipality. The goats had no history of recent deworming.

3.5 Laboratory methods

3.5.1 Anthelmintic Activity of the study plants

3.5.1.1 Egg hatchability inhibition (in vitro)

Isolation of eggs

Faecal samples were collected from the recta of a number of goats with no history of recent deworming. Once in the laboratory, the collected faecal samples were ground and mixed with clean tap water to form a dense homogenous suspension. The suspension was then filtered through tea strainer to remove the faecal debris. The filtrate was filtered through a series of sieves, the eggs being collected on a 25 micron sieve. The retained sample in the 25 micron sieve was washed into the test tubes then centrifuged at 3000 rpm for five minutes. The supernatant was discarded, and the remaining deposit resuspended in saturated sucrose solution, the tubes being filled to the brim and cover slips applied. This was centrifuged at 1000 rpm for one minute, and then the cover slips removed and washed into a 25 micron sieve using distilled water to collect the eggs. The eggs were then repeatedly rinsed with distilled water before being transferred into clean graduated tubes. From this, an estimate of the concentration of the eggs was determined; this was followed by dilution with distilled water to about 50 eggs per 50 µl.

Egg Hatch Assay (EHA)

The method of Coles *et al* (1992) was used, using flat-bottomed microtitre plates. About 50 µl of distilled water was put into each well from the second row to the eighth. Then 100 µl of the test extracts were added into the first wells, by drawing 50 µl from the first well into the second well and repeating this from the second well

onwards until the last well, serial dilutions were carried out to give solutions containing 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml of the aqueous extracts, and 400, 200, 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml of the ethanolic extracts, this was done in triplicates. Control wells containing distilled water were included in the experimental set up. A 50 µl volume containing 50 helminth eggs was added into each well, and then incubated at 27 °C for 48 hours. At the end of incubation, the contents of each well were observed under a compound microscope at 4 power magnification. The number of eggs, which had not hatched and number of live and dead hatched larvae were counted and recorded.

3.5.2 Antischistosomal activity of Salvadora persica

A total of 63 cattle from different owners in Ipogolo, Tagamenda and Kitayawa villages of Iringa district, were screened for schistosome eggs using the miracidia hatching technique; 19 cattle of different ages were found to be positive for schistosomiasis. The egg count per gram from faecal samples from each of 19 animals was obtained using the filtration technique, with slight modification. Out of the 19 animals, 16 were divided into three groups based on the owner of the animals. Animal from Diwani (Tagamenda) and Constantino (Kitayawa) formed group 1 (Test group; n = 6), animals from Mama Chengula (Kitayawa) formed group 2 (Positive control group; n = 4) and animals from Steven (Ipogolo) formed group 3 (Negative control group; n = 6).

Animals in group 1 (Test) were drenched with 10% w/v *S. persica* root bark extract, while those of group 2 were drenched with 10% w/v praziquantel and those in group

3 did not receive anything. All the animals were left to graze with the rest of animals and after 11 days post treatment the faecal samples were collected from the 16 animals and schistosome eggs counted. The formula of Coles *et al* (1992) was used to calculate faecal egg count reduction (FECR);

$$\mathbf{FECR\% = 100 \times (1 - T/C)}$$

Where T and C are the geometric means of the faecal egg count (FEC) of the treated and control groups.

From the same data, the lower confidence limit (95% CI) was determined using the formula;

$$\mathbf{100(1 - (T/C)^{(+2.1\sqrt{\sigma})}}$$

Where T and C are the geometric means of the faecal egg count (FEC) of the treated and control groups and σ is the variance.

3.5.3 Antimicrobial activity of the study plants

3.5.3.1 Agar well diffusion method

The agar well diffusion method was used to test the antimicrobial activity of the study plant extracts. The nutrient agar plates were prepared by pouring 15 ml of warm molten media into sterile Petri dishes and allowed to cool. The bacterial isolates were inoculated into nutrient broth and incubated at 37ºC for about 6 hours after which the nutrient agar plates were seeded with the test microorganisms by the

spread plate technique, and was left for about 30 minutes to dry (Ayo *et al*., 2007). After allowing the plates to dry, wells with 6 mm diameter were punched on each agar plate. The wells were numbered accordingly to match with the code number of test extract (1=*A. anthelmintica*, 2=*S. persica*, 3=*V. amygdalina*), this was done in duplicate. Then, the extracts were poured into wells, while matching the well number with the corresponding code number of the extract. After about 15 minutes on the laboratory bench, the plates were incubated at 37°C for 24 hrs. The assessment of antimicrobial activity was based on measurement of the diameter of the inhibition zone formed around the wells.

3.5.3.2 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was evaluated on plant extracts which showed some antimicrobial activity. MIC, the lowest concentration of a compound that inhibits growth of a microorganism, was determined by the standard two-fold dilution technique using microdilution technique with nutrient broth medium (Obi *et al*., 2007). Serial dilutions of 100 µl of the extracts were made in microtitre wells with 50 µl nutrient broth to obtain a concentration ranging from 500 to 7.8125 mg/ml. A 0.5 McFarland standard suspension of test bacteria was made in nutrient broth, from which 100 µl of the final inoculum containing approximately 1.0 $x10^8$ colony forming units (cfu) was added to the appropriate wells to make a final volume of 200 µl in each well. Inoculated plates were incubated at 37°C for 24 h. One hour before the end of incubation 40 µl of a 0.2% solution of Iodo-Nitro Tetrazolium (INT) (Merck, Germany) was added to the wells and the plates were incubated for another hour. Since the colourless tetrazolium salt is reduced to a red

coloured product by biologically active organisms, the inhibition of growth can be detected when the solution in the well remains clear after incubation with INT (Samie *et al*., 2005; Obi *et al*., 2007). The test was done in duplicates. The mean of the lowest concentrations of each extract showing no visible growth was recorded as the minimum inhibitory concentration (MIC). Gentamicin was used as the positive control while the negative control comprised the test bacteria with DMSO.

3.5.4 Brine shrimp lethality assay of the study plants

3.5.4.1 Hatching the brine shrimps

Brine shrimp eggs (*Artemia salina*, Sanders^{TM} Great Salt Lake, Brine Shrimp Company L.C., U.S.A.) were hatched in simulated seawater prepared from sea salt, which was prepared by boiling seawater to evaporation. The simulated sea water was prepared by dissolving 3.8 g sea salt in one litre of distilled water. Rectangular glass chamber divided into two unequal compartments with holes on the divider was used for hatching. The eggs were sprinkled into the larger compartment that is darkened, while the smaller compartment was illuminated. After 24 hours incubation at room temperature, *nauplii* (larvae) were collected by Pasteur pipette from the lighted chamber, whereas their shells were left in the darkened chamber.

