Screening of some Bangladeshi medicinal plants for in vitro antioxidant, antimicrobial and insecticidal activities

M.S. Thesis

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This thesis entitled "**Screening of some Bangladeshi medicinal plants for in vitro antioxidant, antimicrobial and insecticidal activities"** is submitted by MD. Abu shoyeb in partial fulfillment of the requirement for the degree of Master of Science in Biotechnology, Department of Mathematics and Natural Sciences, BRAC University, Dhaka, was performed at Pharmacy and Biotechnology Laboratories of BRAC University, Dhaka, Bangladesh.

SUPERVISOR

Naiyyum Choudhury

Dr. Naiyyum Choudhury

Coordinator, MS in Biotechnology

Department of Mathematics

and Natural Sciences

BRAC University
Mohakhali, Dhaka

SUPERVISOR

Prof. Dr. Ashik Mosaddik

Chairperson

Department of Pharmacy

BRAC University
Mohakhali, Dhaka

Dedicated to....

My parents

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ABSTRACT

Screening of some Bangladeshi medicinal plants for antioxidant, antimicrobial, insecticidal activity and analysis of some of their chemical contents were undertaken in the present study. The medicinal plants studied were *Mikania cordata* and *Mucuna pruriens.* Literature survey revealed some reports on biological and chemical work done with different parts of these two medicinal plants. However no detailed studies have been reported. In addition previously no work was done on insecticidal activity of these plants and this is also the first report on polyphenol and flavonoid contents in different parts of these plants.

Sixteen methanol and chloroform extracts were obtained from different parts of these two medicinal plants. The antioxidant activity was performed by using DPPH free radical scavenging assay. The methanol extract of *M. cordana* root and shoot showed highest antioxidant activity (19.95 and 28. 18 µg/ml, respectively) compared to that of the reference sample, ascorbic acid (12.58 µg/ml). The antimicrobial activity was conducted by using agar disc diffusion method. Among the 16 extracts, only the chloroform extract of leaf and flower of *M. cordana* exhibited moderate antimicrobial activity against most of Gram positive and Gram negative organisms. None of extracts of *M. pruriens* showed antimicrobial activity compared to the standard kanamycin. But interestingly the chloroform extract of seed of *M. pruriens* showed significant insecticidal activity against *Sitofillus oryzae* within 30 minutes after treatment followed by methanol extract of seed pulp of *M. pruriens.* Analysis of total polyphenol and flavonoid contents of all 16 extracts of two medicinal plants showed that the highest concentration of polyphenol (872.94 ± 4.85 mg/g of galic acid equivalent) was present in the methanol extract of leaf of *M. cordata* and highest concentration of flavonoid (913.39 ± 12.38 mg/g quarcetin equivalent)) was present in the chloroform of the shoot of this plant. The methanol extract of the roots of *M. pruriens* showed the highest concentration of polyphenol ((398.98 ± 10.20 mg/g gallic acid equivalent) and the chloroform extract of the roots of these plant showed the highest concentration of flavonoid (1644.90 ± 11.63 mg/g quarcetin equivalent).

On the basis of the observed bioassay and phenol and flavonoid profiles, different parts of the two plants may be further studied for development antibacterial, antifungal and insecticidal preparations.

ABBREVIATIONS

Abbreviations	Elaborations
WHO	World Health Organization
spp.	Species
NCI	National Cancer Institute, USA
IPCB	International Program of Co-operation for Biodiversity
CIBA	Chemische Industrie Basel
NCEs	New chemical entities
MAP	Medicinal and aromatic plant
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
PG	Propyl gallate
TBHQ	Tert-butyl hydroquinone
UV	Ultra violet
MHRA	Medicines and Healthcare products Regulatory Agency
NSAID	Non-steroidal anti-inflammatory drug
MIC	Minimum inhibitory concentration
DPPH 2,	2-diphenyl-1-picrylhydrazyl
MDCK	Madin Darby canine kidney
MHA	Mueller hinton agar
DMSO	Dimethyl sulfoxide
DMEM	Dulbecco's Modified Eagle Medium
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
UNCTAD	United Nations Conference on Trade and Development
CHO	Chinese hamster ovary

CHAPTER 1

INTRODUCTION

Several diverse lines of evidence indicate that medicinal plants represent the oldest and most widespread form of medication. Until the last century most medicines were derived directly from plant or animal sources. Despite the increasing use of factory-made synthetic drugs, natural organic healing materials have persisted as the "treatment of choice" for a multitude of health problems in populations throughout the world [Halberstein, 2005].

1.1 Historical Overview of Medicinal Plants

The healing properties of various plants are recognized and utilized by other primates. A number of species of monkeys and apes have been observed to repeatedly consume particular botanical species containing chemical components that act as analgesics, anti-microbial, anti-inflammatory, immunostimulants, anti-diarrheal, digestive aids, and fertility regulators [Baker, 1996; Etkin 1994; Plotkin, 200]. A recent review article on this intriguing subject reports that monkeys, gorillas, chimpanzees, and humans select some of the same plants for the management of similar diseases, injuries, and other health problems [Huffman, 1997].

There is also ample archaeological evidence indicating that medicinal plants were regularly employed by people in prehistoric times. In several ancient cultures botanical products were ingested for biomedically curative and psychotherapeutic purposes. Through extensive experimentation the biodynamic activities of the phytochemical plant constituents were gradually discovered and exploited for specific medical and psychiatric applications. Evidence suggests that the early healers were well aware of the mind-body interconnection and the important role of patient relaxation in medical treatments and in health restoration and rehabilitation [Adovasio, *et al.*, 1976; de Montellano, 1975]. In a recent review article Merlin [Merlin, 2003] evaluate different lines of archaeological evidence regarding psychoactive plant usage in a variety of prehistoric cultures in the "Old World" (Eastern Hemisphere).
Some of the earliest known written records also deal with the subject of healing with medicinal substances. The ancient Egyptians of 3000 to 6000 years ago are credited with developing an elaborate and effective pharmacological collection of numerous curing materials obtained from natural resources. [Nunn, 1996] stated that the most common form of treatment recommended in the medical

papyri was the use of drugs, drawn from a very wide range of animal, mineral, and vegetable substances and administered in a variety of ways. The ancient Egyptians were renowned for their skill in this respect. The Egyptian doctors prescribed sedatives, analgesics, gastrointestinal disorder remedies, and medicines for urinary tract diseases and the common cold [Nunn, 1996; Oakes and Gahlin, 2003]. Plant extracts were prepared and taken internally, applied topically, and administered by fumigation and vapor inhalation. The Egyptians are also credited with the early medicinal use of wine, castor oil, marijuana, opium, mints, and beer made from barley and wheat [Selin and Shapiro, 2003]. [Oakes, 2003] pointed out that the Egyptians were the first people to use a number of drugs that modern studies have proved would have been medicinally effective.

