Review of Literature
2. REVIEW OF LITERATURE

2.1 Herbal medicine

Nature has provided many things for mankind over the years, including the tools for the first attempts at therapeutic intervention (Nakanishi, 1999). Neanderthal remains have been found to contain the remnants of medicinal herbs (Holt and Chandra, 2002). The Nei Ching is one of the earliest health science anthologies ever produced and dates back to the thirtieth century B.C. (Nakanishi, 1999). Some of the first records on the use of natural products in medicine were written in cunei form in Mesopotamia on clay tablets and date to approximately 2600 BC (Cragg and Newman, 2001). Indeed, many of these agents continue to exist in one form or another to this day as treatments for inflammation, influenza, coughing, and parasitic infestation.

Initially, the plants were part of folk-medicine practised by ancient man in different parts of the world, which include India, China, the Middle East, Africa and South America. The some herbs, trees and shrubs employed by ancient people have continued to be valued through the ages-by Egyptians, Greeks, Romans and Indians. In the long struggle to achieve mastery over the powerful forces of nature man, has always turned to plants for help. Plants provide vital energy in their growth and seasonal rebirth. When pain, injury or disease struck early man but he had a little choice to turn to plants. Developed empirically by trial and error, many herbal treatments were remarkably effective. But the herbal treatments fell out of favour and branded as ignorant superstition. Now the new medical science is affirming much of the old herbal lore and extending its horizons to botanical medicines.

2.2 Growth of herbal medicine

Even in ancient cultures, tribal people methodically collected information on herbs and developed well-defined herbal pharmacopeia. Traditional herbal medicine gradually evolved over century’s throughout the world, in respect of local flora, culture, and religion (Cassileth, 1998; Lans et al., 2001; Cragg and Newman, 2001). This has resulted in the development of much of the pharmacopeia of scientific
medicine in the twentieth century incorporating herbal lore of native people which in turn evolved into for many systems of traditional medicine all over the world.

2.3 Evolution of herbal medicine to modern medicine

Contributing to this world-wide attention towards formulations based on natural products are their low or absent toxicity, their complete biodegradability, their availability from renewable sources and in most cases, their low-cost if compared with those of compounds obtained by total chemical synthesis. In developed countries this could be connected with the trend favourable to the so-called ‘sustainable development’ and to some extent with the observed decline of patent applications in Organic Chemistry, paralleled by the rise of Life Sciences applications.

Besides their fundamental and historical importance, many are still introduced on the market as approved drugs. In the interval between 2000 and 2006, as many as 26 plant-derived Natural Products were approved or launched on the market, while numerous others were still under clinical trials. In 2005 alone, the combined sales of drugs having vegetal origins were calculated to be in the order of $18 billion. Furthermore, a steady increase of these numbers is expected in the coming years. These plant-derived chemotherapies can be found in numerous applications such as anti-infectious agents, pain alleviation, reduction of inflammation, treatment of cancer and cardiovascular diseases. Furthermore, many other natural sources are emerging as potential sources of new biologically active natural products. This estimate suggests that plant-derived drugs make up a significant segment of natural product based pharmaceuticals.

Owing to this renewed attention to pharmaceuticals, agrochemicals and nutraceuticals (functional foods) obtained from natural sources, the study of bioactive secondary metabolites, traditionally carried out mainly by chemists, has increasingly attracted the attention of pharmacologists, biologists, botanists, agronomists, etc., stimulating cooperative work.

Even in our modern days, natural medicines are widely used, but most modern chemotherapies use pure and well-defined chemical entities. The progression from the
crude herbal remedies to the discovery and use of pure drug entities was a slow process that started with scientists working on it in the beginning of the nineteenth century. Still today, this increase in life quality is giving rise to new challenges such as the emergence of new diseases and the increase in the incidence of others. Chemists have long tried to increase the odds of finding new drug candidates or leads and use these as starting point for drug development.

Experience has persistently and repeatedly demonstrated that nature has evolved over thousands of years a diverse chemical library of compounds that are not accessible by commonly recognized and frequently used synthetic approaches.

