"The art of medicine consists of amusing the patient while Nature cures the disease."

-Voltaire
2.1. Introduction

Despite emphasis being put in research of synthetic drugs, a certain interest in medicinal plants has been reborn, in part due to the fact that a lot of synthetic drugs are potentially toxic and are not free of side effects on the host (Akinpelu and Onakoya, 2006; Balbaa, 1976). This has urged microbiologists all over the world for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents (Hamza et al., 1992). Medicinal plants are well-known natural sources for the treatment of various diseases since antiquity. About 20,000 plant species used for medicinal purposes are reported by World Health Organisation (WHO) (Cos et al., 2006). Furthermore, natural products, either pure compounds, or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (El-Olemy et al., 1994). In the past the wide range of antimicrobial agents from lower organisms and synthetic drugs sufficed in the treatment or control of infectious diseases, but currently there is a problem of microbial drug resistance and there is an increase of opportunistic infections especially with AIDS patients and individuals on immunosuppressive chemotherapy (Faruq et al., 2006). Many antifungal and antiviral drugs are of limited use due to toxicity, while other viral diseases have not yet found a cure. These problems pose a need of searching more potential drug substances that are new (Geddes, 1985; Gullece et al., 2006). Plants are indispensable sources of medicinal preparations, both preventive and curative. China and India are
the leading countries in using medicinal plants. Their traditions of plant remedies date back to at least 7000 years (Harbone, 1973; Hamburger and Hosettmann, 1991). According to WHO, 80% of the World’s population relies on traditional medicine to meet their daily health requirement (Hugo and Russel, 1984). In Africa, the reliance on such traditional medicine is partly owing to the high cost of modern drugs and inaccessibility of modern health institution. Also in Africa, traditional systems are more culturally acceptable and are able to meet psychological needs in a way western medicine does not. Today the huge traditional knowledge of medicinal plants is playing an important role in the development of new drugs. An example of drugs discussed based on information derived from an ethnobotanical investigation is aspirin from *filipendula ulmar*, morphine from *papaver sominiferum*, ephedrin from *ephedra sinica* (Maydell, 1990).

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cos *et al.*, 2006). According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Duraipandiyam *et al.*, 2006). Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs,
men turned to ethnopharmacognosy. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, anti-diarrheal, analgesic and wound healing activity were reported. In many cases the people claim the good benefit of certain natural or herbal products. However, clinical trials are necessary to demonstrate the effectiveness of a bioactive compound to verify this traditional claim. Clinical trials directed towards understanding the pharmacokinetics, bioavailability, efficacy, safety and drug interactions of newly developed bioactive compounds and their formulations (extracts) require a careful evaluation. Clinical trials are carefully planned to safeguard the health of the participants as well as answer specific research questions by evaluating for both immediate and long-term side effects and their outcomes are measured before the drug is widely applied to patients.

According to the World Health Organization (WHO), nearly 20,000 medicinal plants exist in 91 countries including 12 mega biodiversity countries. The premier steps to utilize the biologically active compound from plant resources are extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological evaluation and clinical evaluation. This research work provides details in extraction, isolation and characterization of bioactive compound from plants extract with common phytochemical screening assay, chromatographic techniques, such as HPLC, and HPLC/MS and Fourier Transform Infrared Spectrometry (FTIR).
Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. The basic operation included steps, such as pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples. If the plant was selected on the basis of traditional uses (Fabricant and Farnsworth, 2001), then it is needed to prepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional ‘herbal’ drug. The selection of solvent system largely depends on the specific nature of the bioactive compound being targeted. Different solvent systems are available to extract the bioactive compound from natural products. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. In some instances, extraction with hexane is used to remove chlorophyll (Cos et al., 2006). As the target compounds may be non-polar to polar and thermally labile, the suitability of the methods of extraction must be considered. Various methods, such as sonification, heating under reflux, soxhlet extraction and others are commonly used (United States Pharmacopeia and National Formulary, 2002; Pharmacopoeia of the People’s
Republic of China, 2000; The Japanese Pharmacopeia, 2001) for the plant samples extraction. In addition, plant extracts are also prepared by maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems.

The other modern extraction techniques include solid-phase micro-extraction, supercritical-fluid extraction, pressurized-liquid extraction, microwave-assisted extraction, solid-phase extraction, and surfactant-mediated techniques, which possess certain advantages. These are the reduction in organic solvent consumption and in sample degradation, elimination of additional sample clean-up and concentration steps before chromatographic analysis, improvement in extraction efficiency, selectivity, and/ kinetics of extraction. The ease of automation for these techniques also favours their usage for the extraction of plants materials (Huie, 2002).