Plant-based therapeutic treatments continued to be augmented later by health-care practitioners in ancient Greece 3000 through 1500 years ago. Dioscorides, an authority on herbs who lived in the first century A.D., is noted for assembling 24 detailed books on over 600 curative plants and their proper uses under the title De Materia Medica, the earliest known designation of that terminology [Von Staden, H, Herophilus, 1989; Sigerist, 1967].

Following those developments additional discoveries of useful medicinal plants resulted from experimentations in several early historic cultures 1000 to 2000 years ago in China, India, and Tibet. The herbal specialist was recognized as a powerful and influential professional in these societies [Selin and Shapiro, 2003; Jonas, *et al.*, 1999]. About 1000 years ago healers in the Aztec and Maya Indian cultures of Mexico and Central America were experimenting with natural curing substances. Evans *et al.* [2004] noted that Post-Classic Mesoamericans developed a large and effective pharmacopoeia, formulae for medicines concocted from animals, minerals, and especially plants. According to [Berdan, 2005] the ancient Aztec healers exploited at least 132 medicinal herbs for the treatment of specific ailments ranging from pimples and nosebleeds to gout and epilepsy. Respiratory and gastrointestinal infections were addressed with remedies produced from a combination of different herbal products, and some of the preparations were prescribed to prevent certain diseases.

Another major advancement was achieved in the 18th century with the revolutionary taxonomic work of Swedish naturalist Carolus Linnaeus, whose classifications of thousands of botanical species provided the foundation for the standardized documentation of the relationships and evolutionary histories of medicinal plants. His classic work *Systema Naturae* established the framework for modern biological taxonomy and his famous works *Genera Botanica*, *Critica Botanica* and *Philosophica Botanica* deal with the subject of the precise identification of plants and their characteristics, including catalogues with Latin terminology of all species known at that time. In *Species Plantarum* Linnaeus recorded detailed descriptions of over 5900 plant species. These landmark publications continue to be consulted by botanists, herbalists, horticulturalists, and taxonomists.

Another indication of the lengthy history of botanical medicine is found in its global, cross-cultural distribution. From its original inception in prehistory, medicinal plant exploitation has gradually spread, by both independent discovery and cultural diffusion, to all corners of the earth. Organized, systematic collections of traditional herbal remedies have been described by anthropologists and ethnobotanists in all countries and ethnic groups surveyed so far [Wheelwright, 1974; Anderson, 1977; Tierra, 1988]

1.2 Significance of Medicinal Plants

The use of natural products with therapeutic properties is as ancient as human civilization and, for a long time, mineral, plant and animal products were the main sources of drugs [de Pasquale, 1984]. The Industrial Revolution and the development of organic chemistry resulted in a preference for synthetic products for pharmacological treatment. The reasons for this were that pure compounds were easily obtained, structural modifications to produce potentially more active and safer drugs could be easily performed and the economic power of the pharmaceutical companies was increasing. Furthermore, throughout the development of human culture, the use of natural products has had magical-religious significance and different points of view regarding the concepts of health and disease existed within each culture. Obviously, this approach was against the new *modus vivendi* of the industrialized western societies, in which drugs from natural resources were considered

either an option for poorly educated or low income people or simply as religious superstition of no pharmacological value.

However, even if we only consider the impact of the discovery of the penicillin, obtained from micro-organisms, on the development of anti-infection therapy, the importance of natural products is clearly enormous. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use. Of the 252 drugs considered as basic and essential by the World Health Organization (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors. Examples of important drugs obtained from plants are digoxin from *Digitalis spp.*, quinine and quinidine from *Cinchona spp.*, vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum.* It is estimated that 60% of anti-tumor and anti-infectious drugs already on the market or under clinical trial are of natural origin [Shu, 1998]. The vast majority of these cannot yet be synthesized economically and are still obtained from wild or cultivated plants. Natural compounds can be lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development and the discovery of new therapeutic properties not yet attributed to known compounds [Hamburger, 1991]. In addition, compounds such as muscarine, physostigmine, cannabinoids, yohimbine, forskolin, colchicine and phorbol esters, all obtained from plants, are important tools used in pharmacological, physiological and biochemical studies [Williamson, 1997].

1.2.1 The Search of New Drugs through Ethno medicine

The modern social context and economic view of health services, the needs of the pharmaceutical market and the recognition that research on medicinal plants used in folk medicine represents a suitable approach for the development of new drugs [Elisabetsky, 1987] have led to an increase in the number of publications in this field, and private and governmental institutions are now financially supporting research programs worldwide.

The NCI (National Cancer Institute, USA) has tested more than 50,000 plant samples for anti-HIV activity and 33,000 samples for anti-tumor activity. In 1993, the International Program of Co-operation for Biodiversity (IPCB) was launched in

order to promote natural products in Latin America and Africa, linking universities, industries and governments in a multidisciplinary program for the sustained development and preservation of the environment [Rouhi, 1997]. Large pharmaceutical companies, such as Merck, CIBA, Glaxo, Boehringer and Syntex, now have specific departments dedicated to the study of new drugs from natural sources [Reid, 1993].

However, the potential use of higher plants as a source of new drugs is still poorly explored. Of the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties; in most cases, only pharmacological screening or preliminary studies have been carried out. It is estimated that 5000 species have been studied for medical use [Payne, 1992]. Between the years 1957 and 1981, the NCI screened around 20,000 plant species from Latin America and Asia for anti-tumor activity, but even these were not screened for other pharmacological activities [Hamburger, 1991].

1.2.2 Advantage over Synthetic Medicine

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants [Goldfrank, 1982; Dukes, 1988]. This interest in drugs of plant origin is due to several reasons, namely, conventional medicine can be inefficient (e.g. side effects and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems, a large percentage of the world's population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggest that "natural" products are harmless. However, the use of these substances is not always authorized by legal authorities dealing with efficacy and safety procedures, and many published papers point to the lack of quality in the production, trade and prescription of phytomedicinal products [Rates, 2001].

1.2.3 Biodiversity loss and Impact on Its Plant Medicine

One of the driving factors for the renewed interest in plant antimicrobials in the past 20 years has been the rapid rate of (plant) species extinction [Rates, 2001; IUCN, 2006].