3.5.4.2 The Bioassay

The brine shrimp lethality test was carried out using the standard procedure as described by Meyer *et al* (1982) and McLaughlin (1991), with slight modifications. The stock solution of the study plants extracts were prepared by dissolving 160 mg of the dry extract in 4 ml of DMSO, to get a concentration of 40 mgml^{-1}. Taking 30,

15, 10, 5, 3, and 1µl of the stock solutions, the final concentrations of 240, 120, 80, 40, 24 and 8 μgml^{-1} were obtained by dilution with the 5 ml of the sea salt solution in the vials. Each concentration was tested in triplicate, 18 vials per test extract and one set of 18 vials were prepared using DMSO as a negative control (Moshi and Mbwambo, 2005; Moshi *et al*., 2006). Ten larvae of brine shrimps were transferred into each vial containing test extract with little sea salt solution using Pasteur pipettes, followed immediately with adjusting the volume of the sea salt solution to 5 ml mark. The vials were maintained at room temperature on a laboratory bench. Survivors were counted after 24 h and from these the percentage death at each concentration was determined (Meyer *et al*., 1982; McLaughlin, 1991).

3.5.5 Phytochemical screening of the study plant extracts

Chemical tests were carried out on the aqueous and ethanolic extracts and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993) and modified by Edeoga *et al* (2005).

Test for tannins: About 0.5 g of the dried powdered samples were boiled in 20 ml of water in test tubes and then filtered. A few drops of 0.1% ferric chloride were added to the filtrate and observed for a colour change. A brownish green or a blue-black colouration was taken as the evidence for the presence of tannins.

Test for phlobatannins: A portion of aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid (HCl), and then allowed to cool.

Deposition of red precipitate was taken as evidence for the presence of phlobatannins.

Test for terpenoids (Salkowski test): Five ml of aqueous extracts of the study plants was mixed in 2 ml of chloroform. 3 ml of concentrated sulphuric acid ($H_2S0_{4)}$ was then carefully added to form a layer. Formation of a reddish brown colouration at the inter- face was taken as the evidence for the presence of terpenoids

Test for flavonoids: 5 ml of dilute ammonia solution was added to a portion of the aqueous extract of each study plant, this was followed by addition of concentrated sulphuric acid (H_2S0_4). Formation of a yellow colouration which disappears on standing indicated the presence of flavonoids.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid (H_2S0_4). Formation of a brown ring at the interface indicates the presence of cardiac glycosides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the layer.

Test for steroids: Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample followed with 2 ml concentrated sulphuric acid ($H_2S0_{4)}$. A colour change from violet to blue or green was taken as an indication for the presence of steroids.

Test for saponins: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath, allowed to cool and then filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed. Formation of emulsion was taken as the evidence for the presence of saponins.

Test for anthraquinones: Powdered plant material was boiled with 10% HCl for a few minutes, filtered and allowed to cool. Then equal volumes of chloroform were added to filtrates, followed by addition of 10% ammonia solution. Formation of rose-pink colour in the aqueous layer was taken as the evidence for the presence of anthraquinones.

3.6. Statistical analysis

The percentage hatchability inhibition data from the *in vitro* anthelmintic activity study were calculated using Ms Excel. The results from the antimicrobial activity studies were expressed as mean zone of inhibition (mm). The percent efficacy of the extract against schistosomes between treated and control groups and between treated groups was compared by independent t-test.

The mean results of brine shrimp mortality against the logarithms of concentrations were plotted using the Fig P computer program, which also gives the regression equations. The regression equations were used to calculate LC_{50} values. Extracts giving LC_{50} values greater than 20 μgml^{-1}, were considered not acutely toxic (Yoga

Latha *et al*., 2007). From the same regression equation, the adjustment factor (f) was obtained which was used to calculate the upper and lower confidence limits.

CHAPTER FOUR

4. 0 RESULTS

4.1 Plant identification and collection

Table 1 show the number of plants which were claimed to have medicinal value by the interviewed traditional healers; from this list, three study plants were identified and collected. The plants collected were *Salvadora persica* L. (roots) (Plate 1), *Albizia anthelmintica* Brongn (roots) (Plate 2) and *Vernonia amygdalina* L. (leaves) (Plate 3).

Table 1: Some plants identified to have medicinal value by traditional healers in Magu district.

Botanical name	**Local name**	**Family**
Albizia anthelmintica	Ngh'otabalashi	Mimosaceae
Azadirachta indica	Mwarobaini	Meliaceae
Bidens pilosa	Idasana	Asteraceae
Combretum molle	Mlama	Combretaceae
Salvadora persica	Muche	Salvadoraceae
Securidata longipedunculata	Ng'watya	Polygalaceae
Solanum incanum	Matulatula	Solanaceae
Vernonia amygdalina	Bhukoka/Mbilizi	Asteraceae

Plate 1: *Salvadora persica* plant (top), roots (bottom left) and root bark (bottom right).

Plate 2: *Albizia anthelmintica* plant (top), roots (bottom left) and root bark (bottom right)

Plate 3: *Vernonia amygdalina* plant (top), dried whole leaves (bottom left) and dried chopped leaves (bottom right).

4.2 Anthelmintic activity of the study plants

Tables 2 and 3 below, shows the percentage inhibition of hatchability of strongyle eggs after incubation in aqueous and ethanolic extracts of *A. anthelmintica*, *S. persica* and *V. amygdalina* plants. Comparing the three plants at 10% w/v concentration of aqueous extracts, *A. anthelmintica* inhibited hatching of the eggs by 94.6%, followed by *V. amygdalina* which inhibited hatching of the eggs by 58.0% and lastly is *S. persica* which inhibited hatching of the eggs by only 18.7 % (p = 0.05)(Table 2). For the ethanolic extracts, at 40%w/v concentration, *A. anthelmintica* inhibited hatching of the eggs by 48% and *V. amygdalina* inhibited hatching of the eggs by 28.6% while *S.persica* had no activity on hatchability of the eggs(p = 0.07) (Table 3). The aqueous extracts inhibited hatching of eggs as well as development of larvae from L_1 to L_3. Ethanolic extracts had little effect on the nematode eggs and larval development as compared to that of aqueous extracts, although their concentrations were higher than those of aqueous extracts. Distilled water was included in the experimental set up as negative control.

Table 2: Percentage inhibition of hatchability by aqueous extracts of *A. anthelmintica*, *S. persica* and *V. amygdalina*

Extract	**Extract concentration/percentage inhibition**				
	10%	5%	2.5%	1.25%	0.625%
A. anthelmintica	94.67	86.33	48.67	14.00	2.00
S. persica	18.76	9.67	2.33	0.00	0.00
V. amygdalina	58.00	39.33	16.23	4.00	0.00
Distilled water (Control)	2.00	0.00	0.00	0.00	0.00

Table 3: Percentage inhibition of hatchability by ethanolic extracts of *A. anthelmintica, S. persica and V. amygdalina*

Extract	**Extract concentration/ percentage inhibition**						
	40%	20%	10%	5%	2.5%	1.25%	0.625%
A. antihelmintica	48.00	18.33	13.00	2.00	0.00	0.00	0.00
S. persica	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V. amygdalina	28.67	12.00	4.00	0.00	0.00	0.00	0.00
Distilled water	2.00	1.00	0.00	0.00	0.00	0.00	0.00

4.3 Antischistosomal activity of Salvadora persica root bark

The results for the antischistosomal activity of *S. persica* root bark aqueous extract are presented in Table 4 below. The aqueous extract of *S. persica* reduced the faecal egg count per gram by 78% with a lower confidence limit of 55 % (55 % at 95% CI), while praziquantel, a standard antischistosomal drug reduced the faecal egg count per gram by 96% with the lower confidence limit of 73% (73% at 95% CI).