Due to the fact that plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. It is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as TLC, column chromatography, flash chromatography, Sephadex chromatography and HPLC, should be used to obtain pure compounds. The pure compounds are then used for the determination of structure and biological activity. Besides that, non-
chromatographic techniques such as immunoassay, which use monoclonal antibodies (MAbs), phytochemical screening assay, Fourier-transform infrared spectroscopy (FTIR), can also be used to obtain and facilitate the identification of the bioactive compounds.

Chromatographic techniques: Thin-layer chromatography (TLC) and Bio-autographic methods. TLC is a simple, quick, and inexpensive procedure that gives the researcher a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the Rf of a compound is compared with the Rf of a known compound. Additional tests involve the spraying of phytochemical screening reagents, which cause colour changes according to the phytochemicals existing in a plants extract; or by viewing the plate under the UV light. This has also been used for confirmation of purity and identity of isolated compounds.

Bio-autography is a useful technique to determine bioactive compound with antimicrobial activity from plant extract. TLC bio-autographic methods combine chromatographic separation and in situ activity determination facilitating the localization and target-directed isolation of active constituents in a mixture. Traditionally, bioautographic technique has used the growth inhibition of microorganisms to detect anti-microbial components of extracts chromatographed on a TLC layer. This methodology has been considered as the most efficacious assay for the detection of anti-microbial compounds (Shahverdi, 2007). Bio-autography localizes antimicrobial activity on a
chromatogram using three approaches: (i) direct bio-autography, where the micro-organism grows directly on the thin-layer chromatographic (TLC) plate, (ii) contact bio-autography, where the antimicrobial compounds are transferred from the TLC plate to an inoculated agar plate through direct contact and (iii) agar overlay bio-autography, where a seeded agar medium is applied directly onto the TLC plate (Hamburger and Cordell, 1987; Rahalison et al., 1991). The inhibition zones produced on TLC plates by one of the above bio-autographic technique will be used to visualize the position of the bioactive compound with antimicrobial activity in the TLC fingerprint with reference to Rf values (Homans and Fuchs, 1970). Preparative TLC plates with a thickness of 1mm were prepared using the same stationary and mobile phases as above, with the objective of isolating the bioactive components that exhibited the antimicrobial activity against the test strain. These areas were scraped from the plates, and the substance eluted from the silica with ethanol or methanol. Eluted samples were further purified using the above preparative chromatography method. Finally, the components were identified by HPLC, LCMS and GCMS. Although it has high sensitivity, its applicability is limited to micro-organisms that easily grow on TLC plates. Other problems are the need for complete removal of residual low volatile solvents, such as n-BuOH, trifluoroacetic acid and ammonia and the transfer of the active compounds from the stationary phase into the agar layer by diffusion (Cos et al., 2006). Because bio-autography allows localizing antimicrobial activities of an extract on the chromatogram, it supports a quick search for new antimicrobial agents through
bioassay-guided isolation (Cos et al., 2006). The bio-autography agar overlay method is advantageous in that, firstly it uses very little amount of sample when compared to the normal disc diffusion method and hence, it can be used for bioassay-guided isolation of compounds. Secondly, since the crude extract is resolved into its different components, this technique simplifies the process of identification and isolation of the bioactive compounds (Rahalison et al., 1991).

2.2. High performance liquid chromatography

High performance liquid chromatography (HPLC) is a versatile, robust, and widely used technique for the isolation of natural products (Cannell, 1998). Currently, this technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants (Fan et al., 2006). Natural products are frequently isolated following the evaluation of a relatively crude extract in a biological assay in order to fully characterize the active entity. The biologically active entity is often present only as minor component in the extract and the resolving power of HPLC is ideally suited to the rapid processing of such multi-component samples on both an analytical and preparative scale. Many bench top HPLC instruments now are modular in design and comprise a solvent delivery pump, a sample introduction device such as an auto-sampler or manual injection valve, an analytical column, a guard column, detector and a recorder or a printer.
Chemical separations can be accomplished using HPLC by utilizing the fact that certain compounds have different migration rates given a particular column and mobile phase. The extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase. Generally the identification and separation of phytochemicals can be accomplished using isocratic system (using single unchanging mobile phase system). Gradient elution in which the proportion of organic solvent to water is altered with time may be desirable if more than one sample component is being studied and differ from each other significantly in retention under the conditions employed.

Purification of the compound of interest using HPLC is the process of separating or extracting the target compound from other (possibly structurally related) compounds or contaminants. Each compound should have a characteristic peak under certain chromatographic conditions. Depending on what needs to be separated and how closely related the samples are, the chromatographer may choose the conditions, such as the proper mobile phase, flow rate, suitable detectors and columns to get an optimum separation.