There is a feeling among natural-products chemists and microbiologists alike that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of being lost irretrievably. Plant extinctions are occurring at a rate unmatched in geological history, leaving ecosystems incomplete and impoverished. Current extinction rates are at least 100 to 1,000 times higher than natural background rates, with a quarter of the world's coniferous trees known to be in jeopardy [IUCN, 2006] and as many as 15,000 medicinal plants are under threat [IUCN, 2006]. Over 50% of cycads, used medicinally and the oldest seed plants on earth are threatened with extinction. This makes them one of the most threatened groups of species currently on the IUCN Red List of Threatened Species [Hawkins, 2008].

1.2.4 Challenges in Drug Discovery from Medicinal Plants

Despite the evident successes of drug discovery from medicinal plants, future endeavors are faced with many challenges. Pharmacognosists, phytochemists, and other natural product scientists will need to continuously improve the quality and quantity of compounds that enter the drug development phase to keep pace with other drug discovery efforts [Butler, 2004]. The process of drug discovery has been estimated to take an average of 10 years upwards [Reichert, 2003] and cost more than 800 million dollars [Dickson and Gagnon, 2004]. Much of this time and money is spent on the numerous leads that are discarded during the drug discovery process. In fact, it has been estimated that only one in 5000 lead compounds will successfully advance through clinical trials and be approved for use. Lead identification is the first step in a lengthy drug development process. Lead optimization (involving medicinal and combinatorial chemistry), lead development (including pharmacology, toxicology, pharmacokinetics, ADME [absorption, distribution, metabolism, and excretion], and drug delivery), and clinical trials all take a considerable length of time [Balunas, *et al.*, 2005].

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, 1997]. 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].Fifty kg of raw material are necessary to provide 500 mg of pure compound for bioassays, toxicology, and "in vivo" evaluation while full pre-clinical and clinical studies can require 2 kg of pure compounds obtained from 200 ton of raw material [Rates, 2001].

At NCI, contracts for the collection of plants that have been operating for nearly 20 years in the Americas, Africa, Madagascar, and Southeast Asia were recently suspended due to reallocation of NCI funds for new initiatives aimed at improving diagnosis and prevention, as well as expediting the translation of drugs from the development phase to clinical use. In addition, as academic pharmacy departments redirect their focus towards the production of clinical and community pharmacists, the emphasis on pharmaceutical research and development related to medicinal plant and natural product drug discovery in academic pharmacy departments is gradually declining [Balunas *et al.*, 2005].

1.2.5 Economic Importance of Medicinal Plants

Medicinal and aromatic plants (MAPs) are produced and offered in a wide variety of products, from crude materials to processed and packaged products like pharmaceuticals, herbal remedies, teas, spirits, cosmetics, sweets, dietary supplements, varnishes and insecticides [Ohrmann, 1991; Gorecki, 2002; Lange, 1996]. The use of botanical raw material is in many cases much cheaper than using alternative chemical substances. An estimated number of 70,000 plant species are used in folk medicine worldwide [Farnsworth and Soejarto, 1991]. As a consequence, there is an enormous demand in botanicals - for domestic use and for commercial trade - resulting in a huge trade on local, regional, national and international levels. As the production of botanicals still relies to a large degree on wild-collection, profound knowledge of trade, size, structure and streams as well as of commodities, traded quantities and their origin is essential for assessing its impact on the plant populations concerned [Lange, 2006].

In the period 1991-2003, the reported annual global export of pharmaceutical plants amounted on average to 467,000 tonnes valued at US$ 1.2 billion. A main feature of the international trade is the dominance of only few countries: about 80% of the worldwide imports and exports are allotted to only 12 countries each,

with the temperate Asian and European countries dominating. The countries of temperate Asia are responsible for 41% of the annual global imports and even 48% of the annual global exports. Europe's share of the global import is one third. Regarding single countries the import share of the USA is 12% and of Germany and Japan 11% each. The list of the world's top 12 countries of import shows that Hong Kong is by far the most important importer of pharmaceutical plants with an annual average import of approximately 59,950 tonnes. It is followed by the USA with an average import of about 51,200 tonnes and Japan with 46,450 tonnes a year. Germany follows on 4th place, importing on average 44,750 tonnes per year. No fewer than five European countries, all of them European Union Member States, are among the top 12 countries of import. On the export side, China heads the list of the world's top 12 countries of export. It exported annually on average about 150,600 tonnes of pharmaceutical plants in the period 1991-2003, which is one third of the total global export of pharmaceutical plants. This figure is three times as high as the quantities exported from Hong Kong, about four times as high as the quantities exported from India and from Mexico, and ten times as high as those exported from Germany and the USA. Further important exporters are Egypt, Bulgaria and Chile. Two southeast-European countries, Bulgaria and Albania, are amongst the top 12 countries of export. From 1991 to 2003 the total world's exports increased by 55% from 377,300 to 584,700 tones [Lange, 2006].

1.2.6 Potentials of Medicinal Plants of Bangladesh

Bangladesh has a great treasure of medicinal plants. Spread over an area of about 55,000 square miles and endowed by nature with a very favorable climate, Bangladesh possesses what is perhaps one of the richest floras of all other areas of a similar size on the surface of the globe. A great variety of plants grows in its forests, jungles, wastelands and in the roadsides. It is not surprising therefore that plants containing active and medicinal principles grow abundantly within its bounds. More than 500 plants growing or available in this country have been reported to possess medicinal properties of some description or other and have been enumerated in the literature of indigenous drugs [Yusuf, M *et al.*, 1994]. A good number of the natural drugs included in different pharmacopoeias grow here; many others can be easily grown under cultivation. Almost all these indigenous medicinal plants are

extensively used in the preparation of Unani, Ayurvedic and Homeopathic medicines in Bangladesh. These plants also serve as important raw materials of many modern medicinal preparations. Since there has been no systematic phytochemical survey of the medicinal plants of Bangladesh, it is quite possible that many other potential medicinal plants in this country still remain unexplored and unevaluated. From this rich natural plant resource and the vast array of materia medica of the indigenous systems, phytochemical and pharmacological investigations and research might bring many useful remedies to the scientific world for alleviation of human sufferings.

Bangladesh imports a large number of vegetable drugs and their extracts from abroad spending a huge amount of foreign exchange every year. This foreign exchange could have been saved if the indigenous pharmaceutical raw materials could be identified from these vast resources and properly processed to render them suitable for use in the pharmaceutical industries for the preparation of various pharmaceutical products and for development of new drugs. This is what the developed countries do with their natural products and export to other countries [Gani, 1998].