2.4 Ethnobotanical approach to drug discovery

Most of the medicines of previous centuries were of botanical origin, products of centuries of ethnobotanical lore. These botanical remedies were generally effective, although they contained many inert compounds in addition to the active compound(s). The advent of modern organic chemistry and the reductionist concept of a single active ingredient led to the discovery and exploitation of many single bioactive compounds from plants that are now used for medicinal or pest-management purposes (Carlson et al., 1997). Useful drugs from the ethnobotanical lore include aspirin, quinine, camphor and digitalis. Examples of pesticides discovered by this approach are the pyrethroids, rotenone and strychnine. The ethnobotanical approach is still successfully used. For example, the antimalarial drug artemisinin was relatively recently found by Klayman (1985) to be the active principle from the ancient Chinese malarial remedy qinghaosu, a formulation of *Artemisia annua* L., commonly known as annual wormwood in North America. Artemisinin is now being produced from plants in commercial quantities.

The ethnobotanical lore has not been sufficiently explored. Most large pharmaceutical companies still commit some of their research to this strategy (Shu, 1998) and there are some companies, albeit relatively small ones, that base their entire drug discovery program on ethnobotanical approaches (Carlson et al., 1997). A small portion of our research program involves following up ethnobotanical leads. Although the ethnobotanical approach has been rewarding, it may have reached the
point of diminishing returns. Other approaches for lead identification have been under exploited and perhaps, may offer greater potential at this time.

Historical experiences with plants as therapeutic tool have helped to introduce single chemical entities in modern medicine. Plants, especially those with ethanopharmaceutical uses, have been the primary sources of medicines for early drug discovery. In recent times, developed countries are turning to the use of traditional medicinal systems that involve the use of herbal drugs and remedies and according to the World Health Organization (WHO), almost 65% of the world’s population has incorporated the value of plants as a methodology of medicinal agents into their primary modality of health care. Approximately, one third of all pharmaceuticals are of plants origin.

The spread of knowledge on the natural wealth is more important for a country like India at a time when the synthetic drugs are stealing the economy rates. It is often noted that 25% of all drugs prescribed today come from plants (Farnsworth and Morris, 1976). The recent analysis of Fabricant and Fransworth (2001) showed that 80% of the 122 plants derived drugs were discovered from their original ethno-pharmacological Natural Products knowledge.

2.5 Ethnobotany of Dichrostachys cinerea

2.5.1 Digestive system disorders

In South Africa the inner bark is chewed as a remedy for toothache (Venter and Venter, 1996). Von Koenen (2001) and Van Wyk and Gericke (2000) reported the oral ingestion of leaf for colic and heartburn by the Heikum Bushmen in Namibia. Elujoba et al., (2005) studied the effect of D. cinerea plant for the treatment and care of tooth. The Herero in Namibia put the root in milk and drink it for constipation (Kakujaha-Matundu, 1996). In Botswana the roots are fried with pig fat, applied to top of head to stop fluttering which may lead to vomit and diarrhoea (Hedberg and Staugard, 1989). The Bushmen in Namibia take a root decoction for stomach upset (Von Koenen, 2001). The plant is commonly used for diarrhoea in Thailand (Hedberg and Staugard, 1989). In Ayurveda Dichrostachys cinerea root is an important component in the preparation of decoction to combat diarrhoea (Khare, 2007).
2.5.2 Genitourinary system disorders

In Botswana the tree is well utilised as a good remedy for maladies of the urinary tract (Roodt, 1998; Setshogo and Venter, 2003). Central African tribes used a leaf decoction for vaginal douches and conditions of the urethra (Von Koenen, 2001). In Zimbabwe it is frequently used for the treatment of sexually transmitted diseases (Kambiyoi and Afolayam, 2001). In Sri Lanka also it is commonly as an aphrodisiac (Wijesundara, 2003). The village folk of Rayalascema used *D. cinerea* roots to treat rheumatism, urinary calculi and renal troubles (Thammamna and Narayane Rao, 1990; Vedavathys Mirudula and Sudhakar, 1997). However, Khare (2007) summarises the use of the plants as diuretic, wide use in renal affections and urinary calculi. In some areas the root decoction is taken as an aphrodisiac (Roodt, 1998). In western Uganda the leaves of *D. Cinerea* pounced and given orally for the treatment of sexual impotence and erectile dysfunction (Maud Kavnatenesi *et al.*, 2005).