Identification of compounds by HPLC is a crucial part of any HPLC assay. In order to identify any compound by HPLC, a detector must first be selected. Once the detector is selected and is set to optimal detection settings, a separation assay must be developed. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time
and should be well separated from extraneous peaks at the detection levels which the assay will be performed. UV detectors are popular among all the detectors because they offer high sensitivity (Li et al., 2004) and also because majority of naturally occurring compounds encountered have some UV absorbance at low wavelengths (190-210 nm) (Cannell, 1998). The high sensitivity of UV detection is bonus if a compound of interest is only present in small amounts within the sample. Besides UV, other detection methods are also being employed to detect phytochemicals among which is the diode array detector (DAD) coupled with mass spectrometer (MS) (Tsao and Deng, 2004). Liquid chromatography coupled with mass spectrometry (LC/MS) is also a powerful technique for the analysis of complex botanical extracts (Cai et al., 2002; He, 2000). It provides abundant information for structural elucidation of the compounds when tandem mass spectrometry (MS^n) is applied. Therefore, the combination of HPLC and MS facilitates rapid and accurate identification of chemical compounds in medicinal herbs, especially when a pure standard is unavailable (Ye et al., 2007).

The processing of a crude source material to provide a sample suitable for HPLC analysis as well as the choice of solvent for sample reconstitution can have a significant bearing on the overall success of natural product isolation. The source material, e.g., dried powdered plant, will initially need to be treated in such a way as to ensure that the compound of interest is efficiently liberated into solution. In the case of dried plant material, an organic solvent (e.g., methanol, chloroform) may be used as the initial extractant and following
a period of maceration, solid material is then removed by decanting off the extract by filtration. The filtrate is then concentrated and injected into HPLC for separation. The usage of guard columns is necessary in the analysis of crude extract. Many natural product materials contain significant level of strongly binding components, such as chlorophyll and other endogenous materials that may in the long term compromise the performance of analytical columns. Therefore, the guard columns will significantly protect the lifespan of the analytical columns

**2.3. Phytochemical screening assay**

Phytochemicals are chemicals derived from plants and the term is often used to describe the large number of secondary metabolic compounds found in plants. Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemicals in a mixture serves as an important tool in analysis of bioactive compound analyses. After obtaining the crude extract or active fraction from plant material, phytochemical screening can be performed with the appropriate tests to get an idea regarding the type of phytochemicals existing in the extract mixture or fraction.

**2.4. Fourier-transform infrared spectroscopy (FTIR)**

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract (Eberhardt *et al.*, 2007; Hazra *et al.*, 2007).
In addition, FTIR spectra of pure compounds are usually so unique that they are like a molecular "fingerprint". For most common plant compounds, the spectrum of an unknown compound can be identified by comparison to a library of known compounds. Samples for FTIR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride. The drop forms a thin film between the plates. Solid samples can be milled with potassium bromide (KBr) and then compressed into a thin pellet which can be analyzed. Otherwise, solid samples can be dissolved in a solvent such as methylene chloride, and the solution then placed onto a single salt plate. The solvent is then evaporated off, leaving a thin film of the original material on the plate.

Throughout the ages humans have relied on nature for their basic needs for the production of foodstuffs, shelter, clothing, fertilizers, flavours and fragrances, and, not least, medicines. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years. In industrialized nations at the present time, some fifty percent of all prescribed drugs are derived or synthesized from natural products, the only available sources for which are animals, marine species, plants, and microorganisms (Farnsworth and Morris, 1976). The importance of natural products is also evidenced by the fact that in 1991 nearly half of the best selling drugs were either natural products or their derivatives (O’Neill and Lewis, 1993). It is considered that because of the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable
resource for the discovery of potential new drugs and biological entities (Balandrin et al., 1985; Hamburger et al., 1991; Cox and Balick, 1994; Cordell, 1995; Clark, 1996; Hostettmann et al., 1998; Cordell, 2000). However, only a small percentage of the world’s estimated 250,000–400,000 flowering plants have as yet been analysed for their possible medicinal uses. Moreover, in developing countries, medicinal plants continue to be the main source of medication. It has been estimated that approximately 80% of the world’s inhabitants and 88% of the inhabitants of underdeveloped countries rely mainly on traditional medicine for their primary health care (Farnsworth et al., 1985; Pezzuto, 1997).