Table 4: Antischistosomal activity of aqueous extract of *S. persica* root bark in cattle

S/N	Group	Preparation given	Epgf 0	Mean	Epgf 11	Mean
1	T	*S. persica*	12		0	
2	T		72		12	
3	T		63		15	
4	T		48		6	
5	T		30		12	
6	T		18	**40.50**	3	**8.00**
7	PC	Praziquantel	12		0	
8	PC		39		6	
9	PC		33		0	
10	PC		15	**24.75**	0	**1.50**
11	NC	NA	24		27	
12	NC		33		51	
13	NC		48		63	
14	NC		27		33	
15	NC		15		15	
16	NC		9	**26.00**	24	**35.50**

Key: **Epgf**= egg per gram of faeces; **Epgf 0**= pretreatment egg count; **Epgf 11**= post treatment egg count; **T**= test; **PC**= positive control; **NC**= negative control and **NA**= not applicable.

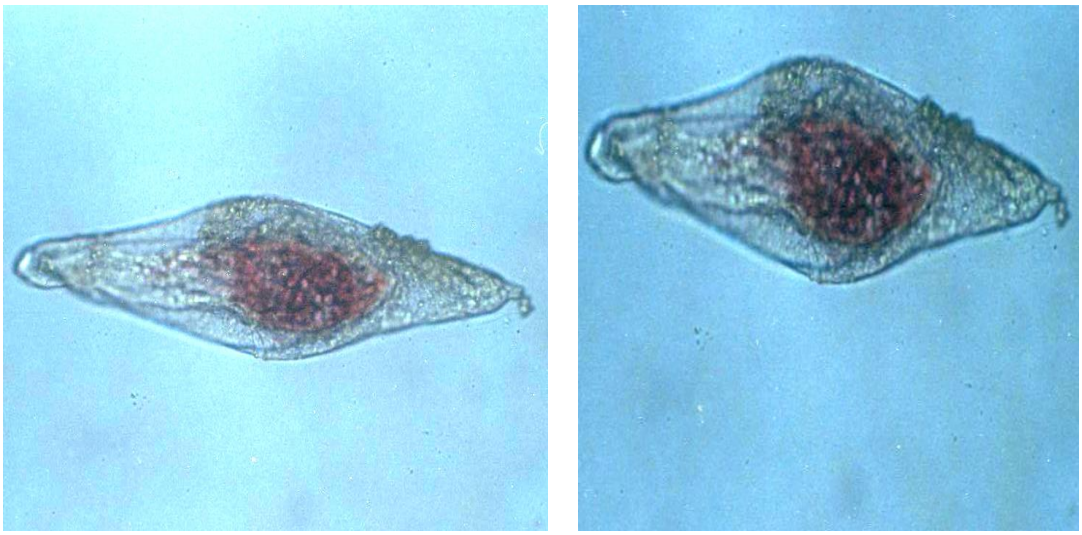

Plate 4: *Schistosoma bovis* eggs

4.4 Antimicrobial activity of the study plants

Tables 5 and 6, show the antimicrobial activity of aqueous and ethanolic extracts of the study plants based on the diameter of zone of inhibition. Gentamycin (30 μg) was included in the experimental set up as the positive control. The aqueous and ethanolic extracts of *Vernonia amygdalina* had activity against *Bacillus subtilis* (Plate 5), *Bacillus anthracis, Staphylococcus aureus* and *Vibrio cholerae* bacteria isolates (Table 5 and 6). The diameter of zone of inhibition being greater than that of a standard broad-spectrum antibiotic, gentamycin. The aqueous and ethanolic extracts of *S. persica* had some activity against *Vibrio cholerae* and *Staphylococcus aureus* (Table 5), while only ethanolic extract of *S. persica* had activity against *B. subtilis*. Aqueous extract of *A. anthelmintica* had little activity only against V. *cholerae*. Of all the extracts, only *S. persica* had some activity against *Candida albicans* with inhibition zone of 12 mm for aqueous extract and 9 mm for ethanolic extract.

Table 5: The antimicrobial activity of aqueous extracts of *A. anthelmintica, S. persica* and *V. amygdalina.*

Bacterial/ fungal isolates plants	**Susceptibility of bacteria to study plants**			
	Zone of inhibition (mm)			
	A	**B**	**C**	**D**
Bacillus subtilis	06	09	23	17
Bacillus anthracis	06	06	20	16
Vibrio cholarae	10	11	21	15
Staphylococcus aureus	06	13	22	15
Pseudomonas aeruginosa	06	06	06	14
Escherichia coli	06	06	06	13
Candida albicans	06	12	06	**NA**

Key: The diameter of zones of inhibition includes the well diameter (6 mm). **A**= *A. anthelmintica*; **B**= *S. persica*; **C**= *V. amygdalina*; **D**= Gentamycin. **NA** refers to Not Applicable.

Table 6: The antimicrobial activity of the ethanolic extracts of the study plants.

Bacterial/fungal isolates	**Susceptibility of bacteria/ fungi to study plants**			
	Zone of inhibition (mm)			
	A	**B**	**C**	**D**
Bacillus subtilis	06	19	22	16
Bacillus anthracis	06	06	26	19
Vibrio cholerae	06	14	25	20
Staphylococcus aureus	06	13	25	16
Pseudomonas aeruginosa	06	06	06	14
Escherichia coli	06	06	06	14
Candida albicans	06	09	06	**NA**

Key: The diameter of zones of inhibition includes the well diameter (6 mm). **A**= *A.anthelmintica*; **B**= *S.persica*; **C**= *V.amygdalina*; **D**= Gentamycin. **NA** refers to Not Applicable.