2.5.3 Infections / infestations

Von Koenen (2001), reports that the bark decoction is a remedy for elephantiasis and other nematode infections among Namibians. Interestingly in Zimbabwe the leaves and roots are smoked for head-colds and tuberculosis (Van Wyk and Gericke, 2000; Von Koenen, 2001).

2.5.4 Injuries

Extracts of the leaves and bark and powdered bark are used to heal wounds (Van Wyk and Gericke, 2000; Von Koenen, 2001). The stem and bark of *D. cinerea* were used for the treatment of wounds (Joshi, 1974). powdered root bark is applied to wounds (Van Wyk, 1972; Van Wyk and Gericke, 2000). In Zimbabwe, the powdered root is sniffed for nose bleeds (Van Wyk and Gericke, 2000).

2.5.5 Muscular-skeletal system disorders

Powder of leaves used in the massage of bone fractures (Maydell, 1983). *Dichrostachys cinerea* roots is used by The village folk of Rayalascema to treat rheumatism (Thammamna and Narayane Rao, 1990; Vedavathys Mirudula and
Sudhakar, 1997). According to Khare (2007) the use of the plant against rheumatism is well documented in various Ayurvedic texts.

2.5.6 Nervous system disorders

The plant is being used for epilepsy (Van Wyk and Gericke, 2000).

2.5.7 Pain

The inner bark is a remedy for toothache (Venter and Venter, 1996). The leaves are taken orally as a tea and externally as an application. They act as an anaesthetic and considered a natural painkiller (Roodt, 1998). The leaves are used for toothache and ear ache (Van Wyk, and Gericke, 2000). Leaf infusions are used for headaches (Van Wyk, 1972) and an excellent cure for toothache. The roots and leaves are boiled and the head put under a blanket with the mouth wide open, thus the steam treats the affected area (Roodt, 1998). In Zimbabwe, the root infusions are used for abdominal pain ((Van Wyk, and Gericke, 2000). The roots are used postnatally to relieve pain (Von Koenen, 2001). External application of dried root paste of *Dichrostachys cinerea* is reported to relieve pain and oedema in rheumatism. Nesamony (1998) also reports the use of leaf infusion to relieve from pain of the eyes in the Indian subcontinent.

2.5.8 Poisonings

The leaves are chewed and directly applied to snake bites (Van Wyk, and Gericke, 2000 ; Setshogo, and Venter, 2003). The roots are chewed and placed on the sites of snakebites and scorpion stings. In Zaire *Dichrostachys* plant traditionally used for the treatment of snake bite. The decoction or maceration of leaves, barks and roots are given internally for snake bite (Kusamba Chifundera, 1987). The leaves are believed to produce a local anaesthesia and are used for the same purpose (Coates Palgrave, 2002).

2.5.9 Pregnancy/birth/puerperium disorders

If goats eat the pods they secrets lot of milk (Sullivan, 1998). The stem and bark of *D. cinerea* were used for the treatment of gynaecological troubles in adults (Joshi, 1974).
2.5.10 Respiratory system disorders

By inhaling the smoke of dried leaves and root, chest complaints and blocked nose can be relieved (Roodt, 1998; Setshogo and Venter, 2003). Dried or fresh roots are boiled with water and one cup taken three times a day for relief to cough (Hedberg and Staugård, 1989). In Zimbabwe, root infusions are used for cough and pneumonia (Van Wyk and Gericke, 2000).

2.5.11 Sensory system disorders

Dried seed-pods soaked in hot water make soothing eyewash. An extract of the leaves mixed with salt is more effective for this purpose (Roodt, 1998; Setshogo and Venter, 2003). The leaves are used for eye afflictions (Von Koenen, 2001). The infusion of tender branches and leaves of Dichrostachys cinerea could cure common eye diseases and is practised in India (Nadkarni, 1976). In Sri Lanka it is commonly used for traditional medicinal purposes to treat eye diseases (Wijesundara, 2003).

2.5.12 Skin/subcutaneous cellular tissue disorders

The powdered bark is used as an application for all kinds of skin conditions (Venter and Venter, 1996; Von Koenen, 2001). The Damara in Namibia cook the small stems and leaves are pounced them into a powder to put onto boils (Sullivan, 1998).