For centuries, the therapeutic properties of various medicinal plants have been used to treat human diseases. It has been estimated that 60-90% of the population of developing countries use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare (WHO, 2002). Consumers are increasingly interested in complementary and alternative medicines, including herbal medicine, as they perceive these forms of healing as being both safe and effective. This trend in use of alternative and complementary healthcare has prompted scientists to investigate the various biological activities of medicinal plants. In the US, a number of medicinal plants have been documented as important source of bioactive compounds (Balunas and Kinghorn, 2005).
Nature has been a source of medicinal agents since time immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Ayurveda is ancient health care system and is practiced widely in India, Srilanka and other countries (Chopra and Doiphode, 2002). Ayurveda system of medicine use plants to cure the ailments and diseases. Despite the availability of different approaches for the discovery of therapeutics, natural products still remain as one of the best reservoir of new structural types. They are used directly as therapeutic agents, as well as starting material for the synthesis of drugs or as models for pharmacologically active compounds (Cowan, 1999). In modern time plants have been sources of analgesics, anti-inflammatory, anti-neoplastic drugs, and medicine for asthma, anti arrhythmic agents and anti hypertensive. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001).

2.7. Phytochemical Screening and Bioactivity of Medicinal Plants

The approach for drug development from plant resources depends on the aim. Different strategies will result in an herbal medicine or in an isolated active compound. However, apart from this consideration, the selection of a
suitable plant for a pharmacological study is a very important and decisive step. There are several ways in which this can be done, including traditional use, chemical content, toxicity, randomised selection or a combination of several criteria (Ferry and Baltassat-Millet, 1977; Soejarto, 1996; Williamson et al., 1996). The most common strategy is careful observation of the use of natural resources in folk medicine in different cultures; this is known as ethnobotany or ethnopharmacology. Information on how the plant is used by an ethnic group is extremely important. The preparation procedure may give an indication of the best extraction method. The formulation used will provide information about pharmacological activity, oral versus non-oral intake and the doses to be tested. However, certain considerations must be taken into account when the ethnopharmacological approach of plant selection is chosen. For instance, each ethnic group has its own concept of health or illness, as well as different healthcare systems (Elisabetsky and Posey, 1986). The signs and symptoms should be translated, interpreted and related to western biomedical concepts, thus allowing a focused study of a particular therapeutic property. Selection based on chemical composition uses phylogenetic or chemotaxonomic information in the search, mainly in certain genera and families, for compounds from a defined chemical class with known pharmacological activity (Gottlieb and Kaplan, 1993; Souza Brito, 1996). The use of medicinal plants for treating diseases is as old as the human species. Maciel (2002) has reported that the use of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even their chemical
constituents are not known. The use of medicinal plants as raw materials in the production of drugs is ever increasing because of their potentials in combating various diseases including the problem of drug resistance in micro-organisms. Demand for medicinal plants is increasing both in developing and developed countries. Research on herbal medicine is one of the leading areas of research globally. However, there is a need to pay closer attention to the issue of conservation of medicinal plants. Many screening tests on medicinal plants are performed in vitro, the fact still remains that the ultimate goal of the researcher is to use the medicinal plants to treat various humans and animals diseases, who has to take the product orally or through other means into the system. There is an urgent need to conserve the medicinal plants to prevent their complete extinction from the nature. This is because the ever expanding trade in medicinal plants has serious implications on the survival of several plant species, with many under serious threat to become extinct. Medicinal plants are plants containing inherent active compounds used to cure disease or relieve pain (Okigbo et al., 2008). The use of traditional medicines in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). The World Health Organization estimated about 80% of the population in developing countries relies on traditional herbal medicines, for their primary basic needs (Schmincke, 2003). Medicinal plants such as Poincianella pyramidalis, Chenopodium ambrosoides, and Mimosa tenuiflora exhibited potential molluscicidal and larvicidal activity (Edilson et al, 2012). Traditionally, herbs have been considered to be nontoxic and have
been used for treating various problems by the general public “and/or” traditional medicine doctors on the globe (Oduola et al., 2007). Although, the literature has documented several toxicity resulting from the use of herbal medicine, still the potential toxicity of herbs has not been recognized by the professional doctors (Jou-fang, 1994; O’Hara et al., 1998). In herbal medicine, crude plant extracts in various forms such as infusion, decoction, tincture or herbal extract are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical molecules (Barnes et al., 2007). Plant derived products contain a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins, lignin etc. (Cowan, 1999). These phytochemicals possess numerous health-related effects such as antibacterial, anti-carcinogenic, etc. (Bidlack et al., 2000). Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables (Okwu, 2005). Diets containing an abundance of fruits and vegetables are protective against a variety of diseases, particularly cardiovascular diseases. Herbs and spices are accessible sources for obtaining natural antioxidants (Okwu, 2004). Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as “secondary metabolities” of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides,
phenols, tannins, terpenes and terpenoids (Harborne, 1973; Okwu, 2004). In addition to these substances, plants contain other chemical compounds. These can act as agents to prevent undesirable side effects of the main active substances or to assist in the assimilation of the main substances (Anon, 2007a). Opium juice, for example from *Papaver somniferum*, contain other chemical compounds in addition to morphine and reports show that it gives fewer side effects than morphine administered on its own (Anon, 2007a). In contrast to synthetic pharmaceuticals based upon single chemicals, many medicinal and aromatic plants exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process. As pointed out by Tyler (1999), these synergistic pharmacological effects can be beneficial by eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body. Kaufman *et al.* (1999) extensively documented how synergistic interactions underlie the effectiveness of a number of Phytomedicines.