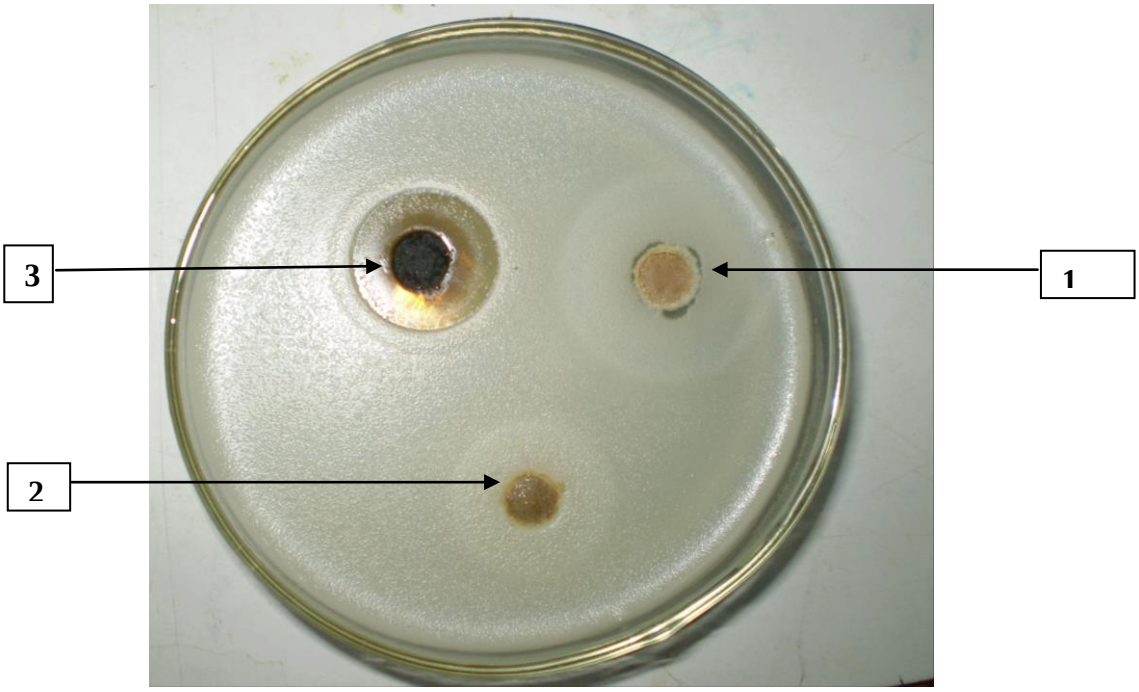

Plate 5: The antimicrobial activity of aqueous extracts of the study plants, 1= *A. anthelmintica*, 2= *S. persica* and 3= *V. amygdalina* against *B. subtilis*. Note the distinct clear zone of inhibition at number 3 *(V. amygdalina)*

4.5. The minimum inhibitory concentration of the study extracts.

The MICs for the plant extracts against the various bacteria are shown in Tables 7 and 8 for aqueous extracts and Table 9 and 10 for ethanolic extracts. Table 11 shows the MICs for a positive control drug gentamycin. The MICs for aqueous extract of *V. amygdalina* ranged from 3.906– 15.625 $mgml^{-1}$ while that of *S. persica* ranged from 15.625 – 31.25 $mgml^{-1}$ (Tables 7 and 8) and that of *A. anthelmintica* was 62.5 $mgml^{-1}$ (*Vibrio cholerae)*. The MICs for ethanolic extract of *V. amygdalina* ranged from 3.906 – 31.25 $mgml^{-1}$ and that of *S. persica* ranged from 7.813 – 62.50 $mgml^{-1}$ (Tables 9 & 10). The MICs of gentamycin (control) ranged from 0.938 – 30.000 $mgml^{-1}$ (Table 11).

Table 7: The minimum inhibitory concentrations of the *S. persica* aqueous extract against sensitive bacteria.

	Concentration of the Extract ($mgml^{-1}$)									
Bacteria	**500**	**250**	**125**	**62.50**	**31.25**	**15.625**	**7.813**	**3.906**	**1.953**	**0.977**
Bacillus subtilis	+	+	+	+	+	+	-	-	-	-
Vibrio cholerae	+	+	+	+	+	-	-	-	-	-
Staphylococcus aureus	+	+	+	+	+	-	-	-	-	-

Key: + = inhibition

\- = no inhibition

Table 8: The minimum inhibitory concentrations of the *V. amygdalina* aqueous extract against sensitive bacteria.

	Concentration of the Extract ($mgml^{-1}$)									
Bacteria	**500**	**250**	**125**	**62.50**	**31.25**	**15.625**	**7.813**	**3.906**	**1.953**	**0.977**
Bacillus subtilis	+	+	+	+	+	+	+	+	-	-
Bacillus anthracis	+	+	+	+	+	+	+	-	-	-
Vibrio cholerae	+	+	+	+	+	+	+	-	-	-
Staphylococcus aureus	+	+	+	+	+	+	-	-	-	-

Key: + = inhibition

\- = no inhibition

Table 9: The minimum inhibitory concentrations of the *S. persica* ethanolic extract against sensitive bacteria.

	Concentration of the Extract ($mgml^{-1}$)									
Bacteria	**500**	**250**	**125**	**62.50**	**31.25**	**15.625**	**7.813**	**3.906**	**1.953**	**0.977**
Bacillus subtilis	+	+	+	+	+	+	+	-	-	-
Vibrio cholerae	+	+	+	+	+	-	-	-	-	-
Staphylococcus aureus	+	+	+	+	-	-	-	-	-	-

Key: + = inhibition

\- = no inhibition

Table 10: The minimum inhibitory concentrations of the *V. amygdalina* ethanolic extract against sensitive bacteria.

	Concentration of the Extract ($mgml^{-1}$)									
Bacteria	**500**	**250**	**125**	**62.50**	**31.25**	**15.625**	**7.813**	**3.906**	**1.953**	**0.977**
Bacillus subtilis	+	+	+	+	+	+	+	+	-	-
Bacillus anthracis	+	+	+	+	+	+	+	+	-	-
Vibrio cholerae	+	+	+	+	+	+	-	-	-	-
Staphylococcus aureus	+	+	+	+	+	-	-	-	-	-

Key: + = inhibition

\- = no inhibition

Table 11: The minimum inhibitory concentrations of gentamycin against sensitive bacteria.

	Concentration of the Extract (μgml^{-1})							
Bacteria	**30**	**15**	**7.5**	**3.75**	**1.875**	**0.938**	**0.469**	**0.234**
Bacillus subtilis	+	+	+	+	+	+	-	-
Bacillus anthracis	+	+	+	+	+	-	-	-
Vibrio cholerae	+	+	-	-	-	-	-	-
Staphylococcus aureus	+	-	-	-	-	-	-	-

Key: + = inhibition

\- = no inhibition

4.6. The brine shrimp Lethality test

Table 12 below shows the findings for the brine shrimp lethality assay. The LC_{50} values and their 95% confidence intervals are given. The extracts from all three study plants had LC_{50} values greater than 20 $\mu g/ml^{-1}$, but half of the extracts have LC_{50} values less than 100 µg/ml which is the cut-off for a compound to be regarded as not acutely toxic.

Table 12: The brine shrimp LC_{50} values for the study plant extracts and 95% confidence intervals (CIs).

Plant extracts	**LC_{50} (µg/ml)**	**95% CIs**
A	60.11	42.9 – 84.2
B	40.30	28.4 – 57.2
C	138.43	80.5 – 237.9
D	132.21	79.7 - 235.3
E	112.62	74.5 – 169.4
F	43.46	31.2 – 60.6

Key: A =*A. anthelmintica* ethanolic extract, **B** =*A. anthelmintica* aqueous extract, **C**=*S. persica* aqueous extract, **D**=*S. persica* ethanolic extract, **E**=*V. amygdalina* aqueous extract, **F=** *V. amygdalina* ethanolic extract.

4.7. Phytochemical screening of the study plants.

The findings of the phytochemical analysis revealed the presence of tannins, saponins, flavonoids, glycosides, steroids, and terpenoids (Table 13). The phytochemical screening of the *A.anthelmintica* root bark (Table 13) showed the presence of saponins, cardiac glycosides and terpenoids (Plates 7 and 8), while it was negative for flavonoids, steroid, tannins, anthraquinones and phlobatannins. *S. persica* root bark was positive for terpenoids (Plate 7), saponins and cardiac glycosides (Plate 8) and negative for the rest. *V.amygdalina* leaves were positive for

tannins (Plate 6), flavonoids, terpenoids (Plate 7), cardiac glycosides (Plate 8), steroids and saponins and negative for the rest.