To conclude, plant possess broadest spectrum of synthetic activities and are the chief source of many useful compounds. Dichrostachys cinerea as per the traditional chains, bark and leaves are the chief source of drug compounds that are active against various ailments such as jaundice, inflammation, rheumatism, fever, asthma, body ache, chest problems, toothache, ulcer, wounds eye disease and have an aphrodisiac property (Igwue and Igoli, 2004). Rheological properties, the moisture and ash contents of the gum resins from D. cinerea was studied by Gundidza (2011). This gum has potential as a product for the cosmetic, pharmaceutical and food industries.

2.6 Pharmacognosy and Reverse Pharmacognosy

Pharmacognosy focuses mainly in the pharmacochemistry of natural raw materials, but not exclusively from plants (Bruneton, 1993) for pharmaceutical, diet and cosmetic purposes. This discipline includes several fields of expertise: in botany
(taxonomy, ethnobotany), in chemistry (extraction, purification and characterization of compounds) and in pharmacology (*in vitro* and *in vivo* biological tests). Ethnopharmacology is considered a part of pharmacognosy that focuses on the study of biological effects of raw materials from traditional medicine. Its goal is the validation or otherwise, of the use of plants, diet or cosmetic purposes.

The subject had developed mainly on the botanical side, being concerned with the description and identification of drugs, both in the whole state and in powder and with their history, commerce, collection, preparation and storage. Such branches of pharmacognosy are still of fundamental importance, particularly for pharmacopial identification and quality control purposes, but rapid developments in other areas have enormously expanded the subject.

Modern Pharmacognosy, the science of the medical application of phytochemicals, can be one source of bioactive compounds for medicinal chemistry programs. The ethnopharmaceutical knowledge (Heilman, 2007) accumulated over thousands of years that is, in traditional Chinese medicine (TCM) or the Indian Ayurveda is being successfully explored to yield the active ingredients as novel candidates for drug development programs (Bhushan and Manish, 2007). Although often dismissed as superstition by Western medicine, a growing number of active ingredients could be isolated and successfully tested for their pharmacological effects (Das *et al.*, 2008; Mali *et al.*, 2007; Sangwan *et al.*, 2008). Especially modern methods such as functional genomics can help to elucidate the mode of action of these traditional medicines (Deocaris *et al.*, 2008). Several drugs including artemisine based antimalarials (Imakura, 1990; Imakura, 1988) or a synthetic analog of schizandrin C for the treatment of chronic hepatitis (White, 2008) have originated from these fields of research. Plant compounds are probably the well-studied source of Natural Products, since they have been easily available, applied, extracted and analyzed for centuries. Although more than 10,000 Natural Products from plants are linked to medical applications, it has been estimated that only up to 200 of these have been translated into Western medicine so far (Liu, 1983). Albeit plant natural products may still hold a number of ‘‘new’’ drugs to be discovered.
The strategies for the identification of plant species or plant parts that is most likely to have bioactive compounds of interest for use as pharmaceuticals or agrochemicals could be ethnobotanical, chemical, ecological and anatomical information. These can be used singly or in combination to provide clues as to what plant species and what tissues of those species might be worth the major investment of time and resources to conduct a careful dereplication. Furthermore, information from these sources can provide valuable hints as to what types of biological activity the active compounds might have. Ethnobotanical leads are more likely to suggest pharmaceutical uses, whereas chemical ecology and anatomical information often lead to potential agrochemical uses. After a plant species is selected, the dereplication process begins. Rediscovery of known compounds has been the most costly aspect of this process.

Characteristically the higher plants consist in the vegetative phase of roots, stems and leaves with flowers, fruits and seeds forming stages in the reproductive cycle. Modifications of the above structures are frequently present rhizomes, stolons, stipules, bracts, tendrils, etc. Certain organs may appear to be missing or much reduced for example, the reduction of leaves in some xerophytic plants. It is most important that students acquire the ability to interpret morphological and anatomical descriptions of crude drugs as found in pharmacopoeias and allied works and also to record adequately the features of whole or powdered drugs and adulterants of commercial significance.