Most of these phytochemical constituents are potent bioactive compounds found in medicinal plant parts which are precursors for the synthesis of useful drugs (Sofowora, 1993). Some of the most common phytochemical classes are described in the following sections and are Phenolic compounds. Phenols are a member of a group of aromatic chemical compounds with weakly acidic properties and are characterized by a hydroxyl (OH) group attached directly to an aromatic ring. The simplest of phenols
derived from benzene is also known as phenol and has the chemical formula C₆H₅OH. The presence of phenols is considered to be potentially toxic to the growth and development of pathogens (Okwu and Okwu, 2004). The structural classes of phenolic compounds include the polyphenolic (hydrolysable and condensed tannins) and monomers such as ferulic and catechol (Okwu, 2005). Polyphenols might interfere in several of the steps that lead to the development of malignant tumours, may play a role in inactivating carcinogens and inhibiting the expression of mutagens (Urquiaga and Leighton, 2000; Okwu, 2004). Flavonoids are 15-carbon compounds generally distributed throughout the plant kingdom. They are known to be synthesized by plants in response to microbial infection and have been found in vitro to be effective against a wide array of microorganisms (Harborne, 1973). Flavone with the molecular formula, C₁₅H₁₀O₂, is a commonly found plant flavonoid (Martindale, 1996). Flavonoids are potent water-soluble super antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anti-cancer activity and protects against all stage of carcinogens. Flavonoids in the body are known to reduce the risk of heart diseases (Urquiaga and Leighton, 2000). In terms of anti-cancer activity, they inhibit the initiation, promotion and progression of tumors (Urquiaga and Leighton, 2000; Okwu, 2004). In recent times, plant flavonoids have attracted attention as potentially important dietary cancer chemo-protective agents (Hertog et al., 1993; Elangevan et al., 1994). Some isoflavones act as allelochemicals widely used in insecticides (Kandaswami et al., 1994). Saponins are glycosides of both triterpenes and
steroids that are characterized by their bitter or astringent taste, foaming property, haemolytic effect on red blood cells and cholesterol binding properties (Okwu, 2005). Saponins have been shown to possess both beneficial (lowering cholesterol) and deleterious (cytotoxic and permeabilization of intestinal epithelium) properties and to exhibit structure dependent biological activity. In medicine, it is used to some extent as an expectorant and an emulsifying agent (Harborne, 1973). Quinones are aromatic rings with two or more ketone substitutions. The natural quinone pigments range in colour from pale yellow to almost black and there are over 450 known structures (Harborne, 1973). These compounds are responsible for the browning reaction in cut or damaged fruits and vegetables and are an intermediate in the melanin synthesis pathway in human skin. Hypercin is an anthroquinone which is an example of quinine obtained from St. John’s wort (Hypericum perforatum), has received much attention as an antidepressant, antiviral and also have several antimicrobial properties (Aarts, 1998). Alkaioids rank among the most efficient and therapeutically significant plant substances (Okwu, 2005). Some 5,500 alkaloids are known and they comprise the largest single class of secondary plant substances which contain one or more Nitrogen atoms, usually in combination as part of a cyclic structure (Harborne, 1973). They are usually organic bases and form salts with acids and when soluble gives alkaline solutions. Examples include nicotine, cocaine, morphine and codeine (Papaver sominfe rum), quinine (Cinchona succirubra), reserpine (Rauwolfia vomitoria), which has a large demand worldwide. Alkaloid production is a
characteristic of all plant organs. They exhibit marked physiological activity when administered to animals (Okwu and Okwu, 2004). Furthermore, alkaloids are often toxic to man and many have dramatic physiological activities, hence their wide use in medicine for the development of drugs (Harborne, 1973; Okwu, 2005). Alkaloids are usually colourless, but often optically active substances. Most are crystalline but a few are liquid at room temperature. Alkaloids have bitter tastes. The alkaloid quinine for example is one of the bitterest tasting substances known and is already significantly bitter at a molar concentration of 1x10^-5 (Harborne, 1973). Pure, isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects (Stray, 1998). Quinine with a molecular formula of C20H24N2O2 is an anti-malarial drug extracted from the bark of a cinchona tree (C. succirubra). Quinine is highly valued in the treatment of unusually resistant strains of malaria. Tannins: Tannin is a general descriptive name for a group of polymeric/phenolic substances capable of tanning leather or precipitating gelatine from a solution, a property known as astringency (Harborne, 1973). They are divided into two groups, namely hydrolyzed and condensed tannins. Hydrolysable tannins are based on Gallic acid, usually as multiple esters with D-glucose, while the numerous condensed tannins (often proanthocyanides) are derived from flavonoid monomers (Harborne, 1973; Okwu, 2005). Many physiological activities such as stimulation of phagocytic cells, host mediated tumour activity and wide range of anti-infective action have been assigned to tannins (Okwu and Okwu, 2004).
Essential oils and triterpenoids Terpenoid essential oils are the main compounds found in the volatile steam distillation fraction responsible for the characteristic scent, odour or smell found in many plants. Some essential oils possess medicating properties and are used in the pharmaceutical industry. They are commercially important as the basis of natural perfumes and also of spices and are used for flavouring purposes in the food industry. Plant families particularly rich in essential oils include the Compositae, Lamiaceae, Liliaceae, Myrtaceae and others. The terpene essential oils can be divided into 2 classes; the mono and sesquiterpenes, C10 and C15 isoprenoids, which differ in their boiling points (Monoterpenes = 140 - 180°C, sesquiterpenes > 200°C) (Harborne, 1973). Triterpenoids are compounds with a carbon skeleton based on six isoprene units and which are derived biosynthetically from the cyclic C30 hydrocarbon, squalene. They are colourless, crystalline, often have high melting points and are optically active substances. The essential triterpenoids are saponins, steroids and cardiac glycolsides which occur mainly as glycosides. Triterpenes occur especially in the waxy coatings of leaves and on fruits such as apple and pear and they may serve a protective function in repelling insects and microbial attack (Harborne, 1973).