Table 13: Qualitative analysis of the phytochemicals present in the study plants

Phytochemicals	**Plants**		
	A. anthelmintica	***S. persica***	***V. amygdalina***
Tannins	-	-	+
Phlobatannins	-	-	-
Flavonoids	-	-	+
Terpenoids	+	+	+
Cardiac glycosides	+	+	+
Steroids	-	-	+
Anthraquinones	-	-	-
Saponins	+	+	+

Key: + = presence of a compound

- = absence of a compound

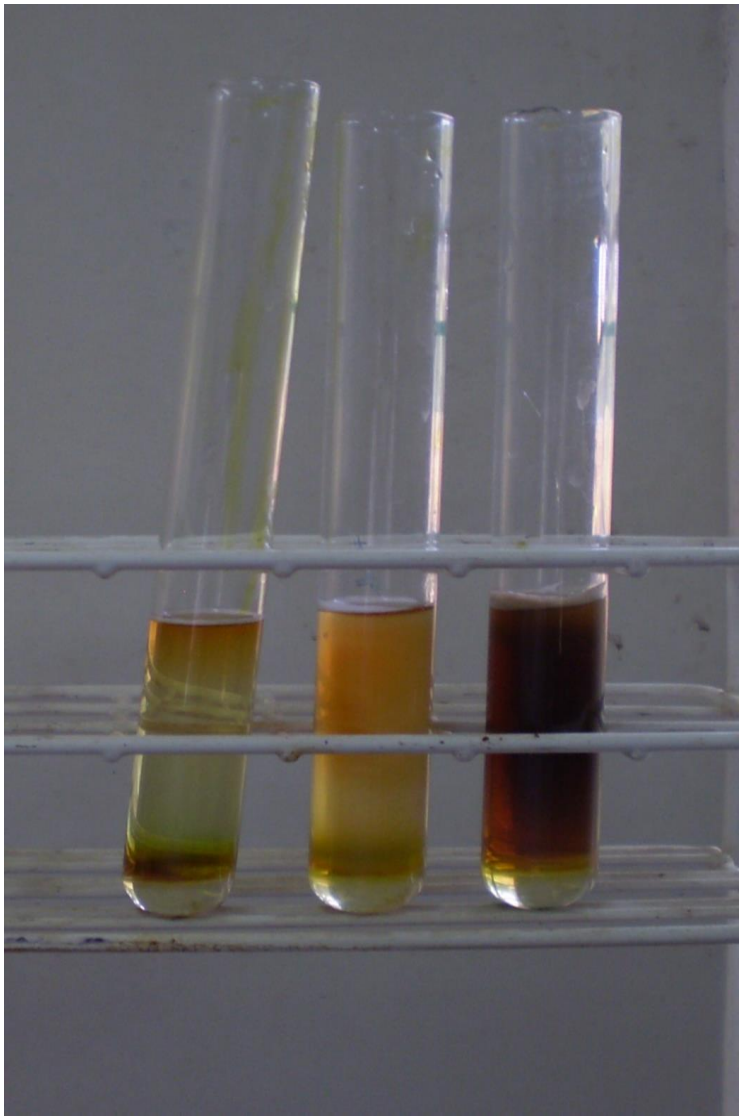

Plate 6: The test for tannins: The extracts are arranged from left to right as *A. anthelmintica, S. persica* and *V. amygdalina*. The test was positive for *V. amygdalina*.

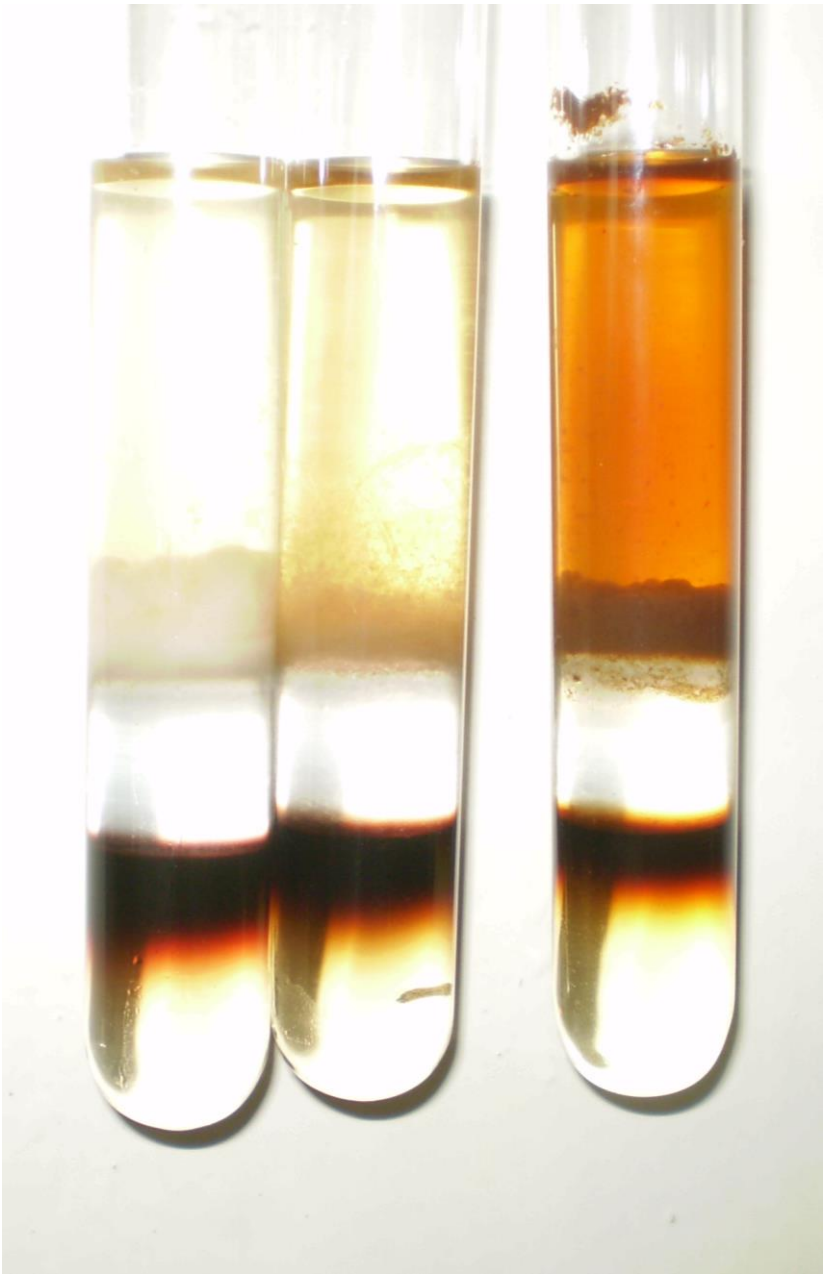

Plate 6: Test for terpenoids: The extracts are arranged from left to right as *A. anthelmintica, S. persica* and *V. amygdalina*. The test was positive for all the three plant extracts.