Reverse pharmacognosy is a new concept that complements pharmacognosy in the research of new compounds or plants in pharmacy or cosmetics (Do and Bernard, 2006). Natural compounds are selected by in-house drug likeness and chemical diversity criteria. They are submitted to a database for prediction of activity then validated in biological tests or the molecules are evaluated directly in biological assays. That is, Pharmacognosy is from plants to molecules and reverses pharmacognosy from molecules to the plants. Integrating pharmacognosy and reverse pharmacognosy in the research process provided an efficient and rapid tool for natural drug discovery.
2.7 Anatomical clues

Plant anatomy provides a realistic interpretation of morphology, physiology, and phylogeny thorough knowledge of the structure of cells and tissues. Furthermore, the knowledge of plant structure is also essential to solve many important everyday problems such as the identification of unknowns, food contaminants and forensic problems. Plants often compartmentalize, sequester or secrete bioactive compounds from specialized tissues or cells. Structures commonly associated with secondary compound accumulation in plants are glandular trichomes, lacticifers, idioblasts, resin canals and nectaries. The primary driving forces for the evolution of these different structures are the needs for efficient synthesis and delivery of secondary products to enhance interaction with other organisms and for avoidance of auto toxicity. In other cases, highly active compounds can be found in specialized cell layers or epidermal cell secretions. Roots possess a plant surface that comes in contact with potentially detrimental organisms. Many highly potent compounds can be exuded by roots. Information on the anatomical specialization at the sub cellular, cellular, tissue or organ level related to the synthesis and storage of these compounds can provide clues as to function and activity.

The anatomical distribution is discovered after the compound was isolated and then chemically and biologically characterized. The reverse sequence of anatomical examination, followed by isolation and characterization of compounds from structures of interest, should be a valid discovery strategy for new compounds. Clearly, the compounds sequestered, compartmentalized, or secreted by plants should be studied for their biological activities. Species that partition relatively large portions of their biomass into such structures and processes might be expected to be good candidates for the discovery process.

2.8 Phytopharmaceuticals

The bioactive constituents are usually secondary metabolites, derived from biosynthetic pathways present within the plant tissue. Out of many families of secondary metabolites or compounds on which the growth of a plant is not dependent, nitrogen-containing alkaloids have contributed the largest number of drugs to the
modern pharmacopoeia, ranging in effects from anticholinergics (atropine) to analgesics (opium alkaloids) and from antiparasitics (quinine) to anticholinesterases (galantamine) to antineoplastics (vinblastine/vincristine). Natural products have revealed the ways to new therapeutic approaches, contributed to the understanding of numerous biochemical pathways and have established their worth as valuable tools in biological chemistry and molecular and cellular biology.

The use of single pure compounds, including synthetic drugs, is not without its limitations, and in recent years there has been an immense revival in interest in the herbal and homoeopathic systems of medicine, both of which really heavily on plant sources.

In conclusion one can state that a large part of nature’s chemical diversity is still waiting to be discovered and can be expected to contain many bioactive natural products. Increased efforts directed toward this goal may be one way to increase the number and scope of new clinical candidates and finally, drugs. That is, drug discovery can be significantly improved through the use of the knowledge to be gained from research into natural products. However, despite the powerful resource that natural products present to us, the knowledge that is locked therein can only be fully realized with proper management of the resources, the parallel development of ancillary technologies, and the fostering of open and shared communication.

2.9 Alkaloids

The alkaloids are characterized by the presence of heterocyclic nitrogen, their basic reactivity and their physiological effects (Waterman, 1998). Analgesics, like morphine (*Papaver somniferum*) and toxins, like aconitine (*Aconitum napellus*) are two examples of this heterogeneous class of substances. Alkaloids were thought to occur only in the plant kingdom; however, they are now known to occur also in some animals, e.g., in the toxic secretions of fire-ants, ladybugs, and toads (Harborne, 1999). Classification is usually based on their chemical structure, e.g., purine alkaloids, tryptophan, ornithin and lysine derivatives. Subclasses may be based on the type of ring system present (Waterman, 1998, Waterman, 1999). The alkaloid literature is extensive. Harborne and co-workers (Harborne, 1999) have written an
overview of publications on this topic: The most comprehensive and up-to-date single reference work is the dictionary of Southon and Buckingham (Southon et al., 1989). The best modern account of the chemistry, biosynthesis, and pharmacology is the monograph by Cordell (1981). Two books which concentrate on alkaloid biochemistry and biology are the works of Robinson (1981) and of Waller and Nowacki (1978). Three series of review publications on alkaloids should also be mentioned (Manske et al., 1950; Grundon, 1976–1983; Pelletier, 1983.)