It is estimated that some 12,500 tonnes of dried medicinal plant material produced and sold in Bangladesh. These products are worth some Tk. 255 million ($4.5 million) to the rural economy and around Tk. 330 million ($5.8 million) at the factory rate/wholesale. The 5,000 tonnes of imported medicinal plants cost around Tk 480 million ($8 million). It is believed that there are around 350 inter-district beparis (traders) who are serviced by 6,000 to 10,000 local collectors, pikers (whole salers) and growers. In total there are said to be around 200 Unani and 200 Ayuverdic registered factories, plus some 70 homeopathic factories. Collectively they employ 2,000 to 4,000 people. In addition, there are around 5,000 qualified and 80,000 unqualified herbal practitioners in the country [Dixie, 2005].

1.3 Plant as a Source of Antimicrobials

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies. For

example, the use of bearberry (*Arctostaphylos uva-ursi)* and cranberry juice *(Vaccinium macrocarpon)* to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm *(Melissa officinalis)*, garlic *(Allium sativum)* and tee tree *(Melaleuca alternifolia)* are described as broad-spectrum antimicrobial agents [Heinrich, 2004]. It has generally been the essential oils of these plants rather than their extracts that have had the greatest use in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin. In the case of Melaleuca alternifolia, for example, the use of the essential oil (tee tree oil) is a common therapeutic tool to treat acne and other infectious troubles of the skin [Vanaclocha, *et al.,* 2003].

1.3.1 Major Groups of Antimicrobial Compounds from Plants

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives [Geissman, 1963]. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total [Schultes, 1978]. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some, such as terpenoids, give plants their odors; others (quinones and tannins) are responsible for plant pigment. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds.

1.3.1.1 Simple Phenols and Phenolic Acids

Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Cinnamic and caffeic acids are common representatives of a wide group of phenylpropane- derived compounds which are in the highest oxidation states. The common herbs tarragon and thyme both contain caffeic acid, which is effective against viruses [Warren, 1994], bacteria [Brantner, 1996; Thomson, 1978], and fungi [Duke, 1985]. Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to microorganisms. Catechol has two OH groups, and pyrogallol has three. The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased

hydroxylation results in increased toxicity [Geissman, 1963]. In addition, some authors have found that more highly oxidized phenols are inhibitory [Rama Raje, 1975].

1.3.1.2 Quinones

Quinones are aromatic rings with two ketone substitutions. In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins [Stern, 1996], often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides, and membrane-bound enzymes. Quinones may also render substrates unavailable to the microorganism. As with all plant-derived antimicrobials, the possible toxic effects of quinones must be thoroughly examined [Cowan, 1999].

1.3.1.3 Flavones, Flavonoids, and Flavonols

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). The addition of a 3-hydroxyl group yields a flavonol [Fessenden and Fessenden, 1982]. Flavonoids are also hydroxylated phenolic substances but occur as a C_6-C_3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection [Dixon, R.A., 1983], it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described above for quinones. More lipophilic flavonoids may also disrupt microbial membranes [Cowan, 1999].

1.3.1.4 Tannins

"Tannin" is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency. Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins [Haslam, 1996].One of their molecular actions is to complex with proteins through so-called nonspecific forces such as hydrogen

bonding and hydrophobic effects, as well as by covalent bond formation [Stern, 1996; Haslam, 1996].

1.3.1.5 Coumarins

Coumarins are phenolic substances made of fused benzene and a-pyrone rings [O'Kennedy, *et al.,* 1997]. Their fame has come mainly from their antithrombotic [Thastrup, 1985], anti-inflammatory [Piller, 1975], and vasodilatory [Namba, 1988] activities. Several other coumarins have antimicrobial properties. R. D. Thornes, working at the Boston Lying-In Hospital in 1954, sought an agent to treat vaginal candidiasis in his pregnant patients. Coumarin was found in vitro to inhibit Candida albicans. As a group, coumarins have been found to stimulate macrophages [Casley-Smith, 1997], which could have an indirect negative effect on infections.

1.3.1.6 Terpenoids and Essential Oils

The fragrance of plants is carried in the so called quinta essentia, or essential oil fraction. These oils are secondary metabolites that are highly enriched in compounds based on an isoprene structure. They are called terpenes, their general chemical structure is $C_{10}H_{16}$, and they occur as diterpenes, triterpenes, and tetraterpenes (C_{20}, C_{30}, and C_{40}), as well as hemiterpenes (C_5) and sesquiterpenes (C_{15}). When the compounds contain additional elements, usually oxygen, they are termed terpenoids. Terpenenes or terpenoids are active against bacteria [Ahmed, 1993; Taylor, 1996], fungi [Taylor, 1996; Ayafor, 1994; Harrigan, 1993; Suresh, 1997], viruses [Fujioka, 1994; Hasegawa, 1994; Pengsuparp, 1994; Xu, 1996], and protozoa. In 1977, it was reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria [Chaurasia and Vyas 1977]. The triterpenoid betulinic acid is just one of several terpenoids which have been shown to inhibit HIV.

1.3.1.7 Alkaloids

Heterocyclic nitrogen compounds are called alkaloids. Diterpenoid alkaloids, commonly isolated from the plants of the Ranunculaceae, or buttercup family, are commonly found to have antimicrobial properties [Omulokoli an Chhabra, 1997]. Solamargine, a glycoalkaloid from the berries of Solanum khasianum, and other

alkaloids may be useful against HIV infection [McMahon, et al., 1995; Sethi, 1979] as well as intestinal infections associated with AIDS [McDevitt, 1996].

1.3.1.8 Lectins and Polypeptides

Peptides which are inhibitory to microorganisms were first reported in 1942. They are often positively charged and contain disulfide bonds [Zhang and Lewis, 1997]. Their mechanism of action may be the formation of ion channels in the microbial membrane [Zhang and Lewis, 1997; Terras, 1993] or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors [Sharon and Ofek, 1986]. Recent interest has been focused mostly on studying anti-HIV peptides and lectins, but the inhibition of bacteria and fungi by these macromolecules, such as that from the herbaceous Amaranthus, has long been known. [De Bolle, 1996]

1.3.2 Requirement of New Antimicrobials

It can be argued that the discovery, development and clinical exploitation of antibiotics were the most significant medical advances of the twentieth century. It is sobering that at the beginning of the twenty-first century articles abound concerning resistance, superbugs and the prospects of a post-antibiotic era [Livermore, D.M., 2003]. Furthermore, the threat of bioterrorism with multi-drug resistant pathogens highlights the need for continued development. With hindsight, it is perhaps not surprising that we find ourselves in this situation. Complacency about the importance of bacterial infections and a feeling that these had been controlled by the development of antibiotics led to a subsequent decline in both academic and industry research and a consequent erosion of the knowledge base that now urgently requires to be redressed [Coates, 2002].