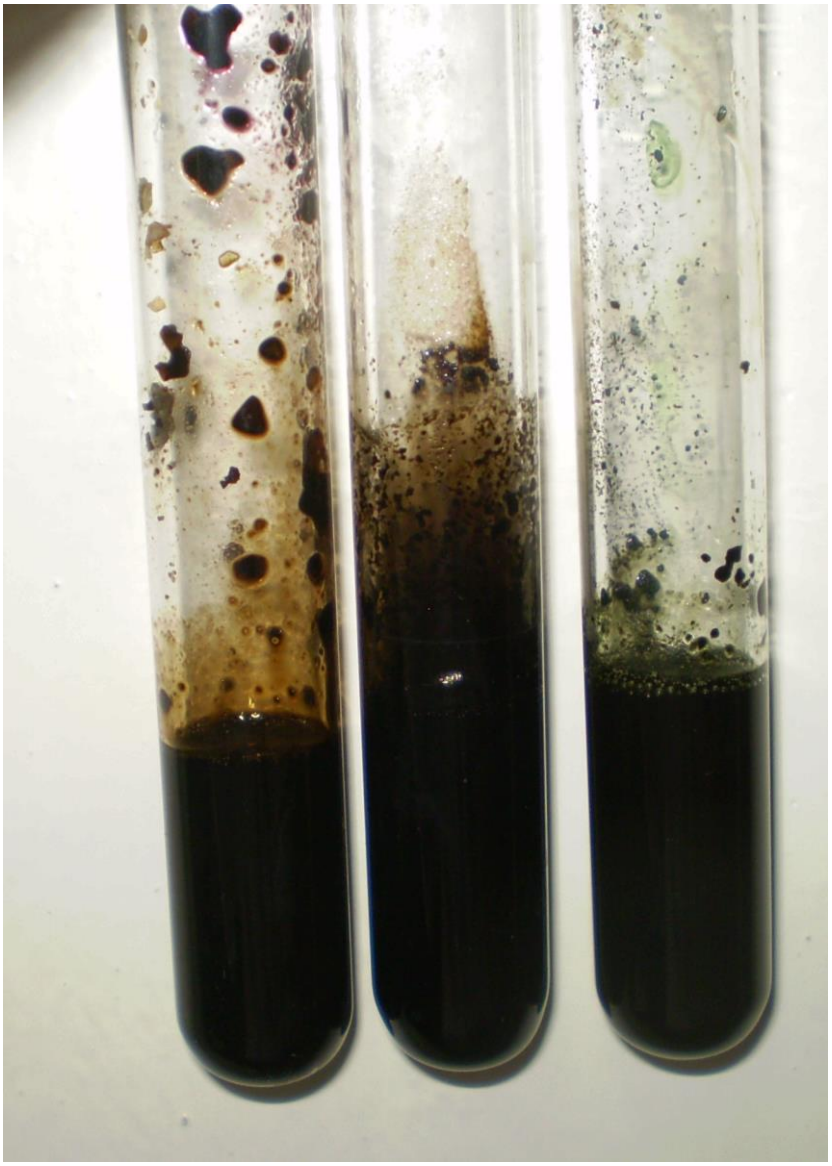

Plate 7: Test for cardiac glycosides: The extracts are arranged from left to right as *A. anthelmintica, S. persica* and *V. amygdalina*. The test was positive for all the three plants.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Anthelmintic activities of the study plants

The anthelmintic activity of the study plants has been shown (Tables 2 and 3). Aqueous extract of *A. anthelmintica* showed high activity on nematode eggs based on the *in vitro* hatchability studies done in this study. Aqueous extract of *V. amygdalina* had activity on the hatchability nematode eggs as well as larval survival (Table 2). The aqueous extract of *S. persica* had very little effect on hatchability of the egg as well as larval survival (Table 2). The ethanolic extracts of all study plants had little effect on hatchability of the eggs and larval survival (Table 3) as compared to aqueous extracts, with *A. anthelmintica* having a high activity compared to the other two plants. The anthelmintic activity of *A. anthelmintica* and *V. amygdalina* is well documented (Galal *et al*., 1991a, b; Koko *et al*., 2000; Thoithi *et al*., 2002; Alawa *et al*., 2003; Hördegen *et a*l., 2003), in which a range of concentrations were reported to inhibit different stages of nematode helminths, both *in vitro* and *in vivo*.

The aqueous extracts inhibited hatching of eggs and had moderate activity in terms of larval mortality. Ethanolic extracts had little effect on the hatchability of the nematode eggs, though their concentration was higher than that of aqueous extracts. This is probably due to the fact that, quality and quantity of phytochemicals present in the extracts, which are believed to be the basis for the claimed biological activities of plants, are affected by the type of solvent used for the extraction (Siddhuraju and Becker, 2003). These results (Tables 2 and 3) are in agreement with the work of others who suggested that the aqueous extracts of the stem and root bark of *A.*

anthelmintica and the leaves of *V. amygdalina* have anthelmintic activity (Galal *et al*., 1991a, b; Koko *et al*., 2000; Thoithi *et al*., 2002; Alawa *et al*., 2003; Hördegen *et al*., 2003). The observations in this study and others, confirm the claims of the traditional healers that these two plants are used to control helminths in animals and man.

5.2 Antischistosomal activity of Salvadora persica

The antischistosomal activity of *S. persica* is probably being reported for the first time; in this study, the aqueous extract of the plant has been shown to reduce faecal egg count in animals infected with *Schistosoma bovis* (Table 4). According to the WAAVP guidelines; the test drug or compound is considered effective if it has the percentage faecal egg count reduction of 95% and above with lower 95% confidence limit of 90% and above. The test drug is considered not effective if it has percentage faecal egg reduction of less than 95% with lower 95% confidence limit of less than 90% (Coles *et al*., 1992). In this study, it implies therefore, the aqueous extract of *S. persica* was not effective against *S. bovis*. Despite this inference, the 78% faecal egg count reduction can not be ignored, though the small sample size per test group might have made the results less statistically significant. Some other medicinal plants have been known to possess antischistosomal activity (Adam *et al*., 2006; Sparg *et al*., 2000) these plants include the *Commiphora molmol* (Myrrh), *Artemisia annua* (Asteraceae) and *Jatropha curcas* (Euphorbiacaea) (Adam *et al*., 2006). The study done in South Africa by Sparg *et al*., (2000) showed that the extracts from *Berkheya speciosa* (Asteraceae), *Euclea natalensis* (Ebenaceae) and *Trichilia emetica* (Meliaceae) were lethal to the schistosomes.

5.3 Antimicrobial activities of the study plants

The antimicrobial activities of V. anthelmintica, V. amygdalina and S. persica have been studied (Tables 5 and 6). Of the three studied plants, V. amygdalina has been shown to possess strong antimicrobial activity compared to the other two plants. Both the aqueous and ethanolic extracts of S. persica had activity against some of tested bacteria namely; B. subtilis, V. cholerae, and S. aureus, though the activity was low compared to that of V. amygdalina which had strong activity against almost all tested bacteria with exception of P. aeuruginosa and E. coli (Table 5 and 6). The aqueous extract of A. anthelmintica has been shown to have little antimicrobial activity against V. cholerae only (Table 5); while its ethanolic extract had no activity against all tested bacteria strains (Table 6). The antimicrobial activity of S.persica and V. amygdalina has been well documented (Darout et al., 2000; Almas and Al-Zeid., 2004; Khalil, 2006; Erasto et al., 2006). The two plants have been shown to be active against almost all the gram-positive bacteria in this study. These findings are in agreement with previous reports related to plant extracts regarding gram-positive bacteria (Cowan, 1999).