Phytochemicals are natural bioactive compounds found in plants. Phytochemicals are divided into two groups; primary and secondary compounds. These classes are according to their functions in plant metabolism. Amino acids, sugars, proteins and chlorophyll are known as primary compounds while secondary compounds consists of alkaloids, terpenoids,
phenolic compounds and many more (Krishnaiah et al. 2009). Herbs and spices are known to produce certain bioactive compounds which react with other organisms in the environment to exhibit antioxidant activity and inhibit bacterial and fungal growth. The majority of the active compounds are phenolics, vitamin C, vitamin E, tannins and carotenes (Aqil et al. 2006; Thitilertdecha et al. 2008). Sources of natural antioxidants are primarily plant phenolics such as flavonoids that exhibit antioxidant, antimicrobial, anticarcinogenicity and other biological activities (Demiray et al. 2009; Mohan et al. 2008; Sengul et al. 2009). Antioxidant properties may also result from the chelation of transition metal ions by flavonoid compounds. The substances that inhibit the growth of pathogens and are least toxic to host cells are considered good candidates for development of new antimicrobial drugs.

Progress in phytochemicals is growing enormously by the development of rapid and accurate methods of screening plants for particular activity (Banso and Adeyemo, 2007). These procedures have taught us; many phytochemicals originally rare in occurrence are of almost universal distribution in the plant kingdom. Medicinal plants contain physiologically active principles that over the years have been exploited in traditional system of medicine for the treatment of various diseases (Adebajo et al., 1983). Banso and Olutimayin (2001) reported that plants contain a wide variety of active molecules. There is a reasonable likelihood that medicinal plants with a long history of human use will ultimately yield novel drug prototypes (Eshrat and Hussain, 2002). There are many standard methods used for the phytochemical screening of medicinal
plants. They are as described for alkaloids (Harborne, 1973), steroids and phlobatannins (Trease and Evans, 1989), phenolics and flavonoids (Awe and Sodipo, 2001), saponins and cardiac glycosides (Sofowora, 1993), tannins (Odebiyi and Sofowora, 1978). Methods for quantitative analysis of phytochemicals are as described for phenolics (Edeoga et al., 2005), flavonoids (Boham and Kocipal-Abyazan, 1974), alkaloid (Harborne, 1973), saponins (Obadoni and Ochuko, 2001) and glycosides (El-Olemy et al., 1994). The most commonly encountered secondary metabolites of plants (phytochemicals) are saponins, tannins, flavonoids, alkaloids, anthraquinones, cardiac glycosides and cyanogenic glycosides. The pharmacological and other beneficial effects of anti-nutritional factors in plants have been reviewed by (Soetan, 2008). The presence of these secondary metabolites in plants probably explains the various uses of plants for traditional medicine. For many of the medicinal plants of current interest, a primary focus of research to date has been in the areas of phytochemistry and pharmacognosy. In the area of phytochemistry, medicinal plants have been characterized for their possible bioactive molecules, which have been separated and subjected to detailed structural analysis. Research in the pharmacognosy of medicinal plants has also involved assays of bio-activity, identification of potential modes of action, and target sites for active phytomedicinal compounds. Screening programmes for biologically active natural products require the right bioassays. Detection of compounds with the desired activity in complex plant extracts depends on the reliability and sensitivity of the test systems used. Bioassays are also essential for monitoring
the required effects throughout activity-guided fractionation. All fractions are tested and those continuing to exhibit activity are carried through further isolation and purification until the active mono-substances are obtained. The search for promising plant extracts and subsequent activity guided isolation put specific requirements on the bioassays to be used. They must be simple, inexpensive and rapid in order to cope with the large number of samples - including extracts from the screening phase and all fractions obtained during the isolation procedure. They must also be sensitive enough to detect active principles which are generally present only in small concentrations in crude extracts (Hostettmann, 1995).

