A PRELIMINARY PHYTOCHEMICAL STUDY OF *DIPLORHYNCHUS CONDYLOCARPON, HOLARRHENA PUBESCENS, PSIDIUM GUAJAVA, SCLEROCARYA BIRREA, STEGANOTAENIA ARALICEA, TRICHILIA EMETICA* AND *VERNONIA GLABRA* USED FOR THE TREATMENT OF DIARRHOEA BY TRADITIONAL HEALERS IN MALAWI

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A thesis submitted to the Chemistry Department of the Faculty of Science, in partial fulfilment of the requirements for the degree of Master of Science (Chemistry)

Chancellor College

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June 2008

DECLARATION BY THE CANDIDATE

I hereby declare that this is my original work except where information is sourced from other authors, acknowledgements have been made, and no part of this thesis has been submitted for any degree in any university and neither is being submitted concurrently.

Signature of the candidate:

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CERTIFICATE OF APPROVAL

We hereby declare that this thesis is from the student’s own effort and that it has been submitted this day with our approval.

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DEDICATION

I dedicate this work to my father who began the journey of education. I also dedicate this thesis to my late sister Etness and late brother Charles who through their demise I have found courage and strength to complete my work.

ACKNOWLEDGEMENTS

I wish to thank my supervisors: Professor J.D.K. Saka and Dr. N. Chokotho of Chemistry Department, Chancellor College for their consistent guidance throughout my study. I would also wish to thank Mr. Fred Masumbu, the chief laboratory technician, and his laboratory team for their support and assistance in the laboratory work in Chemistry Department. I am also grateful to the Chief technician and his team at Zomba Central Hospital Microbiology laboratory for their assistance in carrying out antibacterial activity analyses. I would also like to express my gratitude to botanists from National Herbarium and Botanical Gardens of Malawi for their assistance in sample identification and collection.

Finally, I wish to thank Mzuzu University for the sponsorship of the study.

ABSTRACT

Diarrhoea is one of the common ailments amongst children as well as adults in Malawi. It is mainly caused by bacterial pathogens. These bacterial diarrhogenic problems are treatable using antibiotics available at healthcare facilities. However, healthcare facilities are not readily accessible to majority of rural and urban poor population such that the majority rely on traditional medicine and medicinal plants for health problems such as diarrhoea. Ethnobotanical survey on medicinal plants traditionally used for diarrhoea problems have been carried out. However, phytochemical screening and antibacterial activity of these plants is not done. Therefore, selected seven medicinal plants (*Diplorhynchus condylocarpon, Guajava psidium, Holarrhena pubescens, Sclerocarya birrea, Steganotaenia aralicea, Trichilia emetica* and *Vernonia glabra*), used locally to treat diarrhoea in Malawi were investigated for their classes of phytochemicals and in vitro antibacterial activity.

The presence of alkaloids, saponins, terpenoids, flavonoids and tannins was determined in the leaves, stem and root barks of the plant species using standard phytochemical methods. Plant species were extracted using methanol, ethyl acetate, and dichloromethane by soxhlation, and water using cold extraction. The yields, phytochemical composition and antibacterial activity of the resulting crude extracts were determined and compared. Growth inhibition, using agar disc diffusion method was determined against five gram-negative bacterial entero-pathogens, S*almonella typhi, S. typhimurium, S. enteritidis, Shigella boydii* and *S. flexneri*. The plant species whose crude extracts gave high yields and phytochemical composition, and exhibited significantly high efficacies were selected for further studies. Minimum Inhibitory Concentrations (MIC) were determined for methanolic crude extracts from root and stem barks of *D. condylocarpon* and *H. pubescens*. Yields, thin layer chromatography (TLC) and UV spectra of crude extracts of alkaloids, saponins, terpenoids and flavonoids from the two plant species were also determined and analysed.

Phytochemical composition, yield and antibacterial activity of the crude extracts varied significantly with plant species and the extracting solvent ($p < 0.001$). Methanol was found to be the most effective extracting solvent system such that its crude extracts exhibited the largest number of phytochemicals, and gave the highest yields of the three organic extracting solvents. The yields of crude extracts varied from 3.06 ± 0.12 % to 13.26 ± 0.19 %. Extracts of all the plant species except *T. emetica* were active against at least three pathogens tested, and the mean inhibition diameters of the active extracts varied from 8.3 ± 0.2 mm to 16.6 ± 0.2 mm. However, methanolic crude extracts of root and stem barks of *D. condylocarpon* and *H. pubescens* were the most active against the tested bacterial strains. The strain, *S. flexneri*, was the most susceptible while *S. typhimurium* was the most resistant pathogen.

Alkaloids, saponins and tannins, which are some of the classes of phytochemicals associated with antimicrobial properties in medicinal plants, were observed in the aqueous extracts of the selected plants. However, alkaloids were predominant class of phytochemicals in all four extracting solvents. Only aqueous extracts of root and stem barks of *D. condylocarpon* and *H. pubescens* were active against the tested bacterial strains. Traditional healers commonly use aqueous extracts to treat their patients. Therefore, presence of these phytochemicals in aqueous extracts supports the use of these plants for therapeutically purposes. Hence, aqueous extracts of root and stem barks of *D. condylocarpon* and *H. pubescens* should be promoted. However, proper methods of harvesting these plants should be adhered to in order to ensure sustainability of the species.

The MIC of crude extracts of *D. condylocarpon* and *H. pubescens* ranged from 0.80 to 25.00 mg/ml and 0.20 to 15.00 mg/ml, respectively, indicating that crude extracts of *H. pubescens* were more active than those of *D. condylocarpon*. The yields of alkaloids, saponins, flavonoids and terpenoids in *D. condylocarpon* and *H. pubescens* were significantly different ($p < 0.05$). The crude saponin extracts of *D. condylocarpon* and *H. pubescens* showed at least three spots on the TLC plates using butanol: ethanol: water (5:3:1), revealing that these plants contain more than one type of saponins. Further separation and isolation techniques needs to be explored in order to determine identities of the bioactive agents.

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ACRONYMS

CHSU	Community Health Services Unit
DCB	*Diplorhynchus condylocarpon* stem bark
DCR	*Diplorhynchus condylocarpon* root bark
HPB	*Holarrhena pubescens* stem bark
HPR	*Holarrhena pubescens* root bark
HPL	*Holarrhena pubescens* leaves
PGL	*Psidium guajava* leaves
SBR	*Sclerocarya birrea* root bark
TEB	*Trichilia emetica* stem bark
TER	*Trichilia emetica* root bark
WHO	World Health Organisation

APPENDICES

CHAPTER 1: INTRODUTION

1.1 Background

Diarrhoea, a disease that has caused a health concern all over the world since time immemorial, still remains one of the major health problems today. It accounts for more than 5 – 8 million deaths every year in infants and children under the age of five (Venkatesan et al., 2005). It is also prevalent in adults especially those suffering from diseases related to Human Immuno-deficiency Virus / Acquired Immune Deficiency Syndrome (HIV/AIDS). For instance, opportunistic infections of the gastrointestinal tract accounted for 68-85% of both acute and chronic diarrhoea cases in HIV infected individuals (Thomas et al., 1999). In Africa, it represents a frequent opportunistic disease in people living with HIV and has been described as one of the most distressful and persistent symptoms of HIV/AIDS (Abere and Agoreyo, 2006). In Malawi, like other sub Saharan region countries, diarrhoea leads morbidity and mortality rate at 33% in children (Kandala et al., 2006). Diarrhoea is mainly caused by gastrointestinal infections (Farthing, 2000). Many intestinal infections are largely water and food borne such that the etiological agents are mainly bacterial pathogens such as *Escherichia coli, Vibrio cholerae, Shigella* and *Salmonella* species.

Potential pathogens that cause diarrhoea in general and in HIV/AIDS patients include parasites, fungi, bacteria and viruses. The parasitic pathogens include *Cryptosporidia, Microsporidium, Isospora* and *Entamoeba* (Lebabad et al., 2001). Viral infections that are associated with chronic diarrhoea include Herpes Simplex Virus and *Cytomegalovirus*, which are the main isolates in HIV patients (Thomas et al., 1999; Pollok, 2001). Bacterial pathogens that cause diarrhoea include *Salmonella* and *Shigella* species, *Streptococcus pneumoniae* and *Mycobacterium avium complex*. Though these pathogens are mostly common in sero-positive HIV patients, they are however not limited to these patients (Maartens, 2002).

In Malawi, cases of diarrhoea caused mainly by bacterial infections have been reported. Most common are the outbreaks due to *Vibrio cholera* and *Salmonella* species (Gordon et al., 2001). Diarrhoea episodes caused by *Salmonella typhi*, non-typhoid *Salmonellae* especially in HIV seropositive individuals have been reported too (Gordon et al., 2001; Graham et al., 2000). All these bacterial infections are treatable effectively using antibiotics. Even though these pathogens are treatable by antibiotics, bacterial strains have shown to develop resistance to antibiotics since their inception (Martini, 2001; Molbak et al., 2002). Furthermore, accessibility to modern treatment is limited due to few health institutions and medical practitioners. In Malawi, it is known that there is one doctor for every twenty seven thousand people (Mwanyambo and Nihero, 1998). Consequently, people who have health problems such as diarrhoea rely heavily on traditional medicine.

Even though diarrhoea problems seem to be overshadowed by diseases such as HIV/AIDS and Malaria, interviews with medical practitioners have shown that diarrhoea is ranked amongst the first three health problems encountered in the hospitals and conventional clinics in Malawi (Ndibwami et al., 1998). It is also amongst the diseases for which traditional practitioners are frequently consulted, even though it is not amongst the most frequent disease that traditional practitioners encounter. However, this observation is probably due to the tendency of patients to seek traditional medication mainly for those diseases that are believed to be treatable by traditional medicine alone. For instance, maternity and infertility problems and migraine, which are believed to be successfully cured by traditional medicine only, were amongst the first three health problems for which the traditional healers are most frequently consulted (Ndibwami et al., 1998). In addition, people who have diarrhoea problems in rural areas do not always seek assistance from traditional healers since many possess knowledge of therapeutic properties of medicinal plants for common diseases such as diarrhoea. Hence, diarrhoea is not ranked as the top frequent disease encountered by traditional healers. In the study of knowledge of medicinal plants and the diseases they cured, Mwanyambo and Nihero (1998) found out that 64% of respondents from Zomba and Mangochi had some knowledge of medicinal plants and diseases cured. Thus, rural population does not always consult traditional healers for diarrhoea problems.

In a country where use of medicinal plants and consultation of traditional healers for the health care is an integral part of the people’s livelihood, treatment of diarrhoea problems using traditional medicine should be encouraged. Medicinal plants, unlike modern treatment, are readily accessible by majority of Malawian population. Furthermore, medicinal plants have been used in pharmacology studies that lead to synthesis of more potent drugs with reduced toxicity (Babayi et al., 2004). Hence it is essential that the use of herbal medicine for treatment of diseases such as diarrhoea should be promoted and be sought as an alternative.

1.2 Problem statement

It is estimated that over two thirds of world's population to date relies on plant derived drugs and 7000 medicinal compounds used in the Western pharmacopoeia are derived from plants (Mathekga, 2001). Therefore, Malawi should take advantage of its rich heritage in traditional medicine to make strides towards possible production of drugs based on medicinal plants. There is need to establish information of medicinal plants and their secondary metabolites such as alkaloids, saponins, tannins and flavonoids that are associated with antimicrobial properties. This baseline information will give enough evidence to support their use in treating ailments traditionally and will allow possible production of drugs from medicinal plants. In Malawi, there is little information on studies of some medicinal plants. Available literature on some studied medicinal plants is limited, since most work was done in European laboratories. Some plants that have broad antibacterial activities have been screened. For instance, screening of leaves of S*teganotaenia aralicea Honchst* and root bark of *Cassia petersina* showed that their bioactive agents are active against a broad range of bacteria (Msonthi, 1990). Consequently, baseline scientific information to support the use of medicinal plants is still inadequate, in particularly those that are used traditionally for treating diarrhoea. Therefore, selected plants used traditionally for treating diarrhoea were utilised in this study. The following plants were used: *Diplorhynchus condylocarpon, Holarrhena pubescens, Psidium guajava, Sclerocarya birrea, Steganotaenia araliacea, Trichilia emetica and Vernonia glabra.*

1.3 Hypothesis of the study

Medicinal plants that are used locally to treat diarrhoea possess classes of phytochemicals that are responsible for the antibacterial activity against enterobacterial pathogens such as *Salmonella* and *Shigella* species. Their antibacterial activity depends on the solvent of extraction, which determines the phytochemical composition of the extracts. Further, aqueous extracts of these plants that are commonly used by traditional healers contain these phytochemicals responsible for the antibacterial activity.

1.4 General and specific objectives

The study aimed at screening some medicinal plants for their classes of phytochemicals and their in vitro antibacterial activity on some gram-negative bacteria, *Shigella* and *Salmonella* species, which are commonly isolated from patients with diarrhoea.

The specific objectives were:

i. to identify classes of phytochemicals present in the selected medicinal plants and in crude extracts of methanol, ethyl acetate, dichloromethane and water;

ii. to determine the efficacy of the plant crude extracts of methanol, ethyl acetate, dichloromethane and water on *Salmonella typhi*, *S. typhirium*, *S. enteritidis*, *Shigella boydii*, and *S. flexneri*, and

iii. to extract and characterize alkaloids, saponins, terpenoids and flavonoids from plant species most active against *Salmonella* and *Shigella* species.

1.5 Chapter overview

In chapter 2 of the thesis the literature review is given and chapter 3 describes the materials and general methods that were used to achieve the objectives of this study. Chapter 4 of the thesis provides findings of this study and discussion of the findings. Conclusions and recommendations for further study are presented in the chapter 5.

CHAPTER 2: LITERATURE REVIEW

2.1 Traditional medicine and medicinal plants

Traditional medicine is defined 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, whether verbally or in writing’ (WHO, 1978 as cited in Elujoba et al., 2005). It is not known exactly when human beings started practicing traditional medicine, but one can only speculate that human beings have been using herbal medicine since the beginning of life. The use of herbal medicine goes back to prehistory era such that according to Cowan (1999) as cited in Martini (2001), there is evidence that the Neanderthal man used hollyhock (*Alcea rosea L.*). Although Sub Saharan Africa is one of the continents where traditional medicine is deep rooted, there are no known records of the beginning of traditional medicine in the region. Practice of traditional medicine and use of medicinal plants has been and is still in existence almost throughout the world, but the practice varies from one region to another and from culture to culture (Sofowora, 1993).

2.1.1 Traditional medicine and medicinal plants in the world

In North America, Europe and other industrialized countries, though there is little use of traditional medicine 50% of the population has used alternative medicine. However, research work on medicinal plants is taking place in their universities (Hostettmann et al., 2000). In Asia, traditional medicine is heavily relied upon, Ayurveda in India being one of the examples of formalized systems of indigenous medicine (Sofowora, 1993). Similarly, use and research on traditional medicine and medicinal plants are being carried out in South American countries such as Brazil, Argentina (Nascimento et al., 2000).

2.1.2 Traditional medicine and medicinal plants in Africa

In Africa, traditional medicine has been and remains used to treat many ailments. This is largely due to limited accessibility to conventional medicine by many ordinary Africans. This is compounded by high costs and inadequate medical care facilities. Traditional medicine is relatively cheap, easily accessible and acceptable by rural as well as urban populations (Elujoba et al., 2005). Many African countries have therefore instituted traditional pharmacopoeias to promote use of local medicinal plants (Rukangira, 1998). Many Africans strongly believe that some ailments are curable by traditional medicine as evidenced by the high estimations by WHO that indicate that 80% of African population makes use of traditional medicine (Richter, 2003). The Organisation of African Unity (OAU) in 1968 initiated and spearheaded research and development into traditional medicine and medicinal plants. Since then, most African countries have intensified their research into medicinal plants and traditional medicine (Sofowora, 1993).

2.1.3 Traditional medicine and medicinal plants in Malawi

It is not clear when traditional medicine started in Malawi. It seems that the practice was well established amongst Malawian tribes well before they came and settled in the country. In rural areas of Malawi, people still rely on traditional medicine for mild and common ailments (Morris, 1996).

Although Malawi has abundant plant resources that possess medicinal properties and has deep heritage of herbal medicine, limited research has been undertaken in Malawi. Traditional medicine was first recognized and recommended for incorporation in health care delivery system in 1978 at Alma - Ala Primary Health Care Conference. In Malawi, since then traditional birth attendants (TBAs) have undergone training programmes to support the national health care systems. Further, traditional healers collaborate with conventional medical personnel through patient referrals (Msonthi, 1986; Maliwichi, 2003; Mwanyambo and Nihero, 1998).

Some detailed ethnobotanical surveys have been conducted to establish some of the commonly used medicinal plants of Malawi (Mwanyambo and Nihero, 1998; Morris and Msonthi, 1991; Williamson, 1975). The work that was carried out in the departments of Chemistry and Biology in the University of Malawi in collaboration with National Herbarium and Botanical Gardens of Malawi has concentrated on phytochemical screening of plants believed to possess antifungal, molluscicidal, antimalarial, hypoglycenic and antifeedant activities (Seyani and Chikuni, 1994). Isolation and identification of active compounds and in vitro bioassays of some medicinal plants were carried out with assistance from Universities in Italy, Switzerland and Kenya (Msonthi, 1990; Connelly et al., 1996).