The detection of alkaloids in plant extracts are easily accomplished with general (Dragendorff, Mayer) or specific (Ehrlich) colour reagents. These tests reveal only the presence of alkaloids, but cannot distinguish between particular compounds or subclasses. The extraction of alkaloids takes advantage of their basic character, with the use of acidulated (1 M HCl, 10% acetic acid) alcoholic solvents and subsequent precipitation by concentrated ammonia. Subsequent purification by paper chromatography and TLC is necessary, and further analysis of the spots enables the classification of substances of interest. Detection is performed by fluorescence in UV light and application of various sprays (Mattiivi et al., 1995). Volatile alkaloids are best separated by GLC, whereas higher molecular-weight alkaloids are best examined by TLC (Harborne et al., 1999). An overview of the preparative isolation of alkaloids by preparative centrifugal TLC, flash chromatography, and medium-pressure LC, HPLC, countercurrent chromatography and centrifugal partition chromatography has been presented by Hostettmann et al. (1998). Mattivi et al. (1995) and Hostettmann et al. (1998) summarised methods for the preparative isolation of alkaloids.

2.10 Structure identification of natural products

The chemical structures of natural product compounds are tremendously diverse and can be very elegant in their nature (Mabry, 2001; Nakanishi, 1999; Yu, et al., 2001). Such diversity can present a challenge to the analytical or medicinal chemist attempting to unravel the mystery of the chemical structure of an unknown material presented to him or her. However, it is worth mentioning that some particularly exciting developments in structure determination pertinent to the area of
natural products have come from the field of computer assisted structure elucidation (CASE). Several computer programs have become available for scientists to use and a number of publications reporting on the utility of this technological advancement have been forthcoming (Steinbeck, 2001)

As has been previously described, the elucidation of the structure of a natural product begins with the collection of a crude material. This material is then subjected to a series of separation steps, usually involving chromatography, delivering in the end pure compound(s). Finally, a set of spectroscopic and spectrometric experiments are performed on the pure compound to delineate the structural characteristics

The identification of unwanted compounds or de-replication should occur as early as possible in the natural product isolation and purification process to avoid the loss of time and funds. The development of coupled techniques has permitted the achievement of that goal. Indeed, coupled techniques such as LC/NMR/MS have now evolved and have potent application in the pharmaceutical field.

Advancements in chromatography, spectrometry, and spectroscopy together with breakthroughs in the coupling of these technologies are important steps in the production of a fully automated and integrated natural products structure determination instrument, which will provide significant advantage to the early, rapid, and facile identification of new natural-product based drug opportunities.

2.11 Roles of bioassays

A bioassay measures biological activity of a substance based on the response of a biological test system to the test substance. In the pharmaceutical industry, bioassays are commonly applied to characterize a substance’s biological properties, to study a biological process, to detect the presence and quantity of a substance in a sample and to screen for active molecules from a library of molecules. Properties by physiochemical assays (characterization) and the determination of its biological activities by bioassays. The physiochemical properties of drug substances include its chemical composition, chemical structure, solubility, particle size, crystal property, purity and the like (Wu, 2010).
A substance’s bioactivity can only be indirectly measured by its effect on a predefined biological test system. The observations are made to the test system instead of to the substance. The biological activity of the substance is then inferred from the observed changes in the test system based on prior knowledge about the test system.