Currently, a more realistic view of the ongoing battle against bacteria prevails: i) there is certainty of evolving resistance when antibiotics are used, ii) improvements in medical technology result in more patients in critical and immune suppressed states, thus creating a perpetual need for new antibiotics. Yet if the development of antibiotics is reviewed it is clear that the current rate of discovery is far lower than in the golden age of antibiotics in the 1940s through to the 1960s when all the major families of compounds were identified [McDevitt and Rosenberg, 2001;

Spellberg, 2004; Labischinski, 2001]. There is no doubt that there is a need for new antibiotics, particularly in the hospital setting, and the potential of bio-terrorism should also not be forgotten. The relentless rise of resistance in Gram-positive infections is creating everyday therapeutic challenges in managing these infections and much effort has been directed towards developing new compounds to meet this need [Abbanat, et al., 2003]. However, as the time required to bring an antibiotic from discovery to market generally is 8-12 years, research and development efforts must be focused on compounds that will meet not just current needs but those that will be present 10 years in the future [DiMasi, *et al.*, 2003]. Thus while there are a limited number of Gram-positive agents in late-stage development the majority of which stem from established classes of antibiotics there is a lack of new antibiotics in development to tackle the return of multi-resistant Gramnegative pathogens.

1.4 Plant as a Source of Antioxidants

The adverse effects of oxidative stress on human health have become a serious issue. The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components [Craig, 1999]. Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), tocopherol (vitamin E), glutathione, carotenoids, and flavonoids). This imbalance leads to cell damage [Aruoma, 1998; Lefer and Granger, 2000; Smith, 2003] and health problems [Steer, 2002; Uchida, 2000]. A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases [Shahidi and Wanasundara, 1992], including cardiovascular diseases, cancers [Gerber, 2002], neurodegenerative diseases, Alzheimer's disease [Di Matteo and Esposito, 2003] and inflammatory diseases [Sreejayan and Rao, 1996]. One solution to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources [Knekt, 1996]. These natural plant antioxidants can therefore serve as a type of preventive medicine. Recent reports indicate that there is an

inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human disease [Sies, 1993].

The oxidative deterioration of fats and oils in foods is responsible for rancid odors and flavors, with a consequent decrease in nutritional quality and safety caused by the formation of secondary, potentially toxic, compounds. The addition of antioxidants is required to preserve flavor and color and to avoid vitamin destruction. Among the synthetic types, the most frequently used to preserve food are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ). Reports revealing that BHA and BHT could be toxic, and the higher manufacturing costs and lower efficiency of natural antioxidants such as tocopherols, together with the increasing consciousness of consumers with regard to food additive safety, created a need for identifying alternative natural and probably safer sources of food antioxidants [Sherwin, et al., 1990; Wanasundara and Shahidi, 1998]. The replacement of synthetic antioxidants by natural ones may have benefits due to health implications and functionality such as solubility in both oil and water, of interest for emulsions, in food systems.

The growing interest in the substitution of synthetic food antioxidants by natural ones has fostered research on vegetable sources and the screening of raw materials for identifying new antioxidants. Oxidation reactions are not an exclusive concern for the food industry, and antioxidants are widely needed to prevent deterioration of other oxidisable goods, such as cosmetics, pharmaceuticals and plastics [Moure, A *et al.*, 2001].

Polyphenols represents a large family of compounds having in vitro antioxidant activity. The type and amount of polyphenols produced in plants varies greatly depending on genetic factors, environmental conditions, germination and degree of ripeness, processing and storage. Subdivision of polyphenols into tannins, lignins and flavonoids depends on the different phenolic units derived from the plant secondary metabolism pathway [Dugo, *et al.*, 2001].

The flavonoid family is the major group among the polyphenols, with more than 5000 known compounds. Their roles in plants involve pigmentation, enzyme activity regulation, protection from UV irradiation, chelation of transition metal ion and also reducing activity [Harborne, 1988]. Flavonoids family includes the catechins, antocyanidines, flavones, flavonols, isoflavonols and several other compounds. Important dietary sources of flavonoids are, among others, fruits and vegetables (in particular red and purple fruits, red onion, pulses, etc.), tea, dark chocolate and red wine. Beneficial effects of flavonoids have been largely studied in vitro and in vivo on animal models and small human studies [Lotito and Frei, 2004]. Results of in vitro studies confirm an antioxidant effect of many flavonoid compounds in different experimental settings, especially investigating their potential role in the prevention of cancer and cardiovascular diseases. Effect of these compounds on animals and humans, though, can be strongly affected by the bioavailability of the active compounds, which, in turn, depends on several factors, such as their release from the food matrices, modifications and degradations they can undergo during the digestive process, and their ability to reach cellular targets [Williamson and Manach, 2005].

Another group of antioxidant compounds widely diffused in plants is the carotenoids. They belong to the family of fat-soluble tetraterpenoids and occur naturally in chloroplasts of plant and algae and also in some types of fungi and bacteria [Krinsky, 1998]. Plant and algae use carotenoids to absorb light energy to use during photosynthesis and to protect chlorophyll from light damage. Carotenoids can be classified into carotenes (oxygen-free molecules) and xanthophylls (containing oxygen). Alpha-, beta- and gamma-carotenes and beta cryptoxantine are all dietary carotenoids that have vitamin A activity in humans [Britton, 1995]. Carotenes take their name from the most well-known member of this group, carotene, found in carrot and other orange colored fruits and vegetables [Britton, 1995]. The colors of carotenoids range from pale yellow (xanthophylls) to deep red (e.g. lycopene, abundant in tomato fruits). A high intake of carotenoidrich foods has been correlated with a decreased incidence of serious chronic diseases. However, a recent metaanalysis of several in vivo clinical supplementation trials have indicated an increased incidence of death in smoker patients assuming beta-carotene supplements, suggesting a potential harmful effect for this compound, assumed alone and not as a

diet component [Tanvetyanon and Bepler, 2008]. Human's intake of carotenoids depends entirely on the diet.