Important plants studied for their molluscicidal activities include *Diospyros zombensis, Securidaca longipedunculata, Clerodendrum wildii, Xeromphis obovata* and *Psorospermum febrifugum*. Saponins and flavonoids, naphthoquinones isomers 7-methyl juglone plumgagin and 7-methyl juglone isolated from root bark of *D. zombensis* and triterpenoids saponins isolated from the roots of *C. wildii* were found to show molluscicidal and antifungal properties (Gafner et al., 1987; Toyota et al., 1990). The leaves of *S. longipedunculata*, widely used for the control of schistosomiasis showed presence of sterols, terpenes, saponins anthraquinones and tannins (Kamwendo et al., 1985). Crude extracts of root bark of *X. obovata* and *P. febrifugum* at 100 mg/L killed 100% snails (Chiotha et al., 1986). Anti-fungal compounds falcarin-diol sarisan were isolated from *Heteromorpha trifoliate*, and Pterocarpinoids, sphenostylin A, B, C, and D isolated from *Dolichos marginata* showed weak antifungal activity (Villegas et al., 1998; Gunziger et al., 1988). Roots and leaves of *Cassia abbreviata* and *Senna petersiana* showed high anti-malarial activity (in vitro) (Connelly et al., 1996). Thus, so far medicinal plants used for schistosomiasis and fevers in the country have shown to possess phytochemicals that have molluscicidal and antimalarial activities, respectively.

Some plants such as *S. aralicea, C. petersiana* and *Kigelia pinata* that are not necessarily used by herbalist to treat diarrhoea were found to be active against entero - bacterial pathogens. These plants are used to treat sexually transmitted bacterial infections such as gonorrhea and syphilis (Msonthi, 1990). Phytochemical screening of the root bark and leaves of *C. petersiana* revealed presence of anthraquinones and nor-terpene oxides, colensenone and colensanone, which showed bactericidal activity (Msonthi, 1984). Terpenes from methanol fruit extracts of *K. pinata* also exhibited antibacterial activities against *Salmonella enteritidis*, *S. gallinarium*, *E. coli*, *Pseudomonus fluorescens*, *P. aerogunosa*, and *Staphylococcus aureus* (Msonthi and Magombo 1989). The methanolic extracts from leaves of *S. aralicea* also showed antibacterial activity against *S. aureus, E. coli*, *S. enteritidis* and other bacteria (Msonthi, 1990).

Apparently, plants locally used for treatment of diarrhoea have not been phytochemically screened. Only ethnobotanic surveys have revealed that many different medicinal plants in the country are utilised in diarrhoea problems (Maliwichi, 2003; Morris, 1986; Morris and Msonthi, 1991; Mwanyambo and Nihero, 1998).

2.1.4 Some medicinal plants used for treatment of diarrhoea in Malawi

Important plant materials, which are being utilized in treating diarrhoea problems, include the bark of *Mangifera indica,* roots of *S. aralicea Honchst,* roots of *Hynenocardia acida tul*, leaves of *P. guajava*, bark of *Pterocarpus angolensis*, roots and leaves of *Terminalia Sericea Burch*, leaves of *Camellia sinensis L.,* leaves of *Azedaracht indica L.*, roots and bark of *D. condylocarpon,* roots and bark of *H. pubescens*, roots and bark of *S. birrea,* roots and bark of *T. emetica,* bark of *Ficus natalensis Horchst,* and leaves of *V. glabra* (Maliwichi, 20003; Morris, 1986; Morris and Msonthi, 1991; Mwanyambo and Nihero, 1998). Most of these plants are also used for treatment of several other diseases. Some of these plants have been screened elsewhere and their enterobacterial activities determined. However, in Malawi only leaves of *S. aralicea Honchst* was phytochemically screened and its bacterial activity analysed (section 2.1.3).

2.1.4.1 *Diplorhynchus condylocarpon* (Apocynaceae)

D. condylocarpon has been utilised in tropical Africa for treatment of many bacterial infections and other health problems. For example, its roots have been used for wound dressing, treatment of syphilis and stomach complaints in Zambia, treatment of gonorrhoea, bilharzia, and as vermifuge in East Africa (Watt and Breyer – Brandwijk, 1962; Hedberg et al., 1981). The bark is used for treatment of hydrocele, dysentery, testicle inflammation, snake-bites and sore eyes in East Africa (Watt and Breyer – Brandwijk, 1962). In Malawi, the plant is used for treating pelvic inflammatory disease, infertility in women, tuberculosis, menstrual problems, whooping cough and dysentery. The bark is used for treatment of diarrhoea, and its latex combined with crushed seeds of *V. glabra* is utilized in the treatment of cataract (Mwanyambo and Nihero, 1998; Morris and Msonthi, 1991). The root bark of the plant analysed in Tanzania showed that it contains several types of alkaloids such as stemmadenine, condylocarpine, yohimbine, β-yohimbine, norfluorocurarine mossambine and tombozine (Hedberg et al., 1981). Tombozine was found to possess sympatholytic, anaesthetic and ganglionic properties (Stauffacher, 1961; Goutarel et al., 1961; Monseur et al., 1962 as cited in Hedberg et al., 1981). Despite its wide use, phytochemical screening of the plant has not been carried out in Malawi.

2.1.4.2 *Holarrhena pubescens* (Apocynaceae)

H. pubescens is another medicinal plant found in the tropics and subtropics. Its uses are well known and documented in the tropics especially in India. In India, the plant is used for anemia, epilepsy, stomach pains, cholera, diarrhoea and skin diseases (Ballal et al., 2001). The stem bark has astringent, anti-dysenteric, anthelmintic and tonic properties, and it is used for treatment of amoebic dysentery and diarrhoea (Siddiqui et al., 2001). In Malawi, the roots are used for a wide range of ailments such as impotence in men, chronic headache, syphilis, gonorrhoea, stomach pains and liver disorder (Mwanyambo and Nihero, 1998; Morris and Msonthi, 1991). The leaves, stem bark and roots are known to contain alkaloids. The bark alone is reported to have 29 different alkaloids, the well known one being conessine which has been extensively studied (Ballal et al., 2001). Total alkaloids from the stem bark have shown high antibacterial activity against *Staphylococcus aureus, S. epidermidis, Streptococcus faecalis, Bacillus subtilis, E. coli* and *Pseudomonas aeruginosa* (Chakraborty and Brantner, 1994). Recent work on the bark of *H. pubescens* by Siddiqui et al. (2001) has led to identification of two more steroids, pubadysone and puboestrene, and an alkaloid, pubamide.

2.1.4.3 *Psidium guajava* (Myrtaceae)

P. guajava is an indigenous plant of tropical America. It is used in folk medicine of countries in the tropics to treat several ailments. For instance, in Nigeria it is used for the treatment of fevers, diarrhoea, and as a tonic in psychiatry (Akinpelu and Onakoya, 2006). It is also used for ulcers, bowels and cholera in Brazil (Sanches et al., 2005). In Mexico, the leaves of *P. guajava* are used for diarrhoea, and its activity was attributed to quercetin, a flavonoid and its glycosides present. In Malawi the leaves have a variety of uses. The leaf infusion decoctions are locally used for gastric malaria, dyspepsia, diarrhoea, dysentery, fevers and measles (Morris and Msonthi, 1991; Maliwichi, 2003). The leaf is known to have wide range of biological activities. The leaf extract has been reported to have anti-cough, antibacterial, haemostasis, antidiarrhoea and narcotic properties (Belemtougri et al., 2006; Watt and Breyer – Brandwijk, 1962). Water, alcohol and chloroform extracts of the leaves have shown activity against bacterial species: *Aeromonas hydrophila*, *Shigella* species, *Vibrio* species, *Staphylococcus aureus*, *Sarcina lutea* and *Mycobacterium phlie* (Jaiarj et al., 1999 as cited in Sanches et al., 2005). The leaves are also known to contain triterpenoids, tannins and other flavonoids (Abdelrahim et al., 2002).

2.1.4.4 *Sclerocarya birrea* (Anacardacea)

S. birrea, popularly known as Marula tree in Zimbabwe, is an indigenous to Miombo woodlands of Southern Africa and East and West Africa. In folk medicine of South and East Africa the plant stem bark has been used in treatment of dysentery, diarrhoea, malaria and inflammatory conditions (Watt and Breyer – Brandwijk, 1962). The leaf and its extract have been used for treatment of gonorrhoea, boils, abscesses, burns, stings and wounds (Pratt et al., 2002). In Malawi, all parts of the plant are utilised for medicinal purposes. Infusions of roots and leaves are used for treatment of headache and snakebite. The root bark is used for dysentery, cough and throat infection and malaria. The roots are combined with *Annona senegalensis* bark and *Erythrina abyssinica* roots for treatment of syphilis and lymphogranulom venerum (Mwanyambo and Nihero, 1998). The aqueous extract of the bark has shown analgesic, anti-inflammatory and anti-diabetic properties (Ojewole, 2004). The bark of *S. birrea* is known to contain trace alkaloids and tannins whose concentrations vary with region.

2.1.4.5 *Steganotaenia aralicea Honchst* (Umbelliferae)

S. *aralicea Honchst* is one of the most commonly used medicinal plants in traditional medicine in Africa. In East and West Africa, it is used for the treatment of gastro-intestinal disorders, peptic ulcer, rheumatism and various diseases caused by microbial agents (Alemika et al., 2004). In Southern Malawi, it is the most highly utilised plant for microbial infections as well as psychiatric problems. It is very popular among herbalists and is used for various ailments. The leaves are used for treatment of chronic headache and mental illness. They are also used as a luck charm and for abortion in goats. Some of the ailments where the roots are utilised include syphilis, lymphogramuloma venerum, infertility in women, impotency in men, epilepsy, chronic cough and diarrhoea. The bark is claimed to treat asthma (Morris and Msonthi, 1991; Mwanyambo and Nihero, 1998; Maliwichi, 2003). Investigation of leaves by Msonthi (1990) showed that they have broad-spectrum antibacterial properties. Water and methanol extracts of root bark from Eastern Uganda have shown antibacterial activity against *E. coli*, *Pseudomonas aeruginosa*, and *S. aureus* too (Lino and Deogracious, 2006). Phytochemical screening of aqueous extract of the stem bark has shown presence of saponins that displayed significant anti-inflammatory activity (Alemika et al., 2004).

2.1.4.6 *Trichilia emetica* (Meliaceae)

T. emetica popularly known as natal mahogany in South Africa is another medicinal plant that is commonly used in tropical Africa. In Mali folk medicine, it is used for various ailments including malaria, abdominal pains, dermatitis, haemorrhoids, jaundice and chest pains (Togola et al., 2005). In Senegal, the infusion of the leaf is used for treatment of headache and burns (Togola et al., 2005). In East Africa, the root bark is used as an emetic and a purgative against fever, epilepsy and leprosy. The bark and leaf have been used for dysentery amongst Xhosa and Zulus (Togola et al., 2005; Watt and Breyer – Brandwijk, 1962). In Malawi, the plant is used for malaria especially in the north part of the country (Pratt et al. 2002). Its bark infusion is used for stomachache and dysentery, and as enema (Mwanyambo and Nihero, 1998). Studies have shown that the plant possesses some antimicrobial properties. It has also been found to show anti-plasmodia, anti-inflammatory, anti-pyretic activities (El Tahir et al., 1999; Togola et al., 2005). Hoet et al. (2004) showed that methylene chloride, methanol and aqueous extracts of the leaf have anti-trypanosomal activities, while crude aqueous extracts of the roots exhibited antibacterial activity against bacteria responsible for respiratory infection (Germano et al., 2005). The bark and leaf are known to contain tannins and limonoids (Germano et al., 2005).

2.1.4.7 *Vernonia glabra* (Compositae)

V. glabra is another tropical plant that is frequently utilized for many diseases in Malawi. The roots of the plant are used for treatment of infertility in women, venereal diseases, trichomas and haemorroids. The leaves are utilised in treatment of cataract, bilharzia, rheumatism, backache, headache, diarrhoea, sprain swelling and tumours (Morris and Msonthi, 1996; Mwanyambo and Nihero, 1998). The plant is known to contain vernolepin, vernodalin germacrolides and fifteen sesquiterpenes lactones that have shown anti-tumour activity (Jakupovic, 1985 as cited in Msonthi, 1990)

2.2 Phytochemicals in medicinal plants

Phytochemicals are secondary metabolites present in plants that play important roles in plant growth and defense against predators (Mallikharjuna et al., 2007). There are several classes of phytochemicals, which are classified based on their biosynthesis origin, solubility properties and their key functional groups (Harbone, 1973). Some important examples of phytochemicals include alkaloids, terpenoids, flavonoids, anthraquinones, saponins and tannins. Phytochemicals have been shown to possess pharmacological properties such that they find abundant applications in health care systems (Harbone, 1973). Epidemiological evidence and clinical studies have revealed that diet containing plant food reduce risk of some chronic diseases. For instance, cholesterol, which is associated with heart problems, is reduced by some phytochemicals such as flavonoids and tannins (Prior and Cao, 2000). Their importance in their use as antimicrobial cannot be overemphasized. Compounds belonging to classes of alkaloids, terpenoids and others have been isolated and synthesised.

2.2.1 Alkaloids

Alkaloids have diverse structures such that it is not easy to come up with a systematic definition to describe them satisfactorily. However, alkaloids basically contain one or more nitrogen atoms that usually form part of a cyclic system (Harbone, 1973). These alkaloids are widely distributed in all parts of the plants and exhibit some interesting physiological activities such that plants use them for protection from predators; functioning as growth regulators, insect repellents and attractants (Harbone, 1973). They also find wide application in medicine (Mathekga, 2001). Many alkaloids that possess varied antimicrobial properties have been isolated and characterized. For example, quinine (Figure 1) isolated from cinchona tree is active against plasmodium, a parasite that causes malaria.

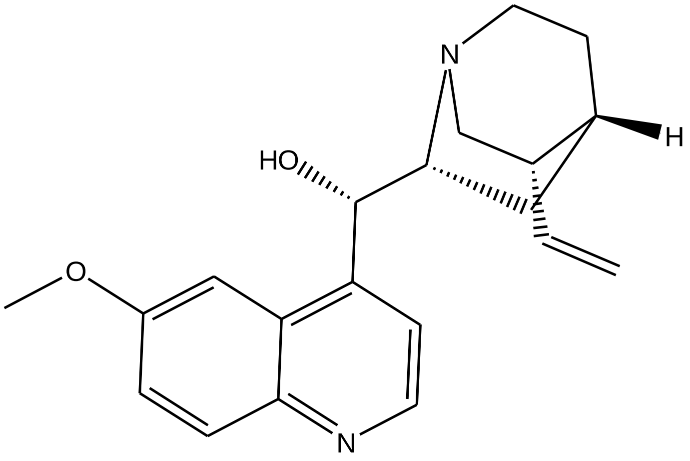

Figure 1: Structure of quinine

Further, caffeine (Figure 2), a purine alkaloid, which is present in plants such as tea, coffee and cocoa, has shown to possess both antibacterial and antifungal properties (Avery et al., 2005).

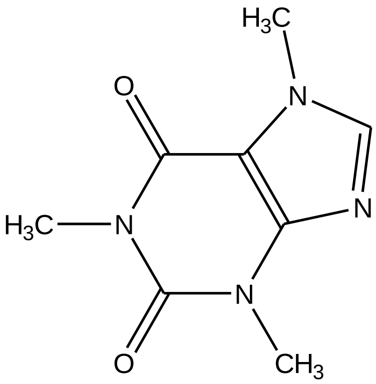

Figure 2: Structure of caffeine

2.2.2 Saponins

Saponins are glycosides of triterpenoids, steroids, flavonoids and steroidal alkaloids present in plants as well as in some marine organisms, and one of their characteristics is that they produce colloidal soapy solutions in water (Harbone, 1973). Saponins also possess a wide range of biological activities such that they play important roles in pharmaceutical properties. For example, *Aloe barbadensis*, a plant with application in treating various ailments contains saponins that possess antibacterial activities (Ferro et al., 2003). Glycoside known as quercetin-3-O-α-L-arabinopyranoside (Figure 3) isolated from *P. guajava*, which commonly occur as flavonoids, was found to be active in vitro against *Streptococcus mutans* (Prabu et al., 2006).