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga et al, 2005). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolics compounds (Hill, 1952). Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu, 1999; 2001). This field of natural products research is currently being carried out intensively, though it remains far from exhaustion. An attempt to obtain bioactive agents from plants is a worthwhile exercise since only 10% of all plants have been investigated in detail (Harborne, 1973). The majority of these bioactive compounds are alkaloids, followed by sesquiterpenes, diterpenes, triterpene saponins,
triterpenes aglycones, flavonoids, sterols, coumarins, quinine’s and monoterpenes. It is imperative that ethnobotanical researches and phytochemical tests lead to some patent-able and industrially exploitable compounds for drug development.

Research into, and development of, therapeutic materials from plant origin is a hard and expensive task (Borris, 1996; Turner, 1996; Williamson et al., 1996). Each new drug requires an investment of around US$ 100–360 million and a minimum of 10 years of work, with only 1 in 10,000 tested compounds being considered promising and only 1 in 4 of these being approved as a new drug. Up to 1992, the NCI had only found 3 plant extracts active against HIV out of 50,000 tested, and only 3 out of 33,000 plant extracts tested were found to have anti-tumour activity (Williamson et al., 1996). Quantitative considerations regarding the average yield of active compounds and the amount of starting crude plant material required for the discovery, development and launch of a new drug on the market were presented by McChesney (1995): 50 kg of raw material are necessary to provide 500 mg of pure compound for bioassays, toxicology, and “in vivo” evaluation; full pre-clinical and clinical studies can require 2 kg of pure compounds obtained from 200 ton of raw material. The process is multi-disciplinary (De Pasquale, 1984; Verpoorte, 1989). The basic sciences involved are botany, chemistry and pharmacology, including toxicology. Any research into pharmacological active natural compounds depends on the integration of these sciences. The way they are integrated and the extent of integration depend on the objectives of the
study. In any case, a particular discipline should not be seen as secondary to another; quite the opposite, as each step must be carried out considering the theoretical and technical background of each of the sciences involved, otherwise the results may not be robust enough and may lead to breakdown of the process.

India is one of the twelve megadiversity countries of the world with a rich diversity of biotic resources. Out of thirty-four hotspots recognized, India has two major hotspots, namely the Eastern Himalayas and the Western Ghats. India harbours about 47,000 species of plants 17,000 of which are angiosperms (Kapaia, 2010). The world is now looking towards India due to its rich biodiversity of medicinal plants and abundance of traditional medicinal systems (Salahuddin et al., 1998). In traditional societies, nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes (Justin et al., 2011). Medicinal plants are natural sources of compounds that can be used against many diseases today (Deshpande and Bhalsing, 2011). Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. The discovery of medicinal plants has usually depended on the experience of the populace based on long and dangerous self-experimentation (Chhetri et al., 2008). Phytochemicals are the natural bioactive compounds found in plants. These phytochemicals work with nutrients and fibers to form an integrated part of defence system against various diseases and stress conditions (Koche, 2010). The most important of these bioactive constituents
of plants are alkaloids, tannins, flavonoids, steroid, terpenoid, carbohydrate and phenolic compounds (Pascaline et al., 2011). Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these chemically active (bioactive) constituents of plants are: alkaloids, tannin, flavonoid and phenolic compounds. Many of these indigenous medicinal plants are also used for medicinal purposes (Edeoga, 2005).