Vegetable oils, nuts and wholegrain cereals are all good dietary sources of tocopherols, organic compounds with vitamin E activity [Rigotti, 2007]. Alpha- and gammatocopherol are the main dietary sources of vitamin E. As tocopherols are present in oils, a very low-fat diet could decrease the intake of vitamin E, also considering that its absorption is best when associated with consumption of meals. Tocopherols are often used, along with other antioxidants, to preserve food itself from oxidation, especially oils from going rancid. Many studies investigated the effects of vitamin E supplementation, and antioxidants are today available on the market as dietary supplements, alone or in combination with other bioactive molecules. Integration of the diet with vitamins and other micronutrients supplements can be beneficial, but can also represent a risk [Dugo *et al.*, 2001].

1.5 Plant as a Source of Insecticide

Plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control [Arnason *et al.*, 1989]. Insecticidal activity of many plants against several insect pests has been demonstrated [Jilani, 1983; Isman, 2000; Carlini *et al.*, 2002]. The deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant growth inhibitor, suppression of reproductive behaviour and reduction of fecundity and fertility. [Yang and Tang, 1988] reviewed the plants used for pest insect control and found that there is a strong connection between medicinal and pesticidal plants. *Sitophillus oryzae* is considered as a major pest of stored grains [Howe., 1965]. Annual post-harvest losses resulting from insect damages, microbial deterioration and others factors are estimated to be 10-25% of worldwide production [Matthews., 1993]. Control of these insects relies heavily on the use of synthetic insecticides and fumigants. But their widespread use has led to some serious problems including development of insect strains resistant to insecticides [Zettler *et al.*, 1990; White, 1995; Ribeiro *et al.*, 2003], toxic residues on stored grain, toxicity to consumers and increasing costs of application. However, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally

friendly. Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides.

1.6 Potential Risk Associated with Medicinal Plants

A general disillusionment with conventional medicines, coupled with the desire for a 'natural' lifestyle has resulted in an increasing utilization of complementary and alternative medicine across the developed world. Herbal medicinal products are being used increasingly by the general public on a self-selection basis to either replace or complement conventional medicines.

As with all forms of self-treatment, the use of herbal medicinal products presents a potential risk to human health [De Smet, 2004; De Smet, 1995]. There are concerns that the patient may be exposed to potentially toxic substances either from the herbal ingredients themselves or as a result of exposure to contaminants present in the herbal product. Furthermore, self-administration of any therapy in preference to orthodox treatment may delay a patient seeking qualified advice, or cause a patient to abandon conventional treatment without first seeking appropriate advice. Emerging evidence suggests that herbal medicinal products may in some cases compromise the efficacy of conventional medicines, for example through herb-drug interactions.

The safety of herbal medicinal products is of particular importance as the majority of these products is self-prescribed and is used to treat minor and often chronic conditions. The extensive traditional use of plants as medicines has enabled those medicines with acute and obvious signs of toxicity to be well recognized and their use avoided. However, the premise that traditional use of a plant for perhaps many hundreds of years establishes its safety does not necessarily hold true [De Smet, 2004; De Smet, 1995; De Smet, 1997]. The more subtle and chronic forms of toxicity, such as carcinogenicity, mutagenicity and hepatotoxicity, may well have been overlooked by previous generations and it is these types of toxicities that are of most concern when assessing the safety of herbal remedies.

A UK Medical Toxicology Unit conducted a study of potentially serious adverse reactions associated with exposure to traditional medicines and food supplements during 1991 to 1995 [Shaw, 1997]. Of 1297 enquiries from healthcare professionals, a total of 785 cases were identified as possible (n = 738), probable (n = 35) or confirmed (n = 12) cases of poisoning caused by traditional medicines or food supplements. The report concluded that the overall risk to public health from these types of products was low. However, clusters of cases were identified that gave cause for concern. Twenty-one cases of liver toxicity, including two deaths, were associated with the use of traditional Chinese medicines, although no causative agent was identified.

Potential hepatotoxicity associated with herbal medicines has been discussed for some time [Chitturi *et al.*, 2000, Pittler and Ernst *et al.*, 2003]. Hepatotoxicity has been reported with a number of herbal medicines. Teucrium species have also been implicated in hepatotoxicity. Following reports of serious cases of liver toxicity associated with the use of Piper methysticum, P. methysticum has been prohibited in unlicensed medicinal products in the UK since January 2003 [The Medicines for Human Use., 2002].

In 2007, MHRA was aware of 79 cases of liver damage associated with the consumption of kava that have been reported worldwide.

1.7 Profiles for Investigated Medicinal Plants

1.7.1 Mikania cordata

Scientific name: *Mikania cordata* (Burm. f.) B.L. Rob.
Local name: Asami lata, Bonno lata.
Vernacular Names:
Malaysia Akar lupang, ceroma, selaput tunggul.

English Mile-a-minute.

Indonesia Brojo lego (Javanese), blukar (Sumatra), hila hitu lama (Ambon).

Philippines Bikas (Bagobo), detidid (Igorot), uoko (Bontok).

Thailand Khi-kaiyan.

Synonym: Heartleaf hempvine.

Scientific classification:

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Asteridae
Order	Asterales
Family	Asteraceae – Aster family
Genus	Mikania Willd.
Species	Mikania cordata (Burm. f.) B.L. Rob.

Fig 1.1 *Mikania cordata* (Burm. f.) B.L. Rob

1.7.1.1 Description

Mikania cordata (Burm. f.) B.L. Robinson is a crawling plant, which groves in forests, especially in fallen fields in the Bangladesh. *Mikania cordata* is a scandent herb and often forms a dense tangled mass. Its stem is nearly cylindrical or irregularly angular, ribbed and measures up to 6 m x 2-3 mm. The internodes are 8-20 cm long, with thickened nodes and sometimes short hairs. [Plant Resources of South-East Asia No.11: Auxiliary plants.] A fast growing, creeping or twining, perennial vine; leaves opposite, cordate or triangular-ovate, blade 3 to 12 cm long, 2 to 6 cm wide, on a slender petiole 1 to 8 cm long, base broadly cordate, tip acuminate, margins crenate, dentate, or entire, surfaces nearly glabrous, three- to seven-veined from base; flowers in small heads in open, nearly flat-topped (corymbose) panicles; axillary and terminal heads 6 to 9 mm long, four-flowered; involucral bracts four, obtuse or acute, 5 to 6 mm long, glabrous or subglabrous with one additional smaller bract about 3 mm long;

corolla white or yellowish white, about 5 mm long; anthers bluish gray or grayish black; style white; fruit an achene, linear-oblong, 2 to 3 mm long, five angled, blackish brown, glandular; pappus of 40 to 45 bristles, about 4 mm long, white at first, reddish afterwards. May be distinguished by the following characteristics: 40 to 45 reddish pappus bristles, corollas white, and heads 7 to 7.5 mm long. [www.hear.org]

1.7.1.2 Distribution

Most species of *Mikania* are native to the Americas. The most widely distributed species of *Mikania cordata*, however, is native to and widespread in Southeast Asia including Hainan Taiwan. and Eastern Africa, but currently invasive in many parts of the world. [Plant Resources of South-East Asia No.11: Auxiliary plants.] In Bangladesh, it grows wild in all districts of the country.