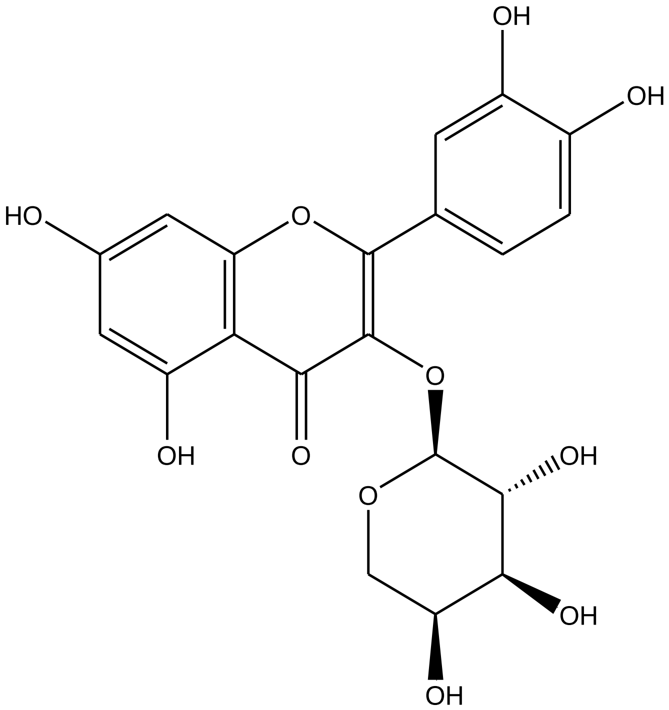

Figure 3: **Structure of quercetin-3-O-α-L-arabinopyranoside**

2.2.3 Terpenoids

These are secondary metabolites that are based on isoprene structure ($CH_2=CCH_2CH=CH_2$). They are a diverse group and widely distributed in plants. Their carbon skeleton is built up from combination of two or more isoprene units (Harbone, 1973). The first in their series has a general chemical structure of $C_{10}H_{14}$ (Figure 4) and is referred to as monoterpenoid. Thus the classification is based on this monoterpenoid such that the terpenoids with C_{20}, C_{30}, and C_{40} are referred to as di-, tri-, and tetra- terpenoids, respectively. Terpenoids with C_{15} are known as sequerterpenoids (Harbone, 1973).

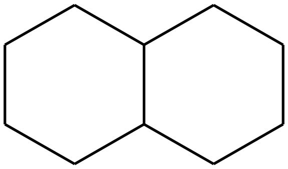

Figure 4: **Structure of part of a monoterpenoid**

Terpenoids have been associated with many important biotic interactions and are involved in physiological functions. For instance, they form part of membrane bound steroids, which are mainly tetraterpenoids (Martini, 2001). Monoterpenoids are the component of essential oils, which find many applications as antimicrobial agents. For instance, essential oils extracted from *Ocimum gratissum* have shown some significant antibacterial activity against *S. aureus*, *S. enteritidis*, *E. coli* and *Klebseilla* species (Nakamura et al., 1999). Other terpenoids such as triterpenoid also have been found to be active against some microbials. One of the examples includes triterpenoid, betulinic acid (Figure 4) that inhibits the HIV activity (Patocka, 2003).

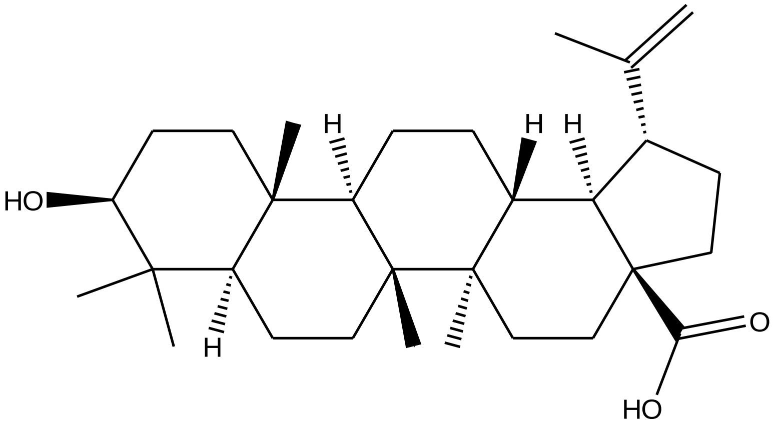

Figure 5: Structure of betulinic acid

2.2.4 Flavonoids

These are secondary metabolites that belong to phenolic compounds, which are made up of conjugated aromatic systems. Flavonoids are generally present as parts of sugars, which exist as glycosides and may also exist in several glycosidic combinations such that they are water- soluble (Mathekga, 2001). There are at least ten known classes of flavonoids namely: anthocyanins, leucoanthocyanidins, flavonols, flavones, glycoflavones, biflavonyls, chalcones, aurones, flavanones and isoflavones (Harbone, 1973). However, flavones and flavonols are commonly present in many plants and isoflavones and biflavonysl have been found in some few plants. Flavonoids are known to function in defense against herbivores as they are normally synthesised in response to microbial infections (Dixon, 1983 as cited in Cowan, 1999). They have also been found to show antimicrobial activity against fungi and bacteria. Examples include some isoflavonoids isolated from *erythrina variegata,* which were found to be active against methicillin-resistance *S. aureus* (Tanaka et al., 2002). Other flavonoids such as quercetin (Figure 5) isolated from the leaves of *P. guajava* have also shown antibacterial activity (Arima and Danno, 2002).

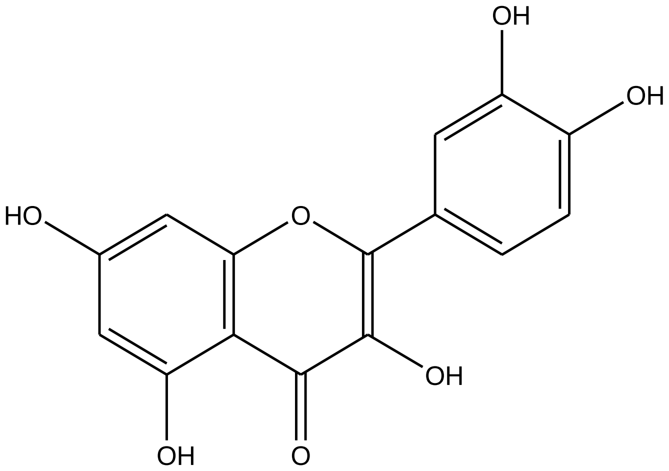

Figure 6: Structure of quercetin

2.2.5 Tannins

Tannins are water-soluble secondary metabolites that belong to phenolic compounds with high molecular weights. They are divided into two groups: hydrolyzed and condensed tannins. The hydrolyzed tannins can be broken down into soluble gallic acid and glucose by acid or enzymes. On the other hand condensed tannins produce more complex insoluble substances when treated with acid or enzymes. Tannins can be extracted from various parts of the plant including fruits, leaves, barks and heartwood of trees (Hagerman, 2001). Tannins in young plant tissues probably play a vital role in protecting the plant from parasitic fungi attacks and grazing animals. Tannins exhibit unique chemical reactivities and biological activities, which are not characteristic to all phenolics. Tannins can also interact with alkaloids and gelatin. They prevent oxidative damage that has been implicated in diseases such as cancer, cardiovascular disease and arthritis. Unlike some low molecular weight phenolics that act as pro-oxidants in formation of free radical, tannins quench free radicals such as hydroxyls thereby reduce the oxidative damage (Hagerman, 2001). Some tannins that show antimicrobial properties have been found too. For instance, 2,2 β-hydroxytingenone and tindenone isolated from Khat Callus cultures showed significant activity against bacterial strain of *S. subtilis*, *S.aueus*, *S. durans* and *Mycobacterium* species (Elhag et al., 1999). Some hydrolysed tannins such as penta-O-galloyl-β-D-glucose (Figure 6) isolated from *Rhus chinensis* have also shown antibacterial activities against *Helicobacter pylori* (Funatogawa et al., 2004).

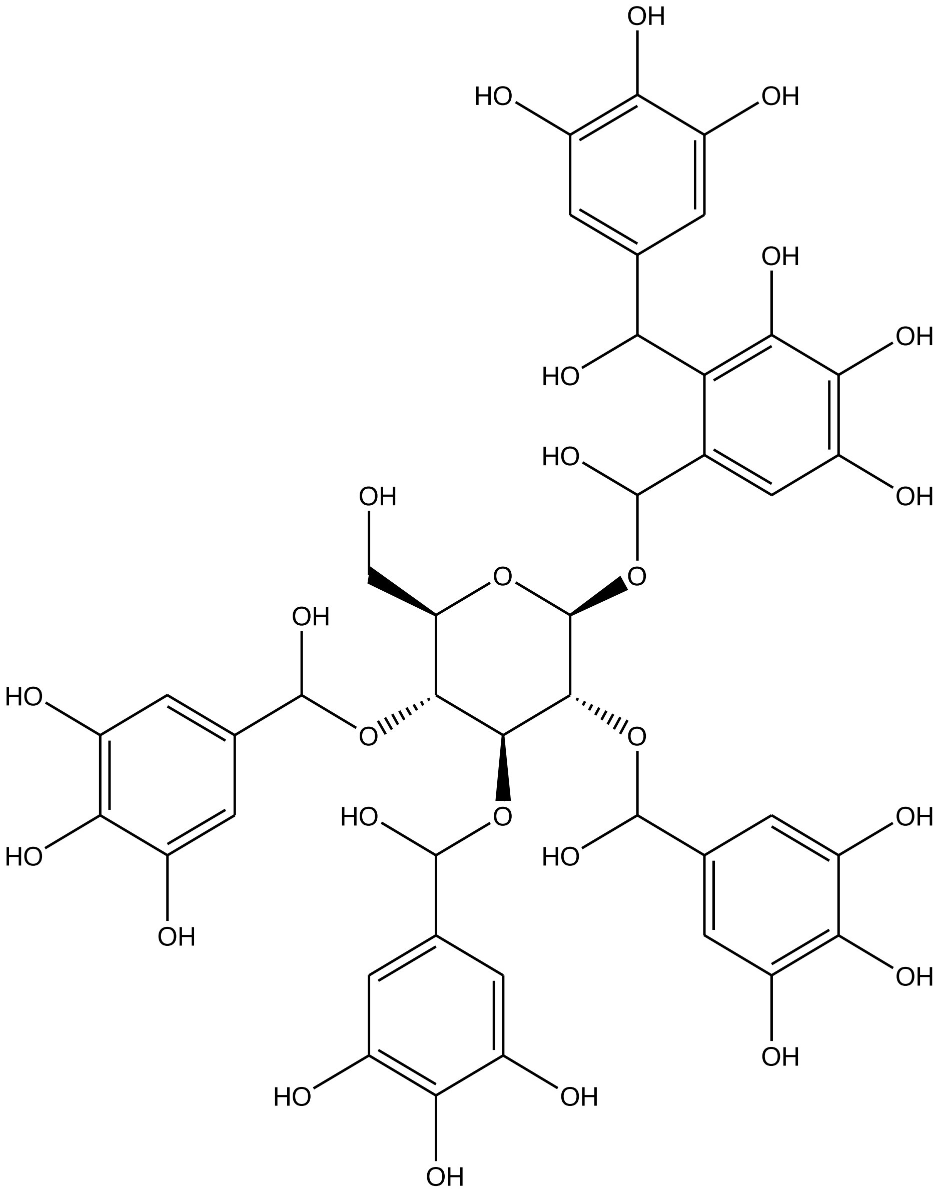

Figure 7: Structure of penta-O-galloyl-β-D-glucose

2.3 Characterisation of classes of phytochemicals

Characterisation of phytochemicals involves sample preparation, separation, purification and identification of individual compounds (Harbone, 1973). The first step is done by solvent partition methods to produce crude extracts of classes of phytochemicals that contain impurities from which individual phytochemicals are separated, purified and identified (Hostettaman et al., 1986).

2.3.1 Isolation of phytochemicals

When crude extracts of class of phytochemicals are obtained, preliminary separation can be done using planar chromatography such as paper chromatography (PC) and thin layer chromatography (TLC) or using column chromatographic techniques such as open column chromatography and high performance liquid chromatography (HPLC). In both PC and TLC, mobile and stationary phases are solid and liquid solvent systems, respectively. The solvent systems range from non – polar solvents such as pet ether to polar solvents such as methanol. These solvents can act as single mobile phases or as mixtures (Hostettaman et al., 1986; Harbone, 1973).

Of these techniques, TLC is commonly used because of its versatility, simplicity and inexpensiveness. In PC, only the mobile phase can be varied and has limited number of solvents that can be employed, whilst in TLC mobile as well as stationary phases can be varied and different solvent systems and adsorbents are utilized (Harbone, 1973). The widely used adsorbent is silica gel in TLC as well as open column chromatography. However, in TLC compounds such as aluminium oxide, calcium hydroxide, cellulose and magnesium phosphate are also utilized as adsorbents (Kalasz, 2002). Separated compounds can be detected by spraying detecting reagents, which vary according class of compounds isolated. For example, alkaloids can be detected by spraying Dragendorff's reagent. Phytochemicals that absorb in UV region, UV radiation lamp set at 254 nm or 365 nm can also be used (Hostettmann et al., 1986). Detection in both techniques allows determination of R_f values where R_f is given by the following expression:

$$R_f = \frac{\text{distance traveled by compound}}{\text{distance traveled by solvent front}}$$

Isolation and purification is mainly done using column chromatographic techniques. However, TLC can also be used for isolation of pure compounds. Commonly used column chromatographic techniques for purification including gas liquid chromatography (GLC), capillary electrophorensis and HPLC (Kalasz, 2002). After these chromatographic separation and isolation, purified compounds are then identified.

2.3.2 Identification of phytochemicals

Identification of pure compounds includes use of biochemical tests and determination of spectroscopic characteristics of ultra violet / visible (UV/vis), infra red (IR), nuclear magnetic resonance (NMR) and mass spectra (MS) (Prabu et al., 2006; Saxena and Albert, 2005; Perez – Amador et al., 2004). The spectroscopic data is obtained from machines that are sometimes connected to column chromatography machines. For example, GLC – MS machines are available (Harbone, 1973). In addition, spectroscopic characteristic can be compared with the already existing data if it is available (Harbone, 1973).

Preliminary identification of classes of the phytochemicals can also be done using chemical tests and UV spectra (Harbone, 1973). In this case the UV spectra give wavelength at which classes of phytochemicals have maximum absorption only. Absorption occurs due to electronic transitions between energy levels especially in π – conjugated systems that are present in phytochemical compounds (Fleming and Williams, 1995). In UV analysis, choice of solvents is critical since some solvent interfere with spectrum of the sample. The most common solvents that can be used with minimum interference include methanol, 95% ethanol and water (Gomez – Garibay et al., 2002; Harbone, 1973)

2.4 Diarrhoea and its bacterial pathogens

Diarrhoea, which is clinically grouped into two categories: bloody diarrhoea also known as dysentery and non-bloody diarrhoea, is mainly caused by intestinal infections (Amarapurkar and Baija, 2000). These intestinal infections are largely contracted through ingestion of contaminated food. Infections of enterotoxin producing organisms such as *E.coli* and *V. cholera* are some of the causative agents of acute diarrhoea (Amarapurkar and Baija, 2000). These infections may also result from enteropathogenic organisms such as *Shigella* and *Salmonella* species.

2.4.1 Enterobacteriaceae

Enterobacteriaceae, is one of the largest groups of bacteria with over 120 known species. These are aerobic or facultative gram-negative rods that ferment glucose. All known enterobacteriaceae except genera *Enterobacter, Erwinia, Klebsika, Xenorhabdus* and *Yersina* give a negative test to oxidase and are capable of reducing nitrates to nitrites. These characteristics serve to differentiate members of this family with other bacterial families (Kelly et al., 1985).

Enterobacteriaceae are one of the most pathogenic families that are associated with human infections such as abscesses, pneumonia, meningitis septicemia, and intestinal urinary and wound infections. They account for a large portion of intestinal infections in humans and animals worldwide. Important Members of this family such as *Escherichia, Salmonella, Shigella* and *Yersinia* have been clearly established as the enteric pathogens and many have been implicated as main causes of diarrhoea. Except *Shigella* species, most enterobacteriaceae can also cause extraintestinal infections (Kelly et al., 1985). The infections caused by *Salmonella* and *Shigella* species are treated using antibiotics such as ciprofloxacin and chlorophenicol (Obi and Besong, 2002).

2.4.1.1 *Salmonella* species

Salmonella species are pathogenic bacteria that belong to enterobacteriaceae family. These gram-negative bacteria have their rod shaped structure surrounded by flagella. Most *Salmonella* strains are characterised by their positive response to motility test. The classification of *Salmonella* species is done according to their surface antigens and there are 22000 known species (Martini, 2001). *Salmonella* infections range from gastroenteritis with mild symptoms of short duration to severe gastroenteritis with and without bacteremia to typhoid fever, which is severe, and sometimes life-threatening (Cheesebrough, 1984).

Common *Salmonella* species include S. *typhi, S. enteritidis S. paratyphi* A and B and *S. typhimurium* and these are transmitted to humans through beef, poultry, pork, eggs, milk, fish, fresh fruit juice and vegetables (Kariuki et al., 2006). However, *S. typhi* and *S.*

paratyphi are usually found only in human beings. *S. typhi* causes various disorders including typhoid fever, nephrotyphoid, osteomylitis meningitis and rarely pneumonia (Cheesebrough, 1984). *S. paratyphi* A and B cause paratyphoid fever accompanied with diarrhoea and vomiting (Cheesebrough, 1984). Although diarrhoea caused by *Salmonella* species is not usually life threatening, *Salmonella* species have most recently been associated with opportunistic pathogens isolated from immunocompromised patients mainly due to HIV.