Traditional medicine is the oldest method of curing diseases and infections and various plants have been used in different parts of the world to treat human diseases and infections (Caceres et al., 1991 Nweze et al., 2004, Vineela and Elizabeth, 2005). Different plant parts have also been used for the treatment of various forms of ailments. The Balanites aegyptiaca plants have been reported to be used in a variety of folk medicines in Africa and Asia. It has been used in the treatment of skin diseases and remedy for stomach ache and jaundice (Hammiche and Maiza, 2006), treatment of cough (John et al., 1990), treatment of diarrhoea and syphilis (Boulos, 1992) and Typhoid fever (Doughari et al., 2007). The root was also reported to be used in treatment of inflammation (Kubmarawa et al., 2007), antidote for snake bite (Inngerdigen, 2004). Earlier studies have shown that B. aegyptiaca contains steroidal saponins, with most of them reporting the presence of saponins as the main cause of these activities. Besides its medicinal uses, Balanites trees are widely used as fodder and for timber purposes (Arbonier, 2002). Medicinal plants are
known to owe their curative potentials to certain biological active substances which exist in parts of the plants. The chemicals which are referred to as active principles or phytochemical substances include terpenses, flavonoid, bioflavonoid, benzophonones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthrax-quinones (Iwu, 1993; Asaolu, 2002). This study is therefore aimed at determining the antimicrobial efficacy of the aqueous and the methanolic extracts of *Balanites aegyptiaca*

2.8. The need for the conservation of medicinal plants

Many medicinal plants are disappearing so fast and some are in the verge of extinction. There is a great need to conserve plants because they contain highly bioactive components which can be developed into bioactive agents. Some of the pharmacological and other beneficial effects of anti-nutritional factors in plants have been reviewed by (Soetan, 2008). Many signs reveal that medicinal plants are gradually facing extinction due to (i) People walking long distances to collect them. (ii) Some medicinal plants are no longer found. (iii) What used to be a thick forest of diverse plant species is reduced to bush and areas that have diverse flora are fast disappearing (iv) Many medicinal plants are not maturing and seeding because the young plants are being harvested before they mature (ITDG and IIRR, 1996). Number of factors like rising human population and animal population are causing pressure on plant survival. Agricultural activities result in clearing of natural habitats for farming and grazing, trees are felled for timber, charcoal and other
commercial uses and lands are used in a way that are not sustainable. Others are involved in inappropriate ways of harvesting medicinal plants, like removing all the bark or uprooting the whole plant without leaving part to grow again, bush burning and commercialization of plant sources, lack of awareness that plants are sources of the conventional medicine, poverty in arid or semi-arid areas and religious factors. For example, some western religions view the use of traditional medicine as a form of evil worship or witchcraft (ITDG and IIRR, 1996). As a result of the numerous potentials of medicinal plants in combating drug resistance by microorganisms, strict conservation measures should be put in place to prevent the total extinction of the medicinal plants from the planet earth. Medicinal plants should be harvested in the proper way to avoid serious damage. Control of overgrazing and deforestation is needed. This could be done by practicing rotational grazing so as to allow plants to rejuvenate/regrow. The Government can reserve some areas strictly for medicinal plants and prevent encroachment by undesirable plant species. Individual small-scale cultivation of medicinal plants should also be encouraged as is done for tree planting that is, medicinal plants should be incorporated in agroforestry and reforestation programmes. Mini-forests on individual farms should be maintained to increase biodiversity. Governments should also have reserve stocks of medicinal plants and encourage community actions to collect, retrieve and plant seeds of medicinal plants. The removal and export of rare and scarce medicinal plants should be discouraged/prohibited by legislation. The intellectual property rights of practitioners with great
discoveries in medicinal plants should be protected so as to encourage them. Scientists and toxicologists should investigate the active components of certain medicinal plants and their toxic potentials as to determine their safe level of consumption. Public education on medicinal plants as a potential source of modern medicine should be promoted in schools and tertiary institutions of learning. Okigbo et al. (2008) reported the need for effective conservation strategies for medicinal plants. They stated that saving Africa’s medicinal plant resources from extinction calls for intensive management and conservation through more research and increased level of public awareness about this vanishing heritage, this is one of the best examples of man pressure on ecosystem. Ex situ conservation of plants – in the form of seed banks, in-vitro collections, field gene banks etc. has proliferated in recent decades. For example, in the area of plant genetic resources for food and agriculture, a mere half million samples of plant genetic material were stored in less than ten gene banks in the 1970s. This has risen to more than 7.4 million samples in more than 1,750 gene banks in the present day (FAO, 2009). , there is a need to pay closer attention to the crisis of conservation of medicinal plants. Research on understanding the metabolism of several pathways in plant tissues and their elements contents is needed. In view of this, an attempt has been made in the present research work that involves phytochemical screening and evaluation of medicinal properties of selected legumes from the Western Ghats of Karnataka, with the following objectives.

1. Identification and documentation of selected medicinal legumes
2. Phytochemical screening of selected medicinal legumes

3. Bioactivity of selected medicinal legumes

4. Separation, isolation and characterization of chemical compounds from the selected medicinal legumes.

5. Conservation of selected medicinal plants for sustainable utilization.