1.7.1.3 Ecology / Cultivation

Mikania cordata is adapted to hot, humid tropical environments with 1500 mm or more annual rainfall and plenty of sunlight, at altitudes ranging from sea level to 1600 m. Hence, it is commonly found in young secondary jungles, forest clearings, abandoned grounds, secondary regrowth areas, ravines, mountain slopes, roadsides, water courses, fallow lands, low-lying areas along streams and rivers and open plantations. However, it can also persist with reduced vigour in plantations. It may even be found under closed canopies of 4-5-year-old rubber and oil palm, but it is then markedly etiolated and weakened. It is rarely found in plantations of 5-15 years old. [www.hear.org]

1.7.1.4 Uses

The plant is used as a cover crop to prevent erosion and the leaves are used in some places as a soup vegetable, and can be used as cattle fodder.

Folkloric use: In southern Nigeria a decoction is given for coughs, and the leaf-juice is a remedy for sore eyes. In Portuguese East Africa, the Tongas use the plant as a remedy for snake and scorpion bite. An infusion of the plant is given in affections of the stomach and intestines. In Malaysia the leaves are used for rubbing on the body

against itches. In Java they are used for poulticing the wound of circumcision and other wounds. In India, leaves used for itchiness and as wound plaster. In Bangladesh, decoction used for treatment of gastric ulcer.In southern Africa, leaves applied to wounds. [www.hear.org].It is widely used as traditional medicine against gastric ulcer and a variety of other diseases. There are several scientific reports on different parts of the plant. [Mandal *et al.,* 1992] reported that the root extract of the plant possessed hepatoprotective activity. The leaves of Mikania species are used in folk medicine have antispasmodic, antiulcerogenic and antirheumatic agents. The plant is also used to treat asthma, respiratory problems and infections.This plant is also used for the treatment of skin diseases. [Kirtikar and Basu, 1980].

1.7.1.5 Parts use

Leaves, leaf juice

1.7.1.6 Biological Studies

- Anticarcinogenic Biological Response: Study showed the intracellular contents of active intermediates of various xenobiotics including chemical carcinogens would be reduced by specific enhancement of drug-detoxifying enzymes in the liver of rats treated with the plant extract.
- Essential Oil: Essential oil of the leaves of Mikania cordata yielded four majors constituents: a-pinene (20%), germacrene D (19.8%), beta-pinene (8.7%) and alpha-thujene (7.1%).
- Anti-Inflammatory: Study of the methanolic fraction of M. cordata root extract showed significant anti-inflammatory effects in exudative, proliferative and chronic phases of inflammation and also showed an antipyretic activity.
- CNS Changes: Study showed root extract induces profound behavioral changes, especially the disappearance of aggressive behavior. It also showed strong narcotic effects and analgesic effects.
- Anti-Stress Activity: Study on stress-induced alterations in central neurotransmitters showed pretreatment with M cordata root extract prevented decreases in adrenaline and noradrenaline and increases in 5-

HT, while dopamine was further increases. Dose-depended biochemical responses may be the possible mechanism of the anti-stress activity of this plant extract.

- Analgesic / Antioxidant: Study of a hydromethanol extract of the leaves of Mikania scandens showed strong analgesic and antioxidant effects.
- Anti-Ulcer: Study of alkaloidal fraction from M. cordata on diclofenac-induced gastric ulcer showed the bioactive principles of M. cordata have anti-ulcerogenic effects. Results validate the use of the plant in Bangladesh for treatment of gastric ulcer. [Bikas, Mikania cordata. Htm/ Philipine Medicinal plant].

1.7.1.7 Chemical Constituents

Phytochemical screening of this plant yielded flavonoids, steroids, alklaloid, tannin, gum and saponin. [Plant Resources of South-East Asia No.11: Auxiliary plants]. From the chemical point of view, *M. cordata* has been reported to contain non-volatile compounds: sesquiterpene dilactones, germacranolides, diterpenes and flavonoids. More recently, the literature has revealed only Geographic Variation in the Leaves Oils Composition of *Mikania Cordata* (Burm.f.) B. L. Robinson from Côte d'Ivoire 573 both works concerning volatile oil composition of *M. cordata*. When, we reported the predominance of oil of *M. cordata* from Abidjan (south of Côte d'Ivoire) in α-pinene (20%) and germacrene D (19.8%) accompanied by β-pinene (8.7%) and α-thujene (7.1%) (Bedi *et al.*, 2003).

1.7.2. *Mucuna pruriens*

Scientific name: *Mucuna pruriens* (L.) DC. – cowitch

Local name: Alkushi, Bichchoti, Bilaiachara.

Common names:

Atmagupta/ Kapikacchu in Sanskrit, Kiwanch or Konch in Hindi, Khaajkuiri in Marathi, Alkushi (Bengali), poonaikkaali in Tamil, Velvet bean/ Cowitch in English, Cowhage Juckbohne (German: "Itch bean"), Naykaranam (Malayalam), Mah Mui in Thai.

Synonyms:

Mucuna prurita Hook.

Scientific classification:

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* – Dicotyledons
Subclass	*Rosidae*
Order	*Fabales*
Family	*Fabaceae* – Pea family
Genus	*Mucuna* Adans. – mucuna
Species	*Mucuna pruriens* (L.) DC. – cowitch

Fig 1.2 *Mucuna pruriens* seed pod

1.7.2.1 Description

Mucuna pruriens is a tropical legume known as velvet bean or cowitch and by other common names. When the plant is young, it is almost completely covered with fuzzy hairs, but when older, it is almost completely free of hairs. The leaves are tripinnate, ovate, reverse ovate, rhombus shaped or widely ovate. The sides of the leaves are often heavily grooved and the tips are pointy. In young *M. pruriens* plants, both sides

of the leaves have hairs. The stems of the leaflets are two to three millimeters long. Additional adjacent leaves are present and are about 5 mm long.

The flower heads take the form of axially arrayed panicles. They are 15 to 32 cm long and have two to three, or many flowers. The accompanying leaves are about 12.5 mm long; the flower stand axes are from 2.5 to 5 mm. The bell is 7.5 to 9 mm long and silky. The sepals are longer or of the same length as the shuttles. The crown is purplish or white. The flag is 1.5 mm long. The wings are 2.5 to 3.8 cm long.