In USA, there have been increases in percentages of total *Salmonella* isolated from blood of persons with AIDS. For instance, men of age group between 25 and 49 with high AIDS incidences the percentage of *Salmonella* isolates increased from 2.3% in 1978 to 17.8% in 1987. *S. enteritidis* and *S. typhirium* were found to be causative agents of increase in bacteremia (Morris and Potter, 1997). Similar occurrences are also observed in the Sub-Saharan Region. For instance, in Zambia, it was found that non – typhoid *Salmonella* and *Shigella* species were the common bacterial isolates in HIV sero – positive patients with persistent diarrhoea (Mwansa et al., 2002). In Malawi, *S. typhirium* and *S. enteritidis* were the commonest isolates from HIV sero – positive children with recurrence of non – typhoid *Salmonella* bacteremia (Graham et al., 2000). *S. typhirium* was also found to be amongst the common bacterial isolates from adult HIV patients in Kenya (Mwachari et al., 1998).

2.4.1.2 *Shigella* species

Shigella species are enterobacteriaceae that cause bacillary dysentery also known as shigellosis, which is characterised by severe abdominal cramps and fever (Cheesebrough, 1984). Unlike *Salmonella* species, *Shigella* species are non-motile and have polymorphic rods shape that occur singly or in pairs. Important species include *S. dysenteriae, S. flexneri, S. boydii* and *S. sonnei*. *Shigella* species are found in human intestinal tract and are transmitted by feacal-oral path. Shigellosis is commonly prevalent in areas with poor sanitation and where the water is polluted (Cheesebrough, 1984). In developing countries shigellosis is responsible for the high death rate especially in young children. Most life threatening dysentery is mainly caused by *S. dysenteriae* and to less extent S. *flexneri* and

S. sonnei, which is less pathogenic and its infections are mild. However *S. flexneri* is more prevalent in patients older than 15 years, and have also been isolated in HIV/AIDS infected adults (Mwachari et al., 1998).

2.5 Antibiotics and bacterial resistance

The discovery of antibiotics beginning with penicillin in the 1940s led to excessive usage of antibiotics. Consequently, widespread resistance of many bacteria to antibiotics had developed by 1955 (Martini, 2001). Despite the development of semi-synthetic antibiotics such as semi-synthetic penicillin in the 1960s, resistance patterns were still observed, and over the years, some bacteria have developed resistance to any group of new antibiotics (Martini, 2001). For instance, *S. typhirium* has been observed to be resistant to ampicillin, chloramphenicol, streptomycin and tetracycline (Varma et al., 2005). *Shigella* species have also been found to be resistant to trimethropin / silphamethoxazole (Gaudreau and Turgeon, 1997). According to World Health Report (2000) on infectious diseases, one of the major concerns of WHO for this millennium is to overcome bacterial resistance to antibiotics. Therefore, the past decade has witnessed an increase in research on plants as an alternative source of human health solution (Raghavendra et al., 2006).

CHAPTER 3: MATERIALS AND METHODS

3.1 Sample collection

Initially three herbalists from three districts of the southern region of Malawi were contacted. Two herbalists were heads of their respective herbalists associations based in Zomba and Blantyre. The third herbalist was head of Andiamo herbal clinic that is run by Roman Catholics and based in Balaka. The herbalists were interviewed to establish medicinal plants, which are used for diarrhoea, common to these areas and to compare with ethnobotanic literature already available. Finally candidate plants were selected on basis of information given by the herbalists and ethnobotanic and ethnopharmacological literature.

Leaves, and / or root and stem barks of the plants, and where possible, flowers were collected. *H. pubescens* and *T. emetica* were collected from the forest of Mwanza, while *P. guajava*, *S. birrea*, *S. araliacea* *V. glabra* and *D. condylocarpon* were collected from the forests of Zomba with assistance from Herbarium botanists. The samples were collected in April 2003.

3.2 Chemicals, reagents and instrumentation

Chemicals of analytical reagent (AnalaR) grade were utilised during all the analyses except for phytochemical screening analyses where chemicals of general purpose reagent (GPR) grades were used. The following chemicals and reagents, which were purchased from British Drug House (BDH) and Associated Chemicals Enterprises were used for phytochemical screening analyses: bismuth subnitrate, potassium iodide, mercuric chloride, concentrated hydrochloric acid (32% v/v), concentrated ammonia (25% v/v), chloroform, diethyl ether, acetic anhydride, concentrated sulphuric acid, ethyl acetate and ferric chloride. In extraction and antibacterial analyses the following chemicals and reagents purchased from SAARCHEM were used: methanol, ethyl acetate, dichloromethane, glacial acetic acid, ethanol (99% v/v), concentrated ammonia (25%), chloroform, concentrated hydrochloric acid (32%), zinc dust, diethyl ether, n-butanol, sodium chloride, potassium hydroxide and acetone. Powders for MacConkey agar, Mueller-Hinton agar, Nutrient agar and Selenite F broth purchased from Oxoid were used in preparation of bacterial strains for antibacterial bioassay analysis. Whatman filter paper No. 1 which was purchased from SAARCHEM was utilised in all the procedures.

The equipments used in the analyses included soxhlet apparatus for obtaining crude extracts of plant samples, Corning vacuum rotary evaporator type 349/2 for evaporation of solvents, centrifuge model Centra CL2, Oerting analytical balance model R41 Mark II for weighing crude extracts of plant samples, extracts of phytochemicals and plant samples used for yield determination, Oerting analytical balance model KB23 for weighing plant samples used for phytochemical screening tests, and UV/VIS spectrophotometer model Jenway 6405 UV for spectra scanning of phytochemicals.

3.3 Phytochemical screening of plant samples

Standard chemical tests with some modification were used to determine the presence of alkaloids, saponins, flavonoids, terpenoids and tannins in plant samples and crude extracts. The presence of these classes of phytochemicals is first step in supporting these medicinal plants for their use as antimicrobial agents since these secondary metabolites are associated with antimicrobial properties.

3.3.1 Preparation of testing reagents

3.3.1.1 Dragendorff's reagent

Potassium iodide (22.7g) was dissolved in de-ionised water (25 ml) and bismuth subnitrate (8g) was mixed with this aqueous solution. The precipitate formed was filtered off and the filtrate was collected into a 100 ml volumetric flask and diluted to the mark with water.

3.3.1.2 Mayer's reagent

Mercuric chloride (1.35g) and potassium iodide (5.0g) were weighed into a 100 ml beaker and dissolved in de-ionised water (40 ml). Then the solution was quantitatively transferred into a 100 ml volumetric flask and filled to the mark with water.

3.3.1.3 Dilute aqueous hydrochloric acid solution (10% v/v)

Concentrated hydrochloric acid (32% v/v, 10 ml) was added to de-ionised water (50 ml) and the solution diluted to 100 ml.

3.3.1.4 Dilute aqueous ammonia solution (0.1% v/v)

De-ionised water (40 ml) was added to 100 ml volumetric flask followed by addition of concentrated ammonia solution (25% v/v, 1 ml) and the solution diluted to the mark with water.

3.3.1.5 Aqueous ferric chloride solution (0.1% w/v)

A sample of ferric chloride (0.1g) was dissolved in de-ionised water (40 ml) and quantitatively transferred into 100 ml volumetric flask and filled to the mark.

3.3.2 Chemical tests of phytochemicals

3.3.2.1 Alkaloids tests

This method was based on the method by Ayoub and Kingstone (1981) in screening for alkaloids from Sudanese medicinal plants.

Air – dried powdered plant material (5.0g) was macerated in aqueous hydrochloric acid solution (10% v/v, 50 ml), left to stand for 24 hours and followed by gravity filtration using Whatman filter paper No. 1. Then an aliquot of filtrate (1 ml) was treated with 10 drops of Dragendorff's reagent and a second portion (1 ml) was treated with Mayer's reagent. Brown and white precipitates for Dragendorff's and Mayer's reagents, respectively indicated the positive results. Confirmatory tests were carried out on those samples that gave preliminary positive results. A portion of filtrate (20 ml) was basified to pH 9 with 10 drops of concentrated ammonia solution (25% v/v) and extracted with chloroform (20 ml). The procedure was repeated with another 20 ml of filtrate. The two extracts were mixed and the chloroform was evaporated to dryness using boiling water bath in a fume hood. The residue was dissolved in aqueous hydrochloric acid (10% v/v, 3 ml). The volume of solution was divided into two equal portions. Ten drops of Dragendorff's reagent were added to one portion, 10 drops of Mayer's reagent were added to the other to confirm the preliminary results. The results were recorded as follows: weak precipitate = (+) immediate and strong precipitate = (++) immediate and very strong precipitate (+++).

3.3.2.2 Saponins test

This method was based on the method by Harbone (1973) in screening for saponins.

Air-dried powdered plant material (1.0g) was macerated in de-ionised water (20 ml) and left to stand for 24 hr. The extract was filtered using Whatman filter paper No. 1, and the filtrate (10 ml) was transferred into a 16 mm by 160 mm test tube and shaken vigorously for 10 seconds. Then it was left to stand for 20 minutes. The persistent foaming after 20 minutes indicated presence of saponins. The depth of froth gave a rough idea of the levels of saponins in the samples. The depth greater than 1 mm was indicative of strong presence of saponins (++).

3.3.2.3 Terpenoids test

This method was based on the method by Ayoub and Kingstone (1981) in screening for terpenoids from Sudanese medicinal plants.

Air –dried powdered plant material (1.0g) is macerated in diethyl ether (20 ml) in a 50 ml stoppered conical flask, left to stand for 48 hr and filtered using Whatman filter paper No. 1. Then 10 drops of the filtrate were transferred into a porcelain crucible and evaporated to dryness on a water bath. Then 10 drops of acetic anhydride were added to the residue followed by 10 drops of concentrated sulphuric acid. The development of pink to purple colour indicated the presence of terpenoids. Very intense purple colour was indicative of strong presence of terpenoids (++).

3.3.2.4 Flavonoids test

This test was based on the method by Edeoga et al. (2005) in screening for flavonoids from Nigerian medicinal plants.

Air-dried powdered plant material (2.0g) was stirred in distilled water (50 ml) for 30 minutes and filtered. Then dilute aqueous ammonia solution (0.1% v/v, 5 ml) was added to the filtrate followed by addition of 10 drops of concentrated sulphuric acid. The presence of flavonoids was indicated by development of yellow colouration that disappeared on standing.

Another test was carried to complement this test. Air-dried powdered plant material (2.0 g) was heated with ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and the filtrate (4 ml) was shaken with dilute aqueous ammonia solution (0.1% v/v, 1 ml). A yellow colouration observed was indicative of presence of flavonoids. Very intense yellow colour was indicative of strong presence of flavonoids (++), and mild colour was indicative of weak presence (+).

3.3.2.5 Tannins test

The tests were based on the method by Edeoga et al. (2005) in screening for tannins from Nigerian medicinal plants.

Air-dried powdered plant sample (1.0g) was boiled in de-ionised water (20 ml) in a boiling tube and then filtered. A few drops of aqueous ferric chloride solution (0.1% m/v) was added to the filtrate and a brownish green or blue-black colouration was indicative of positive results while intense blue – black colouration was indicative of strong presence of tannins (++).

3.4 Extraction of crude extracts and yield determination

Three ground air-dried plant materials (50.000g) each were accurately weighed into different 250 ml round bottomed flasks followed by soxhlet extraction using three different organic solvents namely: methanol, ethyl acetate, and dichloromethane. A fourth extraction was done using water at 25°C for 48 hr. The solvents were removed by rotary evaporation under pressure at 45°C. The extracts were further dried at 40°C to a constant weight and the yield was determined as per equation [1]. The whole process was done three times and extracts were stored at 4°C under refrigeration for further analysis.

Only yields of the extracts obtained from organic solvents were determined. Yields from water extracts could not be determined since it was not possible evaporate the solvent completely on the rotary evaporator.

$$\text{Yield (\%)} = \frac{\text{mass of crude extract x 100\%}}{\text{mass of air – dried sample}} \qquad [1]$$

3.5 Phytochemical screening of crude extracts

The dried crude extracts of methanol, ethyl acetate, dichloromethane and water were screened for the presence of alkaloids, saponins, terpenoids, flavonoids and tannins using the standard chemical tests as described in section 3.3.2

3.6 Antibacterial activity analyses

Analyses of in vitro bacterial activity of crude extracts from different extracting solvents were carried out in order to determine extraction ability of solvents, and test the efficacy of these plant extracts against some of the common bacterial pathogens that cause diarrhoea.

3.6.1 Preparation of reagents

3.6.1.1 MacConkey Agar

A sample of MacConkey agar (25.0g) was weighed and dispersed in distilled water (500 ml). The suspension was allowed to stand for 10 minutes before it was autoclaved at 132^{o}C for 15minutes. The solution was allowed to cool to approximately 55^{o}C - 60^{o}C and portions (25 ml) transferred into petri dishes.

3.6.1.2 Mueller-Hinton Agar

A sample of Mueller – Hinton agar (19.0g) was weighed and suspended in distilled water (500 ml). The suspension was boiled to dissolve the solid. Then the solution was autoclaved at 132^{o}C for 15 minutes. The solution was allowed to cool to about 55^{o}C - 60^{o}C before portions (25 ml) were transferred into petri dishes.

3.6.1.3 Nutrient Agar

A sample of nutrient agar (14.0g) was weighed and suspended in distilled water (500 ml). The suspension was boiled to dissolve the solid. Then solution was autoclaved at 132^{o}C for 15 minutes. The solution was allowed to cool to about 55^{o}C – 60^{o}C before portions (25 ml) were transferred into petri dishes.

3.6.1.4 Selenite F broth

A sample of Selenite F broth (23.0g) was weighed and suspended in distilled water (1 L) at 25^{o}C. The suspension was heated briefly to 60°C in order to completely dissolve. Portions (25 ml) of the solution were transferred into boiling tubes.

3.6.2 Bacterial strains

Clinical isolates of *S. typhi, S. typhirium, S. enteritidis, S. boydii* and *S. flexneri* were obtained from Microbiology Section of Community Health Services Unit (CHSU) in Lilongwe. These were subcultured on nutrient agar and inoculated in the Selenite F broth for enrichment and incubated for 24 hr at 35^{o}C – 37^{o}C.

3.6.3 Determination of diameter of inhibition zone

Analysis was done at Microbiology laboratory of Zomba Central Hospital with the assistance of their technicians. Kirby-Bauer Disc Diffusion Method was utilised in carrying out the antibacterial bioassay tests.

The extracts were dissolved in their respective solvents and diluted to a concentration of 100 mg/ml before use. A small antibiotic disc of ciprofloxacin, two sterile paper discs (diameter, 6 mm) previously impregnated with the extract (0.1 ml) and solvent of extraction (0.1 ml) were placed carefully onto the surface of labeled Mueller-Hinton agar plates which were inoculated with the bacteria of interest. The plates were incubated aerobically at 35^{o}C -37^{o}C and the inhibition zones were measured after 24 hr. Discs impregnated with solvent of extraction were used as blanks, and the antibiotic discs were used in order to compare with the extracts. For each extract, the procedure was carried out three times.

3.6.4 Determination of minimum inhibitory concentration

Serial dilution of 100 mg/ml extracts of root and stem barks *D. condylocarpon* and *H. pubescens* that showed significant activities against at least four bacterial strains were carried out. Extract concentrations ranged from 10 μg/ml to 100 mg/ml in incremental steps of 5 μg. The diameters of inhibition were determined as per section 3.6.3.

3.7 Extraction of classes of phytochemicals

3.7.1 Alkaloids extraction

Three extraction methods were utilised. The first method was a modification of a method by Harbone (1973) followed by Christov et al. (2002) and finally by Siddiqui et al. (2001).

3.7.1.1 Harbone method

This method was carried out based on method by Harbone (1973) in extracting alkaloids.

Air-dried powdered sample (50.000g) was accurately weighed and macerated in ethanolic acetic acid (10% v/v, 600 ml). The mixture was allowed to stand for 18 hr, filtered and the extract was concentrated on a steam water-bath to one-quarter of the original volume. Concentrated ammonia (25% v/v) was added drop-wisely until the precipitation was complete. The whole solution was allowed to settle and the precipitate was suction filtered and washed with dilute ammonia (1% v/v, 50 ml). The residue was dried in the oven at 40°C for 6 hr and weighed. The crude extract was dissolved in chloroform (100 ml), filtered and solvent evaporated to dryness in a fume hood. The crude alkaloid extract was further dried at 40°C to a constant mass. The yield was determined as per equation [2]. A portion of the extract (0.100g) was tested for alkaloids as per section 3.3.2.1 and the remaining extract was kept 4°C under refrigeration for further analysis.

$$\text{Yield (\%)} = \frac{\text{mass of crude alkaloids extract x 100\%}}{\text{mass of sample}} \qquad [2]$$

3.7.1.2 Christov method

This method was done based on the method by Christov et al. (2002) in extracting alkaloids from *Senecio macedonicus* Griesb.