Resistance of gram-negative bacteria to the study plant extracts is not unusual, because this class of bacteria is more resistant than gram-positive bacteria. Such resistance could be attributed to the permeability barrier provided by the thick cell wall or to the reduced membrane accumulation, hence preventing accumulation of the plant extracts in the bacterial cell to effective therapeutic doses (El Astal et al., 2005; Yoga Latha et al., 2007). Pseudomonas aeruginosa, which is a gram-negative bacterium, in this study has been found to be resistant to almost all plant extracts,

which is due to the fact that it has high level of intrinsic resistance to virtually all known antimicrobials. This is due to a combination of a very restrictive outer membrane barrier and reduced membrane accumulation, making it highly resistant even to some strong synthetic drugs (Yoga Latha et al., 2007).

The strong antimicrobial activity of the aqueous extract of the *V. amygdalina* could be attributed to the water-soluble components (tannins, terpenoids, cardiac glycosides, steroids, flavonoids and saponins) found in its extracts used in this study (Nwanjo, 2005). Generally the ethanolic extracts of the study plants showed lower antimicrobial activity, this may be due to little diffusion properties of these extracts in the agar or because fresh plants contain active substances that may be destroyed or may disappear through the effects of extraction methods.

It is well established that, the yields of soluble substances expressed as percentage by weight of the powdered sample, are closely dependent on the type of solvent used in the extraction (Siddhuraju and Becker, 2003). Moreover, observations of antimicrobial activity from this study correspond positively with the claimed ethnomedical and ethnoveterinary uses of the leaves of *Vernonia amygdalina* in the treatment of various infectious diseases (Koshimizu *et al*., 1994; Abosi and Raseroka, 2003; Erasto *et al*., 2007)

5.4 Brine shrimp toxicity of the study plants

The LC_{50} values for the three study plants are shown in (Table 12). The brine shrimp toxicity study shows that, the three plants are not acutely toxic due to their high LC_{50}

values. For the compound to be regarded as acutely toxic to biological systems, it should have an LC_{50} value of not more than 20 μgml^{-1} (Yoga Latha *et al*., 2007; McLaughlin *et al*., 1991; Mbwambo *et al*., 2007). Most of the extracts could be toxic at high doses, because same extracts had LC_{50} values less than 100 μgml^{-1}. It has been established that, for a compound to be considered completely safe, it should have LC_{50} value greater than 100 μgml^{-1} (Carballo *et al*., 2002; Chowdhury *et al*., 2005; Yoga Latha *et al*., 2007). But more elaborate toxicity studies are needed to establish the safety of the extracts from the studied plants

5.5 Phytochemical screening of the study plants

Several secondary metabolites have been found in the study plant extracts; these include saponins, flavonoids, terpenoids, steroids, and cardiac glycosides (Table 13). It has been established that, the presence of secondary metabolites in plants produces some biological activity in man and animals and that these metabolites are responsible for the biological activity of plants (Nwanjo, 2005). Collectively, biological activity of plants encompasses a diverse array of different classes of compounds, including saponins, phenolics, cyclic hydroxamic acids, cyanogenic glycosides, isoflavonoids, sesquiterpenes, sulfur-containing indole derivatives, and many others (Nwanjo, 2005). Some of these chemical compounds have been reported to have inhibitory effects on some gram-negative bacteria such as *Escherichia coli* amongst others. However, some reports have shown that flavonoids, tannins and saponins may play some roles in antioxidative and hypolipidaemic effects (Akah and Okafor, 1992; Igile *et al*., 1995; Babalola *et al*., 2001; Izevbigie *et al*., 2004; Ojiako and Nwanjo, 2006; Erasto *et al*., 2007). These

secondary metabolites also have prominent effects on animal systems and microbial cells. Saponins are glycoside components often referred to as “natural detergent” because of their foamy nature (Seigler, 1998; Edeoga *et al*., 2005), and are reported to posses anticarcinogenic properties, immune modulation activities and regulation of cell proliferation as well as health benefits such as cholesterol lowering activity. Flavonoids have been reported to exert multiple biological effects including antibacterial, antiviral, antitoxic and anti-inflammatory activities. Many of the alleged effects of flavonoids have been linked to their known functions as strong antioxidants (Igile *et al*., 1995; Edeoga *et al*., 2005; Erasto *et al*., 2007), free radical scavengers and metal chelators. The effects of glycosides and cardiac glycosides include increased heart rate (positive chronotropic effect), increased cardiac contractile force (positive inotropic effect), increased sympathetic activity and decreased systemic vascular resistance (Seigler, 1998), they are also important in cases of congestive heart failure and as supportive therapy in cardiac arrhythmias. The presence of some of these substances could however be reduced by various processing techniques (Jimoh and Oladiji, 2005). The presence of some of these chemical compounds in the studied plant extracts, therefore suggests the pharmacological activities shown by the studied plants.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

From this study it can be concluded that, some of the plants used in traditional medicine are effective against a number of disease conditions. The antimicrobial studies of the three plants chosen for this study have shown that, some plants are very effective against bacteria compared to the standard antibiotics. This is true with *V. amygdalina* which had shown greater activity with some bacterial isolates as compared to gentamycin, the standard antibiotic used in this study. The anthelmintic activity of *A. anthelmintica* need not be overemphasized, the results from this study has shown that, the root bark extract have greater activity against helminth eggs hatchability *in vitro*.

The antischistosomal activity of *S. persica* is reported probably for the first time; the aqueous extract of the plant has been shown to be effective in reducing faecal egg count in animals infected with *S. bovis*. From this study on the antischistosomal activity of *S. persica* and other studies, it can be concluded that some plants can be a good source of antischistosomal drugs. Further research may help in the identification and probably concentration or modification of the active ingredient(s) so as to have a new antischistosomal drug, thus minimizing the risk and rate of developing resistance against the currently used agents.

6.2. Recommendations

From the results obtained from the anthelmintic activity of *A. anthelmintica*, antimicrobial activity of *V. amygdalina* and antischistosomal activity of *S. persica*, it can be recommended that, more studies should be carried out to ascertain the active constituents responsible for the shown activities. The antischistosomal activity of *S. persica* ought to be investigated further, as stated above. This should build the foundation for the establishment of efficacy testing mechanisms to help the traditional healers use plant medicines accurately and wisely. The efficacy and toxicity studies should go together, culminating into drug formulation and packaging.

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APPENDICES

Appendix 1: Protocol for Brine Shrimp Toxicity: (BST) Assay

1. Introduction

The BST assay is based on the ability of plant extract to kill laboratory cultured

Artemia nauplii brine shrimp.

- The assay is useful tool for a preliminary assessment of toxicity of plant extract.
- LC_{50} of <20 µg/ml is suggestive of possible cytotoxicity (anti-cancer activity). LC_{50} >100 µg/ml is regarded to be non-toxic.

2. Procedure:

2.1 Preparation of Brine Shrimp Salt Water as follows

(i) Weigh 3.8 g of brine shrimp salt and put it into a 100 ml beaker

(ii) Add distilled water and stir well

(iii) Filter the dissolved salt through filter paper into a measuring cylinder with a capacity of 1 litre.