Chemodiversity in nature, e.g. in plants, microorganisms and marine organisms, still offers a valuable source for novel lead discovery, but rapid identification of the bioactive compounds of natural product mixtures remains a critical factor to ensure that this tool of drug discovery can compete with recent developed technologies such as chemical compound libraries and high-throughput screening of combinatorial synthetic efforts. Rapid screening of natural product mixtures requires the availability of a library of reference of natural compounds and methods for simple identification of putative lead structural classes avoiding, to a large extent, the potential for false-positive results. The introduction of a dereplication step after extraction by using a reproducible pre-separation method would enable the rapid elimination of false positives (Verpoorte, 1998). The effective use of automated procedures and databases in the isolation, identification and biological profiling of bioactive compounds from natural sources will be the best guarantee to the continued discovery of novel chemotypes from nature (Hook et al., 1997).

It is self-evident that in order to exploit natural product mixtures fully, an endeavour should be made to design as many screening programmes as possible. According to Suffness and Pezzuto (1991) four major roles of bioassays can be distinguished, i.e. pre-screens, screens, monitors and secondary testings. In a pre-screen a bioassay is applied to large numbers of initial samples to determine whether or not they have any bioactivity of the desired type. Such bioassays must have high capacity, low cost, and must give rapid answers. They need not be quantitative. A bioassay in a screen is used to select materials for secondary testing, whereas, in a monitor, a bioassay is used to guide fractionation of a crude material towards isolation of the pure bioactive substances. It must, therefore, be fast and cheap, have high capacity, and be readily available to the phytochemist. In the secondary testing, lead compounds are evaluated in multiple models and test
conditions to select candidates for development towards clinical trials. Secondary testing is consequently characterized by a low capacity and expensive and slow bioassays.

The monitor may be the same as the pre-screen or the screen or may be a bench-top bioassay, which can be carried out using simple facilities in a chemistry laboratory. Special considerations should be taken into account for screening and/or monitoring plant extracts. The methodology should be adaptable to highly colored, tarry, in water poorly soluble and chemically complex materials. Besides general requirements, such as validity, lack of ambiguity, accuracy, producibility, simplicity and reasonableness of cost, the bioassays should be highly selective to limit the number of leads for secondary testing, highly specific to eliminate false positives and highly sensitive to also detect low concentrations of active compounds (Vanden Berghe and Vlietinck, 1991).

Most of the aforementioned requirements are better met by in vitro testing, so that in most screening programmes the typical pattern is that in vitro screens feed into in vivo tests, the latter mostly being introduced during the secondary evaluation. In all cases, however, a screening process must reduce the test substances for secondary evaluation to a manageable size, but hopefully not so few that no useful development candidates come out. The methods for the detection of biological activity of natural product mixtures can best be divided into two groups for screening purposes: general screening bioassays and specialized screening bioassays. Depending on the aims of the screening programme, either a general screening which can pick up many different effects, or a specific assay which is directed at finding some effect against a specific disease, has to be performed. A broad screening bioassay is probably most useful if one is randomly screening chosen organisms for any kind of pharmacological activity. The alternative to using broad screening would be the setting up of a battery of specific test methods, which is cumbersome and expensive.

2.12 Need of the present study

Insufficient data exist for most plants to guarantee their quality, efficacy and safety. The idea that herbal drugs are safe and free from side effects is false.
Plants contain hundreds of constituents and some of them are very toxic, such as the most cytotoxic anti-cancer plant-derived drugs, digitalis and the pyrrolizidine alkaloids, etc. (Brinker, 1998). Though the adverse effects of phytotherapeutic agents are less frequent compared with synthetic drugs, well-controlled clinical trials have now confirmed that such effects really exist (Brown, 1992).

A further drive to the study of compounds obtainable from natural sources is the increasing consciousness that destruction or severe degradation of rain forests and other wild habitats, including seas and oceans, will unavoidably result in the loss of unexamined species and consequently of potentially useful compounds. In fact, individual plant species may contain over one thousand chemical substances and only a minor fraction of the estimated total of 250,000 to 300,000 plant species has been studied for their biomedical application. Undoubtedly, the plant kingdom still holds many species of plants containing substance of medicinal value which have yet to be discovered; large numbers of plants are constantly being screened for their possible pharmacological value (particularly for their anti-inflammatory, hypotensive, hypoglycaemic, amoebicidal, anti-fertility, cytotoxic, antibiotic and anti-Parkinsonism properties). Pharmacognosists with a multidisciplinary background are able to make valuable contributions to these rapidly developing fields of study.