In this method, air-dried powdered sample (50.000g) was accurately weighed and macerated in methanol (600 ml) for 24 hr. After filtration the residue was extracted again in fresh methanol (450 ml) and the extracts were combined. The combined extracts were evaporated to dryness on the rotary evaporator. Aqueous hydrochloric acid solution (5% v/v, 50 ml) was added to the dried extract, and the mixture shaken and filtered. The acidic filtrate was extracted with chloroform (200 ml). The acidic aqueous layer was magnetically stirred with zinc dust (1 g) for 24 hr, and then filtered. The filtrate was made alkaline with concentrated ammonia (25% v/v, 8 ml) to pH 9 forming a precipitate in the process. The alkaline solution was extracted with chloroform (2 x 150 ml) to obtain the required crude alkaloids extract. The Chloroform was evaporated from the combined extracts on rotary evaporator to remain with white residue of crude alkaloids. The crude alkaloids extract was further dried at 40^{o}C to a constant mass and the yield was determined as per equation [2]. A portion of the extract (0.100g) was tested for alkaloids as per section 3.3.2.1 and the remaining extract was kept at 4^{o}C under refrigeration for further analysis.

3.7.1.3 Siddiqui method

This method is a modification of Siddiqui et al. (2001) which was used to extract alkaloids from stem bark of *H. pubescens* bark from Pakistan.

Air-dried powdered sample (50.000g) was accurately weighed and macerated repeatedly in Methanol (3 x 600 ml) at 26^{0}C for 48 hr. The extracts were combined and evaporated under vacuum using rotary evaporator to obtain crude extract. To the crude extract, dilute aqueous acetic acid (10% v/v, 90 ml) was added and the resulting mixture shaken and filtered. The filtrate was extracted with ethyl acetate (70 ml), and the ethyl acetate layer was collected and treated with aqueous acetic acid (10% v/v, 2 x 50 ml). The acidic solutions were mixed, treated with concentrated ammonia (25% v/v, 7 ml) until pH was

10. The precipitate formed was collected. Fresh ethyl acetate (70 ml) was added to the mixture, stirred for 10 min, and the mixture was washed with ethyl acetate, which resulted in separation of aqueous layer from organic layer. The organic layer, which contained some suspended solids, was removed from the separating funnel. The aqueous acidic layer was re-extracted with fresh ethyl acetate (2 x 70 ml) volumes. The suspended solids were suction filtered and the filtrates of combined ethyl acetate extracts were dried on anhydrous sodium sulphate (2 g). The ethyl acetate filtrate was evaporated to dryness on rotary evaporator to obtain crude alkaloid extract. The extract was further dried at 40^{o}C to a constant mass and the yield was determined as per equation [2]. A portion of the extract (0.100g) was tested for alkaloids as per section 3.3.2.1 and the remaining extract was kept at 4^{o}C under refrigeration for further analysis.

3.7.2 Saponins extraction

The method is based on Obadoni and Ochuko (2001), which was used in determination of saponins in medicinal plants from Nigeria.

Air-dried powdered sample (50.000 g) was accurately weighed and suspended in aqueous ethanol (20% v/v, 200 ml) at 55^{o}C for 4 hr using a hot water bath. The mixture was filtered and the residue re-extracted with fresh aqueous ethanol (20% v/v, 200 ml). The combined extracts were reduced to 40 ml over a hot water bath in a fume hood. The concentrate was transferred into a 250 ml separating funnel followed by addition of diethyl ether (20 ml) and shaken vigorously for 5 minutes. The aqueous layer was recovered while the organic layer was discarded. The purification process was repeated. The aqueous layer was extracted with n-butanol (2 x 60 ml). The combined n-butanol extracts were washed twice with aqueous sodium chloride (5% m/v, 10 ml) and the aqueous layer discarded. The remaining organic layer was heated in a water-bath to evaporate the solvent. After evaporation the extracts were dried at 40^{o}C to constant mass in the oven and its yield calculated as per equation [3]. A portion of extract (0.100g) was tested for saponins as per section 3.3.2.2 and the remainder stored at 4^{o}C under refrigeration for further analysis.

$$\text{Yield (\%)} = \frac{\text{mass of crude saponins extract x 100\%}}{\text{mass of sample}} \qquad [3]$$

3.7.3 Terpenoids extraction

The method utilised is based on one used by Cordeiro et al. (1999) for extracting terpenoids from *Maytenus* species.

Air-dried powdered sample (50.000g) was accurately weighed and soaked with ethyl acetate (100 ml) at 40°C for 15 minutes under water bath. The residue was further extracted in fresh ethyl acetate (2 x 50 ml). After filtration, the combined extracts were treated with aqueous potassium hydroxide (5% m/v, 100 ml) three times to extract acidic compounds. Then the basic compounds were also extracted thrice with aqueous hydrochloric acid (5% v/v, 100 ml) three times. The organic fraction that contained the neutral compounds was washed with de-ionised water (100 ml) and concentrated on the rotary evaporator. The concentrated solution (30 ml) was centrifuged at 1000 rpm for 10 minutes to remove any suspended solids. The supernatant was collected and evaporated to dryness using rotary evaporator. The residue was dried at 40^{0}C to constant mass in the oven and its yield calculated as per equation [4]. A portion of extract (0.100g) was tested for terpenoids as per section 3.3.2.3, and the remainder stored at 4^{o}C under refrigeration for further analysis.

$$\text{Yield (\%)} = \frac{\text{mass of crude terpenoids extract x 100\%}}{\text{mass of sample}} \qquad [4]$$

3.7.4 Flavonoids extraction

A modified method by Krastev et al. (2004) was used for extracting flavonoids from *Astragalus corniculatus* Bieb.

Air-dried powdered sample (50.000g) was accurately weighed and aqueous methanol (80% v/v, 500 ml) added. The mixture was stirred at 25^{o}C for 8hr. The extract was filtered, and the residue re-extracted twice. Methanol extracts were combined and dried at

60^oC using rotary evaporator. The remaining aqueous residue was consecutively treated with chloroform (150 ml) and ethyl acetate (150 ml). The chloroform extract was discarded and ethyl acetate extract was collected and ethyl acetate evaporated on a rotary evaporator. The residue was dried in the oven to a constant mass and its yield calculated as per equation [5]. A portion of extract (0.100g) was tested for flavonoids as per section 3.3.2.4 and the remainder stored at 4^0C under refrigeration for further analysis.

$$\text{Yield (\%)} = \frac{\text{mass of crude flavonoids extract x 100\%}}{\text{mass of sample}} \qquad [5]$$

3.8 Characterisation of some phytochemicals

To further confirm the presence of phytochemicals extracted, the crude extracts were subjected to thin layer chromatography and UV analyses.

3.8.1 Thin layer chromatography (TLC) of crude extracts

Thin Layer Chromatography (TLC) plates were prepared from calcium sulphate. Initially the extracts of the phytochemicals (0.010 g) were dissolved in their appropriate solvents (100 ml) to give concentrations of 0.1mg/L. Spots were made on silica gel plate sizes 2.5 x 7.5 cm using different solvent systems. Those extracts and solvent systems that showed some separation on these small plates were subjected to TLC analysis on larger plates sizes 20.0 x 5.0 cm. R_f values for the separated spots were measured and recorded.

3.8.1.1 TLC for alkaloids

The following solvent systems were used for all extracts of alkaloids: methanol: chloroform (1:1), (4:6), (6:4), and (8:2); methanol: ammonia (25:1) and (50:3); methanol: chloroform: acetone (1:1:1), (10:7:2) and (10: 7: 4).

3.8.1.2 TLC for saponins

The following solvent systems were used for all extracts of saponins: n-butanol: ethanol (1:1), (3:2) and (4:1); n-butanol: ethanol: water (1:1:1), (5:3:1) and (5: 4:2).

3.8.1.3 TLC for terpenoids

The following solvent systems were used for all extracts of terpenoids: diethyl ether: methanol (1:1), (1:2), (2:1), (2:3), (3:2) and (3:7).

3.8.1.4 TLC for flavonoids

The following solvent systems were used for all extracts of flavonoids: ethyl acetate: acetic acid (1:1), (3:7), (4:6), (6:4), (7:3); and ethyl acetate: acetic acid: water (5:3:1) and (10:1:4).

3.8.2 UV analysis

The crude extracts (0.01g) were dissolved in appropriate solvent (20 ml) and diluted to 100 ml. A portion of this solution (1 ml) was diluted further to 100 ml. For crude extracts of alkaloids, saponins and flavonoids methanol was used as solvents, and chloroform was utilised for terpenoids extracts. These solutions were scanned on Jenway 6405 UV/ VIS Spectrophotometer to give spectra.

3.9 Statistical analysis

The Microsoft Excel was used for calculating theoretical yields of crude extracts for plants and the phytochemicals. Genstat Discovery Edition was used for analysis of variance. Comparison between groups was made by one-way analysis of variance (ANOVA). Statistical significance was tested at 95% confidence level for yields of extracts and 99% confidence level for antibacterial bioassays.

3.10 Limitation of the study

This study was limited by two factors. Firstly, the allocated research grant was inadequate considering the number and nature of analysis carried out, such that some tests were not exhausted because of failure to purchase all the necessary chemicals. Inadequacy of equipment also prevented analyses of some very critical determinations. For instance, Yield of crude water extracts could not be done because of the unavailability dry – freezer machine, which is used for complete evaporation of water from an extracts.

CHAPTER 4:RESULTS AND DISCUSSION

4.1 Presence and yields of phytochemical in selected plant species

The presence and intensity of five classes of phytochemicals in different plant parts of the same and different plant species are shown in Table 1.

Table 1: Relative presence of five classes of phytochemicals in plant species

Plant species	**Plant Part**	**Alkaloids**	**Saponins**	**Terpenoids**	**Flavonoids**	**Tannins**
D. condylocarpon	RB	+++	++	+	+	+
D. condylocarpon	SB	+++	++	++	-	+
H. pubescens	L	+++	++	++	+	++
H. pubescens	RB	+++	+	++	+	++
H. pubescens	SB	+++	++	++	-	++
P. guajava	L	-	+	++	++	++
S. birrea	RB	++	++	+	+	+
S. araliacea	L	-	++	-	+	++
S. araliacea	RB	+	++	++	+	+
T. emetica	RB	-	+	++	++	-
T. emetica	SB	-	+	-	++	+
V. glabra	L	-	+	++	-	+

Key

RB: Root Bark; **SB:** Stem Bark; **L:** Leaves

+++: very strongly positive; **++:** strongly positive; **+:** positive; **-:**negative

The results revealed that saponins were the only class of phytochemicals that were found in all the plant species analysed; the intensity of saponins varied from positive to strongly positive. The analysis also revealed that over 80% of the plant species contained tannins and terpenoids, and strong presence of alkaloids were observed in root and stem barks of *D. condylocarpon* and *H. pubescens*, and root bark of *S. birrea*.

The root and stem barks and leaves of *H. pubescens* all exhibited strong presence of alkaloids, saponins, terpenoids and tannins. Strong presence of alkaloids in *H. pubescens* agrees with earlier work done on the same plant where alkaloids such as pubamide, holarricine and conessine in stem bark of *H. pubescens* have been isolated (Siddiqui et al., 2001). Strong presence of alkaloids and saponins in root and stem bark of *D. condylocarpon* also confirm the results of earlier studies (Stauffacher, 1961; Goutarel et al., 1961; Monseur et al., 1962 as cited in Hedberg et al., 1982). The presence of alkaloids in barks of *H. pubescens* and *D. condylocarpon* is not surprising since these plants belong to the same family of Apocynaceae and many plants of this family are known to contain alkaloids (Msonthi and Morris, 1991). These plants are some of the most frequently used medicinal plants in Malawi (Msonthi, 1990), but have limited local phytochemical data. Thus, the present work provides preliminary knowledge of classes of phytochemical compounds present in these plants. Strong presence of alkaloids in the root bark of *S. birrea* has also been recorded by Watt and Breyer – Brandwik (1962).

The leaves of *P. guajava* showed presence of all analysed phytochemicals except alkaloids. This is consistent with earlier studies by Begum et al. (2002). The presence of saponins in *S. aralicea* and tannins in *S. birrea* collaborates with earlier works carried out on these plants by Ojewole (2004) and Watt and Breyer – Brandwik (1962), respectively. Presence of tannins and flavonoids in the stem bark of *T. emetica* also agrees with Germano et al. (2005) work, who also found that the stem bark of *T. emetica* contains tannins and flavonoids. Further, terpenoids and saponins have also been isolated from *V. glabra* too (Jakupovic, 1985). Thus, the findings of this study are in agreement with work done by earlier researchers in Malawi and elsewhere. The presence of phytochemicals, which are associated with antimicrobial properties in plants, is the first positive evidence to support the use of these plants for therapeutical purposes.

4.2 Yields and phytochemical composition of crude extracts

Crude extracts for five selected plant species were obtained from three organic solvents and water, but it was possible to determine yields of extracts from the organic solvents only. It was not possible to determine yields of water extracts since water could not be completely evaporated using a rotary evaporator. The yields are presented in Table 2.

Table 2: Yields of rude extracts using three extracting organic solvents

Plant Species	**Plant Part**	**Yields (%) of crude extracts**		
		Methanol	**Ethyl acetate**	**Dichloromethane**
D. condylocarpon	RB	9.84 ± 0.19	3.80 ± 0.11	3.06 ± 0.12
D. condylocarpon	SB	11.13 ± 0.25	3.75 ± 0.22	3.46 ± 0.13
H. pubescens	L	13.08 ± 0.16	4.73 ± 0.15	3.71 ± 0.13
H. pubescens	RB	10.61 ± 0.21	4.15 ± 0.14	3.60 ± 0.15
H. pubescens	SB	13.26 ± 0.19	5.43 ± 0.19	4.73 ± 0.21
P. guajava	L	11.68 ± 0.18	5.90 ±0.10	5.14 ± 0.16
S. birrea	RB	5.88 ± 0.09	3.982 ± 0.13	3.39 ± 0.09
T. emetica	RB	8.90 ± 0.13	3.57 ± 0.10	3.08 ± 0.11
T. emetica	SB	9.35 ± 0.15	3.77 ± 0.14	3.482 ± 0.06
LS.D$_{(0.05)}$				
Sample	0.15			
Solvent	0.09			
Sample x Solvent	0.25			

The results revealed that both the plant material and the extracting solvent have an influence on the yield potential of the crude extracts (Table 2). The mean percentage yields of the crude extracts varied significantly with plant sample and the solvent used for extraction ($p< 0.001$) (Appendix 1A); methanol gave the highest yields for all plant samples extracted and dichloromethane achieved the lowest. Methanol extracts of leaves and stem bark of *H. pubescens* gave the highest mean yields (13.06 ± 0.16 % and 13.26 ± 0.19 %, respectively), which are not significantly different. Dichloromethane extracts of root bark of *D. condylocarpon* and *T. emetica* gave the lowest mean yields of 3.06 ± 0.12 % and 3.08 ± 0.11 % (Table 2). Thus methanol exhibited the highest extraction capacity of the three organic solvents. These results seem to suggest that methanol, which has hydroxyl group, has ability to dissolve compounds of diverging structures.

Presence and intensity of five classes of phytochemicals in crude extracts of plant species obtained from four extracting solvents are presented in Tables 3 and 4.

Table 3: Relative presence of alkaloids, terpenoids and flavonoids in crude extracts

Plant species	**Plant Part**	**Alkaloids**				**Terpenoids**				**Flavonoids**			
		H_2O	MeOH	EtOAC	CH_2Cl_2	H_2O	MeOH	EtOAC	CH_2Cl_2	H_2O	MeOH	EtOAC	CH_2Cl_2
D. condylocarpon	RB	-	+++	++	+	+	+	+	+	+	+	+	-
D. condylocarpon	SB	++	+++	++	++	-	+	+	-	-	-	-	-
H. pubescens	L	++	++	++	++	-	+	++	++	-	-	-	-
H. pubescens	RB	+	+++	+	++	+	+	+	+	+	-	+	+
H. pubescens	SB	++	+++	+	+	-	++	+	+	-	-	-	-
P. guajava	L	-	-	-	-	-	+	+	+	++	+	+	-
S. birrea	RB	+	++	++	++	-	+	+	+	-	-	-	-
T. emetica	RB	-	-	-	-	-	-	-	-	+	+	+	+
T. emetica	SB	-	-	-	-	-	+	+	+	+	+	+	-

Table 4: Relative presence of tannins and saponins in crude extracts

Plant species	**Plant Part**	**Tannins**				**Saponins**			
		H_2O	MeOH	EtOAC	CH_2Cl_2	H_2O	MeOH	EtOAC	CH_2Cl_2
D. condylocarpon	RB	-	+	-	-	++	+	-	-
D. condylocarpon	SB	+	+	+	-	+	+	-	-
H. pubescens	L	+	+	+	+	++	+	-	-
H. pubescens	RB	++	+	+	+	+	+	-	-
H. pubescens	SB	++	++	+	-	++	+	-	-
P. guajava	L	++	+	+	-	+	+	-	-
S. birrea	RB	+	+	-	-	++	+	-	-
T. emetica	RB	+	+	-	-	+	+	-	-
T. emetica	SB	-	-	-	-	+	+	-	-

The presence of alkaloids in crude extracts from leaves, root and stem barks of *H. pubescens*, stem bark of *D. condylocarpon* and root bark of *S. birrea* varied with extracting solvent. However, relative presence of alkaloids from methanolic extracts was comparable with their presence in the plant species and higher than those from the other crude extracts. Saponins, which are generally water-soluble, were extracted by water and methanol. Due to higher solubilities in water than in methanol, saponins and tannins were generally higher in water than in methanol and ethyl acetate; dichloromethane was the poorest extractant of tannins. Interestingly, extracting capacity of terpenoids by the three organic solvents were comparable. This is largely because of similar intermolecular forces (dipole - dipole interactions) present in terpenoids and the three organic solvents.