(iv) Add some more water into the beaker. Stir well and filter again.

(v) Repeat step (iv) above until all the salt has dissolved

(vi) Filter more distilled water until you get 1L solution

2.2 Hatching the Nauplii

- Put the Brine shrimp salt water into hatching tank. (at least prepare 2L solution of brine shrimp salt water since more than 1L solution will be used for hatching brine shrimps).
- Take a maximum of 1g of brine shrimp eggs and put it into the dark side of the hatching tank.
- Switch on the light to allow the hatching tank to have two sides: light and dark sides. This will allow the hatched nauplii to move from the dark side to the light side, therefore making it easier to collect the nauplii.
- Leave the eggs for overnight to allow hatching.

2.3 Preparation of Extract Stock Solution

- Dissolve 40 mg of the crude extract into 1 ml DMSO or water (depending on the nature of extract)

2. 4 Experimental setup

- Arrange vials in duplicates. Mark them as 1:1, 1:2, 2:1, 2:2, 3:1, 3:2, 4:1, 4:2, 5:1, 5:2 and 6:1, 6:2.
- Put 2 ml of Brine Shrimp salt water into each vial
- Take ten nauplii from the light side of the hatching tank by using Pasteur Pipette and put them into each of the of the 12 vials (Note: each extract will be tested by using a set of 12 vials) with more than 5 ml capacity
- Draw 30 µl of the extract stock solution and put it into the 1:1 labeled vial. Take another 30 µl of the extract stock solution into the 1:2 labeled vial. This will complete the first set.

- Draw 15 µl for the 2nd set, 10 µl for the 3rd set, 5 µl for the 4th set, 3 µl for the 5th set, and 1µl for the 6th set.
- Adjust the volume to 5 ml once you have added the stock solution to each of the vials.
- Leave the nauplii in the vials for up to 24 hours for the extracts to exert their effects.
- Record the number of survivors for each vial after overnight incubation by using this table.

Volume(µl)	Conc. (µg/ml)	Set No.	Number of Survivors				
			Extract 1	Extract 2	Extract 3	Extract 4	Extract n
30	240	1:1					
		1:2					
15	120	2:1					
		2:2					
10	80	3:1					
		3:2					
5	40	4:1					
		4:2					
3	24	5:1					
		5:2					
1	8	6:1					
		6:2					

3.0 Data Analysis

- Analyze the data by using fig P computer program
- Calculate percent mortality for each set of vials
- Feed your data into the program
- The Program calculates a regression equation after plotting a graph of concentration (µg/ml) against percent mortality
- Use the regression equation to calculate LC_{84}, LC_{50}, and LC_{16}.
- y = A (log c) B
- LC_{84} = (84- B)/ A
- LC_{50} = (50- B)/ A
- LC_{16} = (16- B)/ A
- S_1= LC_{84}/LC_{50}
- S_2 = LC_{50}/LC_{16}
- Average S = (S_1 + S_2)/2
- f= average S $^{2.77/\sqrt{n}}$ where n= number of nauplii per test, f is a correction factor
- Lower Limit = LC_{50} /f
- Upper Limit = LC_{50} x f Where

Appendix 2: Miracidia hatching technique

Introduction

Viable eggs and miracidia of the schistosomes can be harvested from either tissues of the infected definitive hosts or their faeces.

Materials

cool box, ice packs, examination gloves, refrigerator, wide-mouthed plastic or glass containers with screw cap(100ml), tea strainer, specimen flask(300ml), wash bottles, applicator sticks, freshwater, normal saline, weighing balance and a microscope with 10-40x magnification.

Sample collection.

Faecal samples collected from the rectum of suspected infected cattle using polythene long-sleeved gloves. Transported in a cool box to the laboratory. These should be examined within 2 weeks after collection.

Procedure

1. Weigh 5g of faecal sample in a 100ml container; add 50ml of normal saline.
2. Apply screw cap on the container and shake the mixture thoroughly till thin suspension of saline and faecal material is obtained.

3. Add another 50ml saline and pour the mixture through tea strainer into the specimen flask. Use wash bottle to spray the sieve with additional saline to ensure that no eggs remain on the mesh.
4. Fill up the specimen flask with saline and leave it for 30min in the dark.
5. After 30min, pour off the supernatant without disturbing the deposit.
6. Fill up the specimen flask with saline and resuspend the deposit in the saline using the applicator stick and leave for another 30min in the dark.
7. repeat steps 4-6 until the supernatant becomes clear (usually 3-4 times)
8. pour off the clear supernatant without disturbing the deposit
9. refill the specimen flask with freshwater and resuspend the deposit in the freshwater using the applicator and leave for 30min in the dark
10. Pour off the clear supernatant without disturbing the deposit.
11. Pour the deposit into plastic/ glass Petri dishes and use wash bottle to spray the specimen flask to ensure that all of the deposit is transferred into Petri dishes(transfer only a little amount of the deposit into each Petri dish)
12. Place the Petri dish under a strong artificial illumination at room temperature (20-25°C) for 30 min.
13. Use a microscope with x 10-40 magnification to check the emergence of the miracidia.

Appendix 3: Modified Bell filtration technique

Introduction

This is the technique for detection and enumeration of schistosome eggs in animal faecal samples.

Materials

In additional to materials in the miracidia hatching, the following should be included; Sieve(mesh size 250µ), vacuum pump, Whatman filter paper no. 541, normal saline, sedimentation flask, Ninhydrin solution, stereomicroscope with x10-40 magnification.

Procedure

1. Weigh 3g of faecal sample in a 100ml plastic container and add 50ml of saline
2. Apply a screw cap on the container and shake the mixture thoroughly until a thin suspension of the saline and faecal material is obtained
3. Add another 50ml of saline and pour the mixture through a tea strainer and sieve (250µ) into a sedimentation flask. Use a wash bottle to spray the container and the mesh with additional saline to ensure that no eggs remain on them
4. Fill the sedimentation flask with saline and leave for 30min in the dark
5. After 30min pour off the supernatant without disturbing the deposit

6. Fill the sedimentation flask with saline gain and leave in the dark for another 30min. pour off the supernatant after 30min.
7. repeat step 6 above several times (3-4 on average) until the supernatant becomes clear
8. Use the vacuum apparatus to filter the deposit. Place a Whatman No. 541 filter paper on top of the holder, followed by the filtration funnel on top of the filter paper.
9. Use an applicator stick to resuspend the faecal deposit in the saline and pour little suspension in the filtration funnel. Then, start the vacuum pump and stop when all the suspension has passed through the filter paper, ten to fifteen filter papers may be used per sample.
10. Stop the pump and remove the filtration funnel, use a forceps to remove the filter paper with eggs upward and place it on the a few drops of 10% Ninhydrin solution on a glass plate
11. Allow the preparations to dry under illumination for 4-5hr or longer
12. For detection and enumeration of schistosome eggs, place the filter paper in a Petri dish and rewet it with saline. Examine using stereomicroscope with 40x magnification. Schistosome eggs stained with Ninhydrin appears deep purple in colour.
13. The filter papers can be stored, as long as are kept in dark and dry. Once they have been rewet, they have to be examined. Count eggs in all filter papers and express the total number of eggs found a egg per gram of faeces (epgf)