Methanolic extracts gave the largest number of classes of phytochemicals. Though numbers of classes of compounds were generally comparable in aqueous, ethyl acetate and dichloromethane extracts, phytochemical composition were not always the same. For instance, water-soluble saponins could not be extracted by either ethyl acetate or dichloromethane, but terpenoids from some plant species such as root barks of *D. condylocarpon* and *H. pubescens* were extractable by water, ethyl acetate and dichloromethane. This trend confirms that these classes have diverse structures.

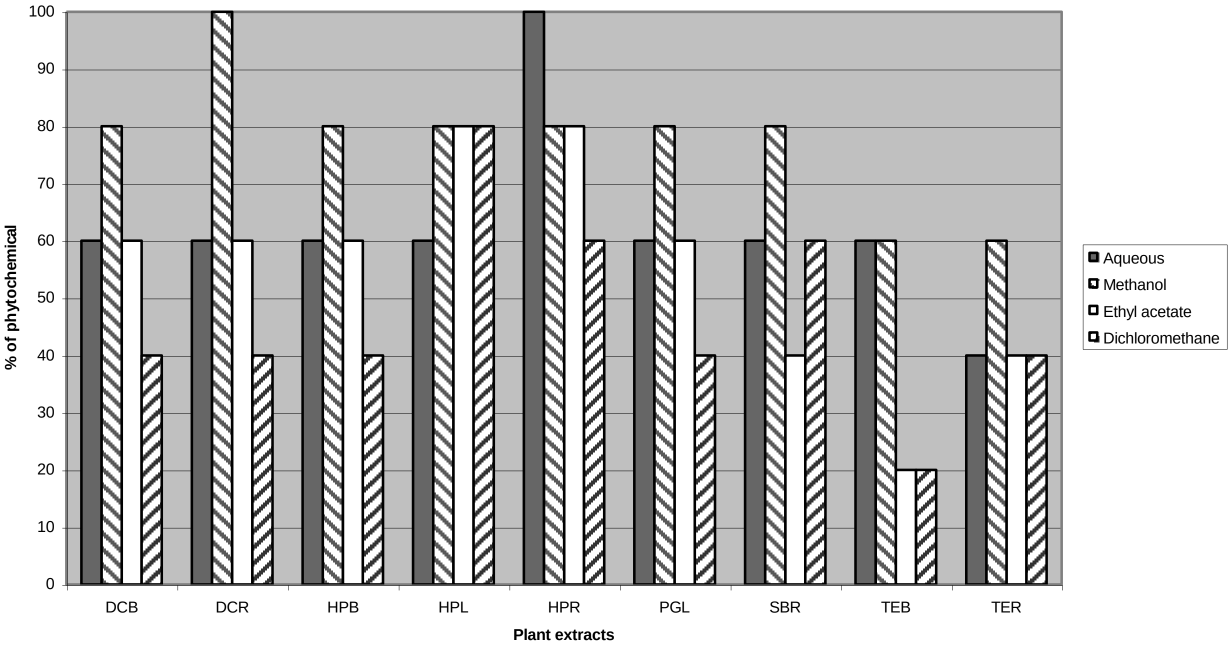

Figure 8: Composition of phytochemicals present in crude extracts using four extracting solvents

4.3 Effect of solvent on yield and composition of phytochemicals in crude extracts

Extracting solvent system significantly affected the yields ($p < 0.001$) and composition of phytochemicals in the crude extracts. The yield was directly related to composition of phytochemicals present. For instance, methanolic extracts, whose yields were consistently high, showed highest relative presences of phytochemicals (Figure 7). This shows that methanol extracted many and diverse compounds. This is because methanol has a higher polarity than ethyl acetate and dichloromethane. Hence, phytochemicals such as terpenoids and alkaloids, which contain electronegative atoms such as oxygen and nitrogen become more soluble. Further, saponins, flavonoids and tannins contain hydroxyl groups that form strong hydrogen bonds with metanol.

In contrast, water was not as efficient as methanol. This is probably because of the predominant lipophilic nature of some classes of phytochemicals (Jones, 1997). Therefore, most phytochemicals could not dissolve in highly polar solvent such as water.

Hence, all saponins, terpenoids, flavonoids and tannins extractable by water were influenced by dipole – dipole interactions and possible formation of hydrogen bonds due to presence of non – hydrocarbon atoms and hydroxyl groups in these compounds, respectively. Extractable alkaloids were obtained from all plants except root bark of *D. condylocarpon*. This observation suggests that the presence of nitrogen atom and other non – hydrocarbon atoms contained in these alkaloids outweighed the influence of the carbon rings and chains. This is possible because in addition to other non – hydrocarbon groups, nitrogen atoms in alkaloids are associated with dipole – dipole interactions and weak hydrogen bonds (Abere et al. 2005). Traditionally, plants extracts are obtained usually by soaking the necessary plant parts in cold water (cold extraction). Sometimes it is obtained by boiling in water. This implies that only phytochemicals that dissolve in water under these conditions are extractable and are responsible for the activity of those extracts.

Ethyl acetate and dichloromethane showed comparable extracting properties; and similar phytochemicals were detected. This is because they have similar solvation properties due to their similar intermolecular forces. For instance, ethyl acetate and dichloromethane have predominantly dipole – dipole interactions and dispersion forces (Bruice, 2001). Therefore, because of influence of their glycosidic parts that promote hydrogen bond formation, saponins did not dissolve in these two solvents. However, terpenoids, alkaloids, flavonoids and tannins were extractable by both ethyl acetate and dichloromethane from some of the plant species probably due to the predominance of dipole – dipole interaction and dispersion forces in these compounds.

4.4 Mean diameters of inhibition zones of crude extracts

Efficacies (measured as mean diameters of inhibition zones in mm) of crude extracts of five plant species against five gram-negative bacterial strains were obtained. Efficacies of crude extracts of all plant species except *T. emetica* are presented in Tables 5 and 7.

Table 5: Mean diameters of inhibition (in mm) for aqueous and methanolic crude extracts

Plant species / Reference		**Aqueous**					**Methanolic**				
	Plant Part	***S. typhi***	***S. typhimurium***	***S. enteritidis***	***S. boydii***	***S. flexneri***	***S. typhi***	***S. typhimurium***	***S. enteritidis***	***S. boydii***	***S. flexneri***
Ciprofloxacin		23.9±1.2	22.6±0.8	23.6±0.9	30.7±0.6	27.4±1.1	23.1 ± 0.5	22.8 ± 0.6	25.5 ± 0.7	31.2 ± 0.8	26.3 ± 1.2
D. condylocarpon	RB	0.00	14.2 ± 0.3	0.00	0.00	0.00	11.5 ± 0.2	10.1 ± 0.3	12.4 ± 0.2	8.9 ± 0.2	0.00
D. condylocarpon	SB	0.00	13.4 ± 0.2	0.00	10.1 ± 0.2	12.3 ± 0.3	9.67 ± 0.2	8.4 ± 0.3	11.9 ± 0.1	10.7 ± 0.2	12.2 ± 0.3
H. pubescens	L	11.7 ±0.3	0.00	0.00	0.00	10.6 ± 0.3	8.3 ± 0.1	0.00	0.00	9.1 ± 0.2	12.3 ± 0.2
H. pubescens	RB	12.3 ±0.3	0.00	0.00	11.4 ± 0.2	9.1 ± 0.2	10.1 ± 0.2	0.00	16.6 ± 0.2	13.1 ± 0.2	12.6± 0.2
H. pubescens	SB	13.6 ±0.2	0.00	13.2 ± 0.3	12.1 ± 0.2	9.2 ± 0.3	9.3 ± 0.3	0.00	14.1 ± 0.2	10.5 ± 0.2	12.1 ± 0.2
P. guajava	L	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.4 ± 0.2	9.1 ± 0.2	10.4 ± 0.2
S. birrea	RB	0.00	0.00	0.00	0.00	0.00	8.9 ± 0.3	8.0 ± 0.1	0.00	8.9 ± 0.2	9.1 ± 0.2

Table 6: Mean diameters of inhibition (in mm) of ethyl acetate and dichloromethane crude extracts

Plant Species / Reference		**Ethyl Acetate**					**Dichloromethane**				
	Plant Part	***S. typhi***	***S. typhimurium***	***S. enteritidis***	***S. boydii***	***S. flexneri***	***S. typhi***	***S. typhimurium***	***S. enteritidis***	***S. boydii***	***S. flexneri***
Ciprofloxacin		24.1 ± 0.5	23.0 ± 1.4	22.5 ± 0.7	29.2 ± 0.8	27.3 ± 1.2	22.7 ± 0.5	22.6 ± 0.4	25.5±1.7	27.2 ± 0.8	26.3 ± 1.2
D. condylocarpon	RB	10.2 ± 0.3		11.5±0.1	10.3±0.2	0.00	0.00	0.00	9.8 ± 0.1	11.0 ± 0.2	12.2±0.2
D. condylocarpon	SB	0.00	0.00	10.1±0.2	10.7±0.2	12.4±0.3	0.00	0.00	8.4 ± 0.1	9.8 ± 0.1	11.9 ± 0.2
H. pubescens	L	7.4 ± 0.2	0.00	0.00	9±0.1	12.5±0.2	7.3 ± 0.1	0.00	0.00	9.1 ± 0.2	13.1±0.2
H. pubescens	RB	10.3 ± 0.2	0.00	0.00	13.2±0.2	12.5±0.2	10.3 ± 0.2	0.00	0.00	13.2 ± 0.2	12.5±0.2
H. pubescens	SB	9.2 ± 0.2	0.00	0.00	10.3±0.1	11.9±0.3	9.4 ± 0.2	0.00	0.00	10.4 ± 0.2	11.8±0.2
P. guajava	L	0.00	0.00	9.8±0.1	11±0.2	12.2±0.2	0.00	0.00	10.1 ± 0.2	11.1 ± 0.2	12.1±0.3
S. birrea	RB	0.00	8.0 ± 0.2	0.00	0.00	8.8±0.1	0.00	9.2 ± 0.3	0.00	0.00	10.5 ± 0.2

The results revealed that the mean diameters ranged from zero to a maximum of 16.6 ± 0.2 mm for the crude extracts; mean value of 4.9 mm. The mean diameters of active extracts ranged from 8.3 ± 0.2 mm to 16.6 ± 0.2 mm; the methanolic extract of root bark of *H. pubescens*, gave the highest value. When compared with the efficacy of ciprofloxacin (standard antibiotic), the extracts showed significantly lower antibacterial activity ($p < 0.001$). For instance, the difference between the highest mean diameter (16.6 ± 0.2 mm) of crude extract and the minimum mean diameter of the antibiotic (22.6 ± 0.40 mm) was much greater than the least significant difference (0.327 mm) (Appendix B1). This difference is not unexpected since ciprofloxacin is one of the antibiotics that is still effective against these gram – negative bacteria (Varma et al., 2005). Further, crude extracts have lower concentrations of the possible bioactive agent(s) and are also impure, hence their lower efficacies.

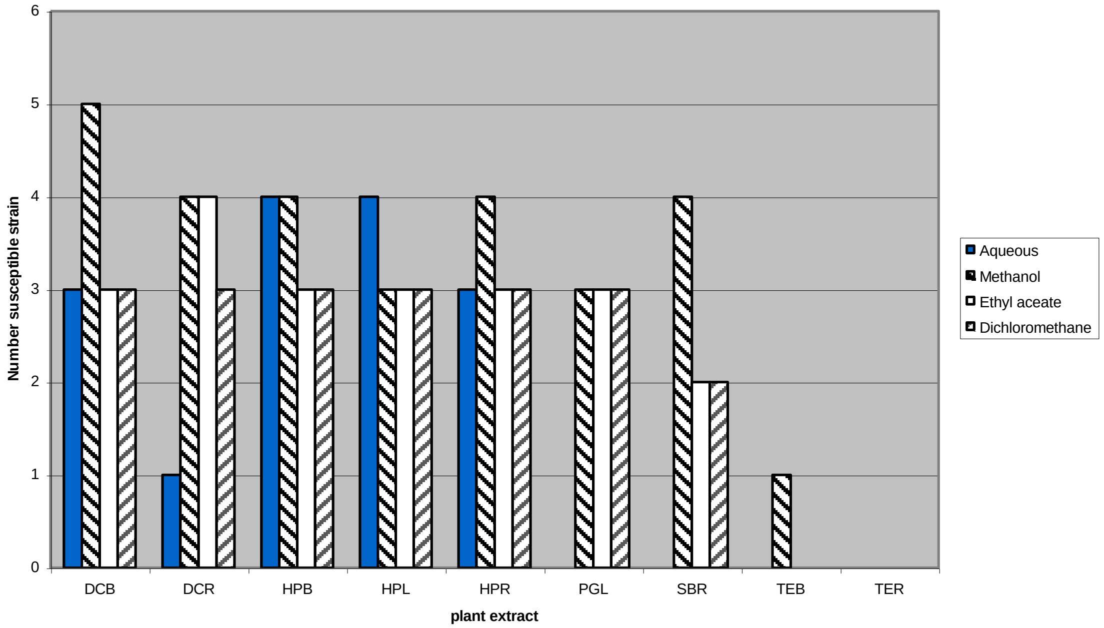

Figure 9: **Number of bacterial strains that were susceptible to plant extracts in four extracting solvents**

The data of mean diameters of inhibition of the crude extracts varied significantly ($p< 0.001$) with plant species (Appendix B1). The results revealed that all crude extracts from all plant species were active to varied extent; all extracts of *T. emetica* were ineffective against all the tested bacterial strains except its methanolic extract which was active against *S. flexneri* alone (8.8 ± 0.1 mm). Generally, crude extracts from *H. pubescens* and *D. condylocarpon* in all extracting solvents showed greatest activity and their activities were comparable (Figure 8). Therefore, the aqueous and organic extracts of *H. pubescens* and *D. condylocarpon* could be used to treat diarrhoea problems caused by some *Salmonella* and *Shigella* species. Further studies in identifying and isolating the active agents are necessary.

Antibacterial activity of some of the plant species are comparable with studies done elsewhere. For instance, Ballal et al. (2001) reported that aqueous and alcoholic extracts (200 mg/ml) were active (mean diameter > 20 mm) against *S. typhirium, S. enteritidis, S. flexneri* and *S. boydii*. Differences in antibacterial activity obtained indicate probably the varying concentration of active components due to environment (Gerson and Kelsey, 1998). Further, working concentrations (100 mg/ml) in our study were lower than Ballal et al. (2001) work.

All extracts of *P. guajava*, except aqueous extracts, were active against some of the *Salmonella* and *Shigella* species. The inactivity of aqueous extracts against *Salmonella* and *Shigella* species suggests that the active phytochemicals were not extractable by water, therefore *P. guajava* leaves extracts obtained using cold extraction cannot be used for treating diarrhoea caused by these gram-negative bacteria. The antibacterial activity of methanolic extracts of *P. guajava* leaves against *Shigella* species is similar to results obtained by Jaiarj et al. (1999). The inactivity of aqueous extracts of *P. guajava* leaves against other gram – negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* have also been recorded by Sanches et al. (2005).

The data of mean diameters also indicated that activities of the crude extracts were influenced by the bacterial strain ($p < 0.001$) (Appendix B1). Results revealed that generally *Shigella* species were more susceptible to the extracts than *Salmonella* species. Over 58% of crude extracts were active against *Shigella* species (Figure 9). *S. flexneri* was most susceptible strain; and was prone to 66% of all the extracts. The most susceptible *Salmonella* species (*S. typhi*) was destroyed by less than 45% of all the extracts; *S. typhimurium* was found to be the most resistant strain, and was susceptible to 22% of all the extracts.

The resistance by *Salmonella* species specifically *S. typhimurium* against plant extracts has been observed previously. For instance, cold and hot water extracts of *Ocinum gratissium* were inactive against both *S. typhimurium* and *S. typhi*. *O. gratissium* is widely used for treatment of diarrhoea among many diseases in Nigeria (Adebolu and Oladimeji, 2005). More recently, Indu et al. (2006) found that *S. typhirium* was the most resistant strain among all the *Salmonella* serotypes against aqueous extracts of *Allium sativum* (garlic), *A. cepa* (onion) *Myristica fragrans* (nutmeg), *Zingiber officinale* (ginger) and *Piper nigrum* (pepper).

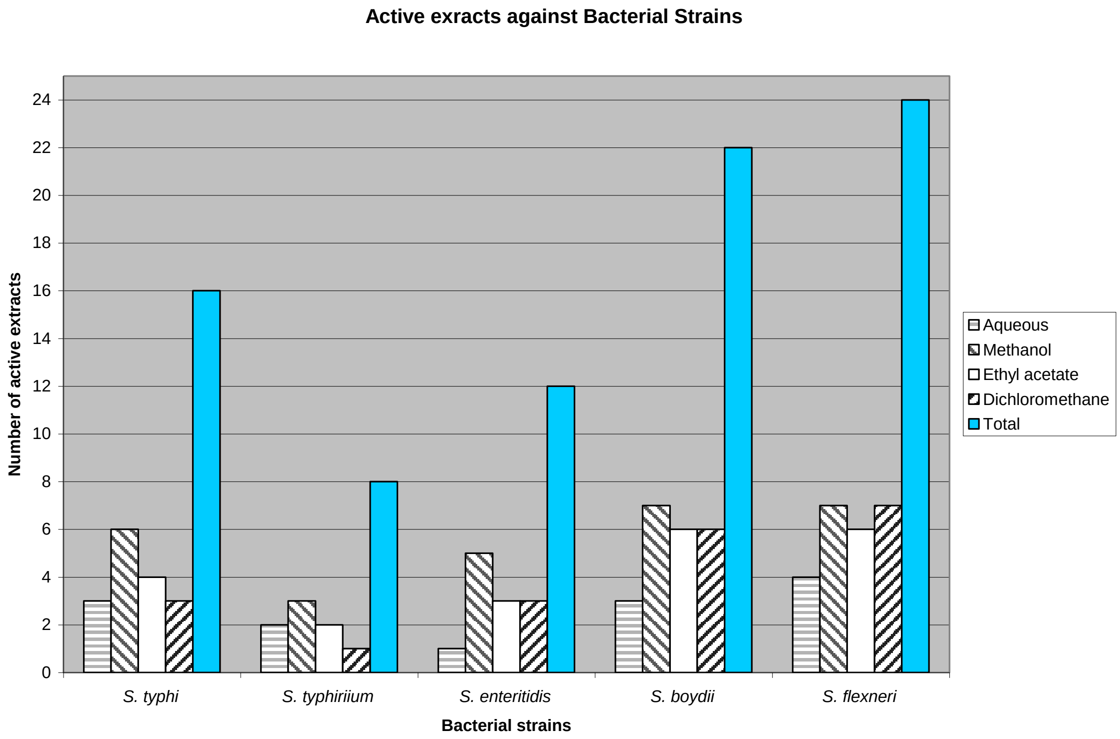

Figure 10: Percentage of active extracts against the five bacterial strains

Solvent of extraction significantly ($p < 0.001$) influenced the activity of the crude extracts (Appendix B1). These results seem to indicate that the activities of extracts were directly related to yield and phytochemical composition. For instance, methanol extracts, which consistently exhibited highest extracting capacity and gave high yields, were the most active, while ethyl acetate and dichloromethane that showed comparable extracting capacities, gave comparable activities (Figure 10). Further, activity of the extracts was strain specific. For example, despite that the same classes of phytochemicals were detected in their extracts, ethyl acetate and dichloromethane extracts from root bark of *D. condylocarpon* were active against different *Salmonella* and *Shigella* strains, in some cases (Tables 3, 4 and 6).

Aqueous extracts showed the least activity (Figure 10), such that significant activity of aqueous extracts was observed in *D. condylocarpon* and *H. pubescens* only. Therefore, for these plant species under study except those from *D. condylocarpon* and *H. pubescens*, use of water extracts obtained by cold extraction cannot be effectively used to treat diarrhoea problems caused by these gram – negative bacteria.

The data of mean diameters further revealed that extracts that contain alkaloids from *H. pubescens* regardless of the extracting solvent were significantly active (Tables 3, 4, 5 and 6). In earlier studies Chakraborty and Brantner, (1999) found that total alkaloids from the stem bark from *H. pubescens* showed high antibacterial activity against *Staphylococcus aureus, S. epidermidis, Streptococcus faecalis, Bacillus subtilis, E. coli* and *Pseudomonas aeruginosa*. Hence, these results seem to suggest that alkaloids from *H. pubescens* stem bark were the classes of phytochemicals responsible for the activity.

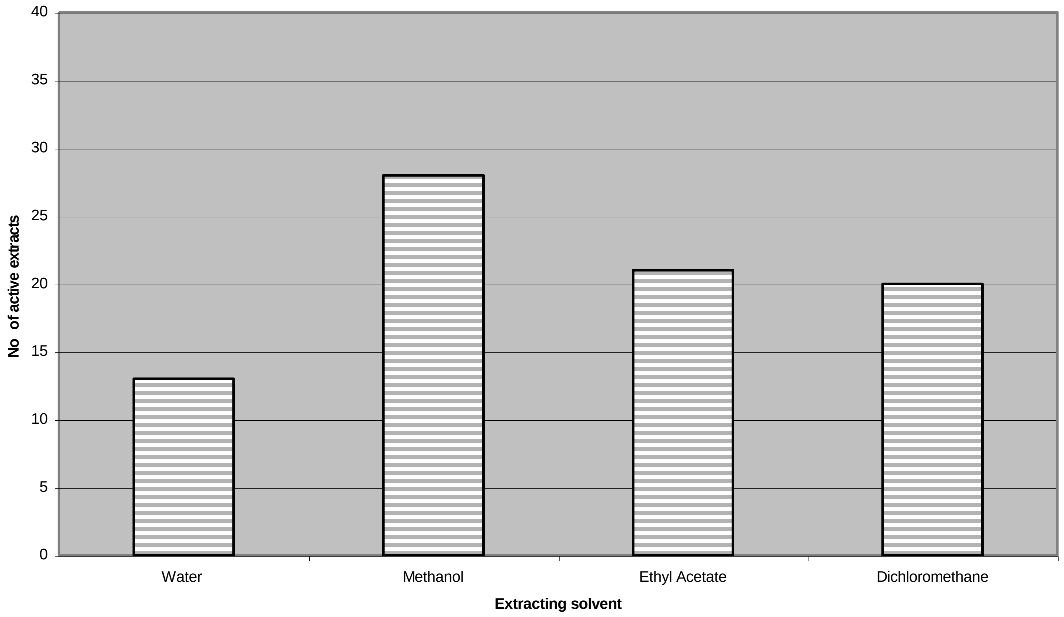

Figure 11: Number of active extracts in four extracting solvents

4.5 Minimum Inhibitory Concentration

In order to determine their minimum inhibitory concentration (MIC), methanolic extracts from root and stem barks of *D. condylocarpon* and *H. pubescens,* which were the most active against the tested strains (Figure 8), their activity were further studied, The MIC results are presented in Table 7.

Table 7: Minimum Inhibitory Concentration (mg/ml) of methanolic extracts

Plant species	**Plant Part**	***S. typhi***	***S. typhimurium***	***S. enteritidis***	***S. boydii***	***S. flexneri***
D. condylocarpon	RB	25.0	30.0	12.0	18.0	-
D. condylocarpon	SB	5.0	45.0	0.80	1.5	5.0
H. pubescens	RB	10.0	-	0.75	2.5	15.0
H. pubescens	SB	5.0	-	9.0	0.20	8.0

The data for their MIC showed that in general, MIC of *H. pubescens* extracts were lower than those of *D. condylocarpon*. The MIC values of *D. condylocarpon* and *H. pubescens* ranged from 0.8 to 45.0 mg/ml and 0.20 to 15.0 mg/ml, respectively. This trend seems to indicate that the *H. pubescens* extracts would be more effective in treating diarrhoea problems associated with the pathogens under study than *D. condylocarpon* extracts. The high resistance of *S. typhimurium* to plant extracts is still reflected by high values of MIC (30.0 and 45.0 mg/ml) of *D. condylocarpon*.

4.6 Yields of crude alkaloids, saponins, terpenoids and flavonoids from *D. condylocarpon* and *H. pubescens*

Using the most active plants, *D. condylocarpon* and *H. pubescens*, yields of crude extracts of alkaloids, saponins, terpenoids and flavonoids were determined, and are presented in Tables 8 and 9. Table 9 gives the yields of crude alkaloids using three extraction methods.

Table 8: Yields of crude alkaloids using three extraction methods

Plant species	**Plant Part**	**Yield (%)**		
		Harbone Method	**Christov et al. Method**	**Siddiqui et al. Method**
D. condylocarpon	RB	0.28 ± 0.05	0.29 ± 0.05	0.26 ± 0.06
D. condylocarpon	SB	0.34 ± 0.02	0.28 ± 0.04	0.27 ± 0.02
H. pubescens	RB	0.44 ± 0.03	0.52 ± 0.07	0.33 ± 0.04
H. pubescens	SB	0.45 ± 0.04	0.42 ± 0.04	0.29 ± 0.05
L.S.D $_{(0.05)}$				
Sample (S)	0.042			
Method (M)	0.037			
S x M	0.073			

The results indicated that the method of extracting alkaloids significantly affected the yield ($p < 0.05$). The yield was also significantly affected ($p < 0.001$) by the plant species (Appendix C1). While methods by Christov et al. (2002) and Harbone (1973) gave similar yields, the method by Siddiqui et al. (2001) afforded the lowest. This probably suggests that chloroform, which was used as the final extracting solvent in the two methods (Harbone and Christov et al.), was a better extracting solvent than ethyl acetate employed by Siddiqui et al. (2001). The higher yields from *H. pubescens* indicate greater concentration of alkaloids than in *D. condylocarpon*. Our yields (0.29 ± 0.05 %) for stem bark of *H. pubescens* are comparable to those reported by Siddiqui et al. (2001). Siddiqui et al. (2001) reported 0.2% crude yield, indicating that the type of environment affects concentration of alkaloids: 5% higher values were for the Malawi plant.

Table 9: Yields of crude extracts of saponins, terpenoids and flavonoids

Plant species	**Plant Part**	**Yield (%)**		
		Saponins	**Terpenoids**	**Flavonoids**
D. condylocarpon	RB	0.79 ± 0.05	0.72 ± 0.06	0.53 ± 0.04
D. condylocarpon	SB	0.90 ± 0.04	0.83 ± 0.03	-
H. pubescens	RB	0.59 ± 0.03	1.82 ± 0.07	0.07 ± 0.01
H. pubescens	SB	1.07 ± 0.05	0.70 ± 0.04	-
L.S.D $_{(0.05)}$				
Sample (S)	0.036			
Phytochemicals (Py)	0.031			
S x Py	0.063			

The results showed clearly that the yields varied significantly ($p < 0.001$) with plant species and class of phytochemicals (Appendix C2). The yields of terpenoids were consistently high for all the four plant samples with the mean yield of 1.02 %, and the extracts of flavonoids gave the lowest yields. Yields of saponins and terpenoids from stem bark of *D. condylocarpon* were significantly different from the yields from plant's root bark (l.s.d. = 0.0625). However, corresponding yields from stem and root barks of *H. pubescens* did not vary significantly. Comparison with other plants further verifies that phytochemical composition vary with plant species. For instance, the yields of saponins in this study are higher than those from another Nigerian plant, *Hyptis suaveolens*, whose yield was 0.300 %. In contrast, *H. suaveolens* afforded higher yield of flavonoids (12.54 %) than the plants in this study (Edeoga et al., 2006).

4.7 Chromatographic and UV spectroscopic identification of classes of phytochemicals

4.7.1 Thin layer chromatography (TLC) of crude alkaloids, saponins, terpenoids, and flavonoids

An attempt to separate the four classes of compounds on TLC using different combination of solvent systems was impossible except for saponins; R_f values of separated spot of saponins using butanol: ethanol: water (5:3:1) are presented in Table 10.

Table 10: R_f values for the components of crude saponins extracts separated on the TLC plates

Plant species	Plant Part	Spot Position	R_f values	Colour of spot of fluorescence at 365 nm
D. condylocarpon	SB	Spot 1	0.18 ± 0.01	Deep blue
		Spot 2	0.30 ± 0.02	Deep blue
		Spot 3	0.41 ± 0.03	Deep blue
		Spot 4	0.62 ± 0.01	Light blue
		Spot 5	0.89 ± 0.02	Light blue
D. condylocarpon	RB	Spot 1	0.37 ±0.02	Purple
		Spot 2	0.48 ±0.02	Purple
		Spot 3	0.67 ±0.03	Purple
		Spot 4	0.78 ± 0.02	Deep blue
		Spot 5	0.90 ± 0.03	Deep blue
H. pubescens	SB	Spot 1	0.26 ± 0.03	Deep blue
		Spot 2	0.42 ± 0.02	Deep blue
		Spot 3	0.61 ± 0.01	Deep blue
		Spot 4	0.80 ± 0.02	Purple
H. pubescens	RB	Spot 1	0.35 ± 0.02	Light blue
		Spot 2	0.58 ± 0.03	Light blue
		Spot 3	0.72 ± 0.02	Deep blue

The results seemed to reveal that both stem and root barks of *D. condylocarpon* contain a minimum of five different saponins. However, only the spot 5 of extract from stem bark and from root bark are the same within the experimental errors. These observations may suggest that the saponins of these spots have similar structures. The R_f values also indicate that saponins extracts from stem and root bark of *H. pubescens* contained at least four and three saponins, respectively.

4.7.2 UV spectra and absorption maxima of crude extracts of crude alkaloids, saponins, terpenoids and flavonoids

UV spectra for crude extracts of alkaloids, saponins, terpenoids and flavonoids were obtained. The absorption maxima and UV spectra for crude alkaloids obtained from the three extracting methods solvents are given in Table 11 and Figure 11 to 13, respectively.

Table 11: Absorption maxima peaks for crude extracts of alkaloids

Plant species	**Plant Part**	**λ_{max}/ nm (MeOH)**		
		Harbone	**Christov et al.**	**Siddiqui et al.**
D. condylocarpon	RB	246	249, 295	248, 297
D. condylocarpon	SB	243, 283	241, 287	247
H. pubescens	RB	242	243	247
H. pubescens	SB	248, 305	249	249, 284

The values of absorption maxima of all the plant materials fall within the ranges of some alkaloids such as steroidal alkaloids isolated from *Sarcococca saligna* which showed λ_{max} (MeOH) values ranging from 209 to 279 nm (Rahman et al., 2002). Evidently, the highest absorption maxima of alkaloids extracts from stem bark of *H. pubescens* were not affected by the method of extraction (λ_{max} = 249 nm). The observed peak at 249 nm probably indicates support of the presence of similar alkaloids to pubamide, which was isolated by Siddiqui et al. (2001) from stem bark of *H. pubescens* (λ_{max} (MeOH) = 245 nm). However, these values alone are not sufficient to confirm the presence of alkaloids.

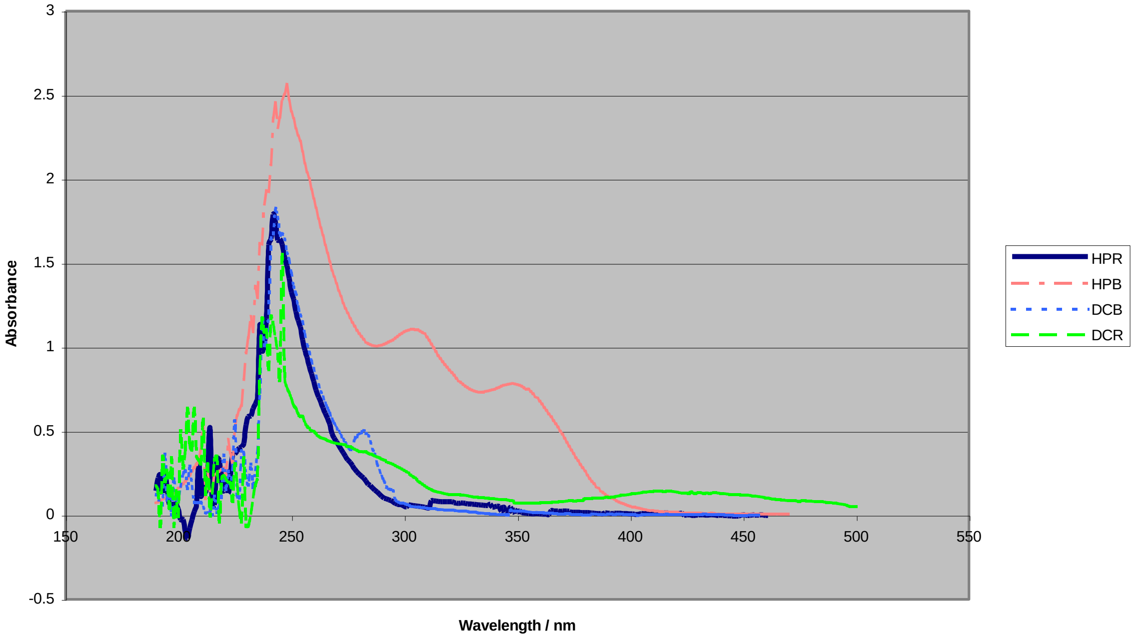

Figure 12: UV spectra for extracts of alkaloids from *D. Condylocarpon* and *H. pubescens* using Harbone method

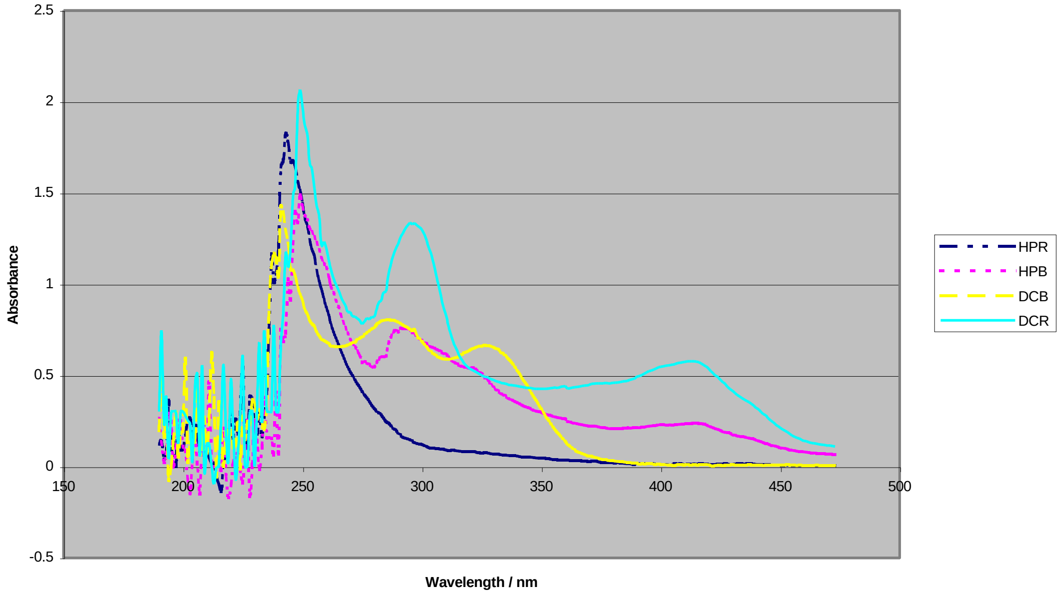

Figure 13: UV spectra for extracts of alkaloids from *D. Condylocarpon* and *H. pubescens* using Christov et al. method

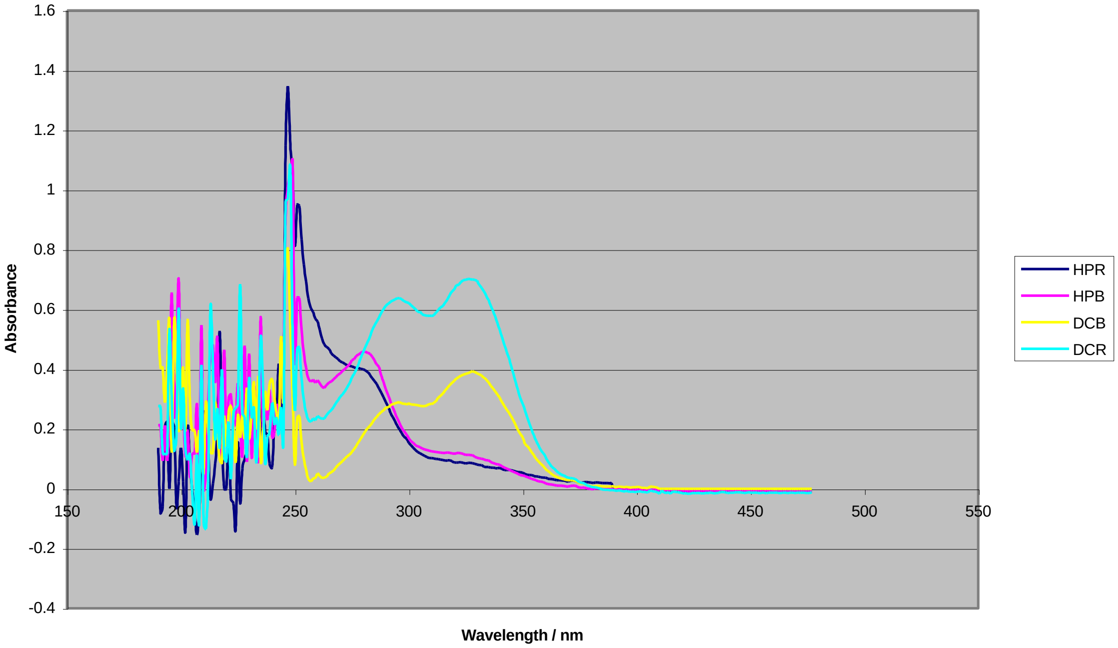

Figure 14: UV spectra for extracts of alkaloids from *D. condylocarpon* and *H. pubescens* using Siddiqui et al. method

The absorption maxima and UV spectra for crude extracts of saponins, terpenoids and flavonoids are presented in Table 12 Figures 14 to 16, respectively.

Table 12: Absorption maxima for crude extracts of saponins, terpenoids and flavonoids

Plant species	**Plant Part**	**λ$_{max}$/ nm (MeOH)**		**λ$_{max}$/ nm (CHCl$_3$)**
		Saponins	**Flavonoids**	**Terpenoids**
D. condylocarpon	RB	225	248	237
D. condylocarpon	SB	227	-	202
H. pubescens	RB	229	252	241
H. pubescens	SB	247	-	241

The absorption maxima of saponins from stem and root bark of *D. condylocarpon* are comparable. This may suggest that the saponins responsible for these absorption maxima are similar in structure. This further supports the results of R_f values for spot five of saponins on the TLC plates (Table 10). Crude extracts of terpenoids from stem and root bark of *H. pubescens* also gave the same λ_{max} (241 nm).

Terpenoids and flavonoids present in root bark of *D. condylocarpon* and *H. pubescens* produced absorption bands that were comparable with those of terpenoids and flavonoids from other plants. For instance, absorption maxima of flavonoids extracts in the present study (248 and 252 nm) are comparable with flavonoids such as chrysoeriol, tricin, azaleatin and luteolin that have λ_{max} at 252, 248, 254 and 255 nm, respectively (Harbone 1973). Absorption bands of terpenoids extracts from *H. pubescens* at 241 nm are compared with those of two campestrine isomers (243 and 244 nm), isolated from roots of *Peritassa Campestris* (Liao et al., 2002). These results seem to support further the presence of these classes of compounds in the two plant species. However isolation and further spectroscopic methods are required to provide further evidence.

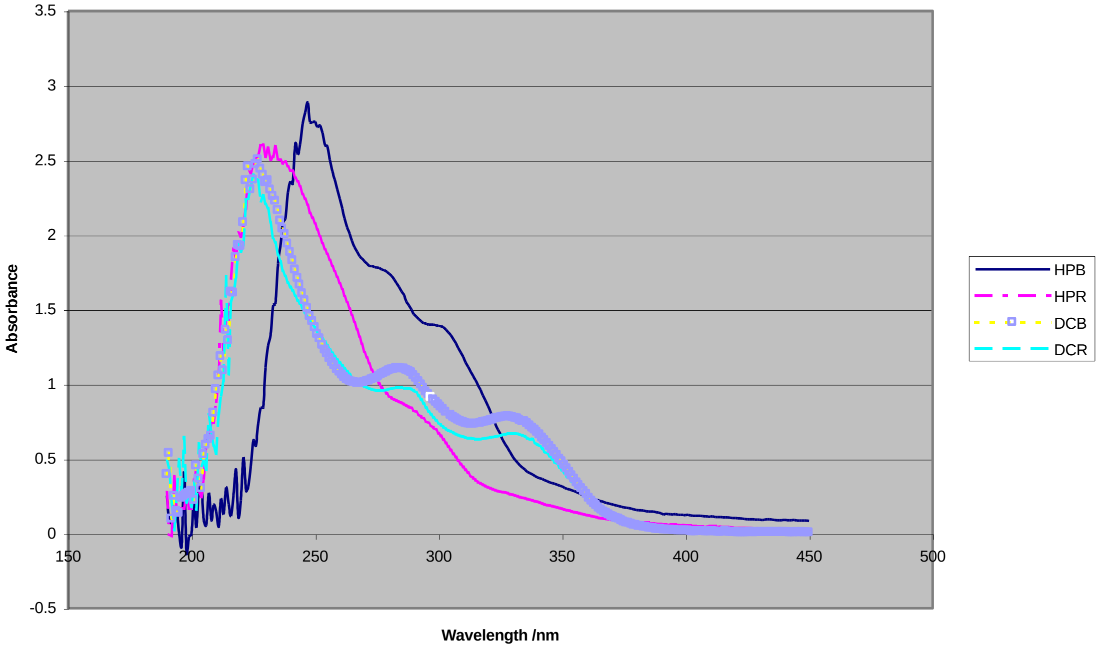

Figure 15: UV Spectra for extracts of saponins from root and stem barks of *D. condylocarpon* and *H. pubescens*

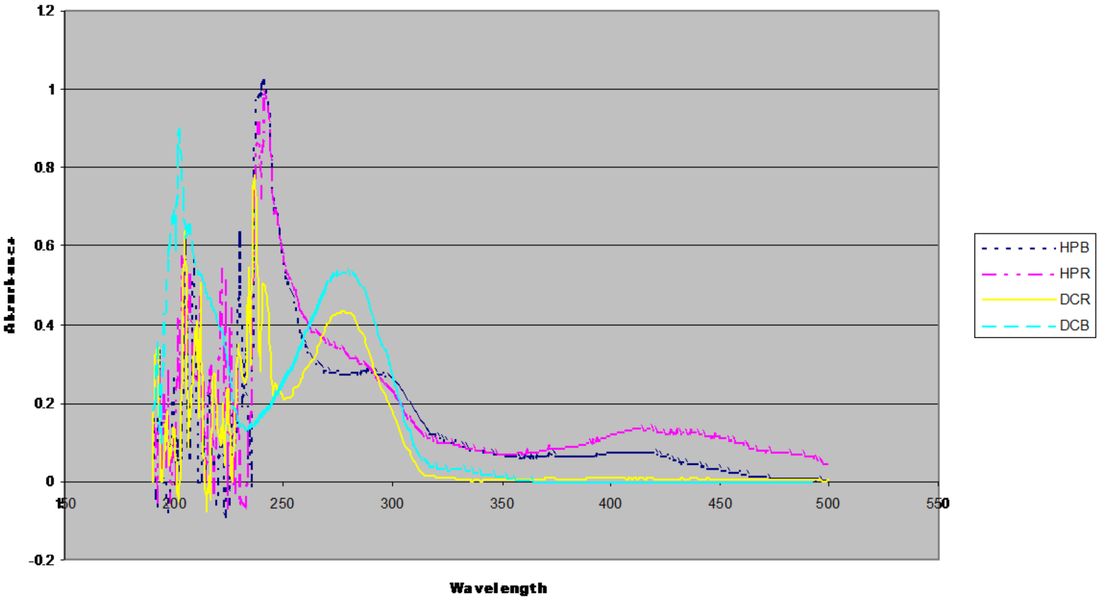

Figure 16: UV spectra for extracts of terpenoids from root and stem barks of *D. condylocarpon* and *H. pubescens*

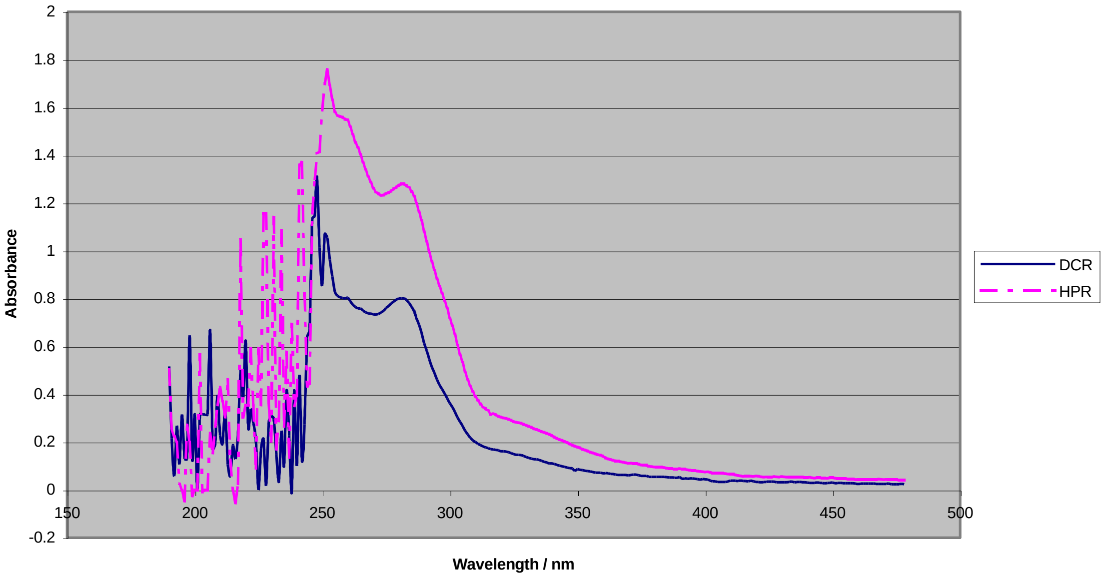

Figure 17: UV spectra for extracts of flavonoids from root and barks of *D. condylocarpon* and *H. pubescens*

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study has revealed that yields and classes of phytochemicals varied with the extracting solvent system and plant species. Using four extracting solvents (water, methanol, ethyl acetate and dichloromethane) methanol was the most effective solvent in extracting many classes of phytochemicals, and in giving high yields. Alkaloids were the most extractable class of phytochemicals by the four solvents.

Extracting solvent and susceptibility of the bacterial strain influenced the antibacterial activity of the crude extracts of the plant species. All plant species from different extracting solvents, except *T. emetica*, showed varied degrees of antibacterial activity. However, methanolic crude extracts from *D. condylocarpon* and *H. pubescens* exhibited the highest significant efficacy against the tested pathogens. Therefore, the efficacy of crude extracts from root and stem barks of *D. condylocarpon* and *H. pubescens* against the gram – negative bacteria showed that diarrhoegenic problems caused by these bacteria could be controlled using these plant species. Hence these plants could be potential sources of antibacterial agents to be considered for drug development.

5.2 Recommendations

In Malawi, conventional medicine is inaccessible to majority of the population whilst use of medicinal plants and consultation of traditional healers is the integral part of livelihood. It is therefore important that utilisation of medicinal plants should be encouraged. The use of root and stem barks of *D. condylocarpon* and *H. pubescens* for addressing diarrhoegenic problems caused by *Salmonella* and *Shigella* species should be exploited and promoted. Further, it is recommended that

- Antibacterial activity of crude aqueous extract obtained by soxhlation be carried out
- antibacterial activity of alkaloids extracts from these plants against *Salmonella* and *Shigella* species be elucidated
- in order to determine the identities of the compounds robust separation and isolation techniques should be further exploited

- bioassay - guided fractional be studied to isolate the active alkaloids, and establish effects of diarrhoea.

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APPENDICES

Appendix A1 Analysis of variance for percentage yields of crude extracts

Source of variation	d.f.	s.s.	m.s	F	Sign. level
Replicate	2	0.02637	0.01318	0.55	
Sample	8	94.29538	11.78692	490.88	<0.001
Solvent	2	737.54427	368.77213	1.53×10^4	<0.001
Sample. Solvent	16	59.71996	3.73250	155.44	<0.001
Error	52	1.24861	0.02401		
Total	80	892.83459			

Appendix B1 Analysis of variance for antibacterial activity

Source of variation	d.f.	s.s.	m.s	F	Sign. level
Replicate	2	0.0916	0.0458	1.72	
Sample	8	24230	3029	113600	<0.001
Solvent	3	704.1	234.7	8799.35	<0.001
Bacteria	4	2962	740.6	27770	<0.001
Sample. Solvent	24	842.6	35.11	1316.29	<0.001
Sample. Bacteria	32	3792	11.85	4443.23	<0.001
Solvent. Bacteria	12	790.9	65.91	2471.13	<0.001
Sample. Solvent. Bacteria	96	3476	36.21	1357.47	<0.001
Error	358	9.548	0.0267		<0.001
Total	539	36810			<0.001

Appendix C1 Analysis of variance for yields (%) of alkaloids

Source of variation	d.f.	s.s.	m.s	F	Sign. level
Replicates	2				
Sample	3	0.14107	0.04702	2.00	0.143
Method		1.06770	0.53385	22.71	<0.001
Sample. Method	6	0.65864	0.10977	4.67	0.003
Error	24	0.51715	0.02351		
Total	35	2.39281			

Appendix C2 Analysis of variance for yields (%) of saponins, terpenoids and flavonoids

Source of variation	d.f.	s.s.	m.s	F	Sign. level
Replicates	2	0.011747	0.005873	4.31	
Sample (S)	3	0.364494	0.121498	89.24	<0.001
Phytochemical (Py)	2	5.044199	2.522099	1852.48	<0.001
S*Py	6	3.211374	0.535229	393.12	<0.001
Error	22	0.029952	0.001361		
Total	35	8.661766			