In the fruit ripening stage, a 4 to 13 cm long, 1 to 2 cm wide, unwinged leguminous fruit develops. There is a ridge along the length of the fruit. The husk is very hairy and carries up to seven seeds. The seeds are flattened uniform ellipsoid, 1 to 1.9 cm long, 0.8 to 1.3 cm wide and 4 to 6.5 cm thick. The hilum, the base of the funiculus (connection between placenta and plant seeds) is a surrounded by a significant arillus (fleshy seeds shell). *M.pruriens* bears white, lavender, or purple flowers. Its seed pods are about 10 cm long and are covered in loose orange hairs that cause a severe itch if they come in contact with skin. The chemical compounds responsible for the itch are a protein, mucunain,[Rätsch and Christian] and serotonin.

1.7.2.2 Distribution

The seeds are shiny black or brown drift seeds. It is found in tropical Africa, India and the Caribbean.It also found in all over place in Bangladesh.

1.7.2.3 Uses

In many parts of the world *Mucuna pruriens* is used as an important forage, fallow and green manure crop.[Factsheet-M. pruriens, 2008] Since the plant is a legume, it fixes nitrogen and fertilizes soil.

M.pruriens is a widespread fodder plant in the tropics. To that end, the whole plant is fed to animals as silage, dried hay or dried seeds. *M. pruriens* silage contains 11-23% crude protein, 35-40% crude fiber, and the dried beans 20-35% crude protein. It also has use in the countries of Benin and Vietnam as a biological control for problematic Imperata cylindrica grass. [Factsheet-M. pruriens., 2008] *M.pruriens* is said to not be invasive outside its cultivated area [Factsheet-M. pruriens., 2008]. *M.pruriens* is sometimes used as a coffee substitute called "Nescafe" (not to be confused with the commercial brand Nescafé). Cooked fresh shoots or beans can also be eaten. This

requires that they be soaked from at least 30 minutes to 48 hours in advance of cooking, or the water changed up to several times during cooking, since otherwise the plant can be toxic to humans. The above described process leaches out phytochemical compounds such as levodopa, making the product more suitable for consumption. If consumed in large quantities as food, unprocessed *M pruriens* is toxic to nonruminant mammals including humans.

Medicinal Uses

Traditionally, *M. pruriens* has been used as an effective aphrodisiac [Amin *et al.*, 1996]. It is still used to increase libido in both men and women due to its dopamine inducing properties and in Ayurvedic medicine it is said to increase sperm count. Dopamine has a profound influence on sexual function. [Giuliano *et al.,* 2001] Use of *Mucuna pruriens* is well documented in Siddha medicine for a host of uses. [Dr.J.Raamachandran,"Herbs of Siddha Medicines"]

The plant and its extracts have been long used in tribal communities as a toxin antagonist for various snakebites. Research on its effects against Naja (Cobra), [Medicinal Plants: Chemistry And Google Book Search 2006] Echis (Saw scaled viper),[*Medical Toxicology - Google Book Search* 2004] Calloselasma (Malayan Pit viper) and Bangarus (Krait)[Yerra Rajeshwar *et al.*, 2005] have shown that it has potential use in the prophylactic treatment of snakebites.

M.pruriens seeds have also been found to have antidepressant properties in cases of depressive neurosis when consumed. [G. V. Joglekar *et al.*, 1963] and formulations of the seed powder have shown promise in the management and treatment of Parkinson disease.[Manyam *et al.*, 2004] Dried leaves of *M.pruriens* are sometimes smoked. [Rätsch, *et al*] *M .pruriens* has also recently become popular among lucid dreaming enthusiasts: when combined with other supplements it stimulates the cholinergic system.

1.7.2.4 Parts use

Shoot, Root, Seed and Seed pulp.

1.7.2.5 Previous Studies

- *M. pruriens* seeds contain high concentrations of levodopa, a direct precursor of the neurotransmitter dopamine. It has long been used in

traditional Ayurvedic Indian medicine for diseases including Parkinson's disease. [Lieu CA. *et al.,* 2010; Katzenschlager *et al.,* 2004] In large amounts (e.g. 30 g dose) it has been shown to be as effective as pure levodopa/carbidopa in the treatment of Parkinson's disease, but no data on long-term efficacy and tolerability is available.

- In addition to levodopa, it contains serotonin (5-HT), 5-HTP, nicotine, N,N-DMT (DMT), bufotenine, and 5-MeO-DMT. As such, it could potentially have psychedelic effects, and it has purportedly been used in ayahuasca preparations. [Erowid *Mucuna pruriens* Vault 2008]
- The mature seeds of the plant contain about 3.1-6.1% L-DOPA, with trace amounts of 5-hydroxytryptamine (serotonin), nicotine, DMT-n-oxide, bufotenine, 5-MeO-DMT-n-oxide, and beta-carboline. One study using 36 samples of the seeds found no tryptamines present in them.
- The leaves contain about 0.5% L-DOPA, 0.006% dimethyltryptamine (DMT), 0.0025% 5-MeO-DMT and 0.003% DMT n-oxide. [The phytochemistry, toxicology, and food potential of velvet bean 2008]

1.8 Rationale of the Research

Although there has been a relentless increase in resistance to antimicrobial agents amongst important bacterial pathogens throughout the world, it is well known that the number of new antimicrobial agents being brought to the market has undergone a steady decline in the past several decades. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies [Rios *et al.*, 2005]. The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used antimicrobials and may have a significant clinical value in treatment of resistant microbial strains [Eloff, 1998]. The current study will contribute to the pursuit and development of new antimicrobial.

There is a growing interest in natural antioxidants plants because of the worldwide trend toward the use of natural additives in food and cosmetics. Plants are one of the most important targets to search for natural antioxidants from the point of view of safety [Yanishlieva *et al.*, 2006].

Annual post-harvest losses resulting from insect damages, microbial deterioration and others factors are estimated to be 25% of worldwide production (Matthews. 1993) Medicinal plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control (Arnason *et al.*, 1989).

The study will contribute towards the growing database of knowledge on herbal medicines and help to advocate the safe and effective use of traditional herbal remedies. The discovery of medicinal plants in different parts of the world is also important both to the agricultural and medicine sectors, helping in establishment of new directions towards propagation of alternative medicinal crops that offer better economic and social benefits.

1.9 Objective of the Research

The major aim of the proposed study was to investigate the antibacterial, antioxidant potential and to execute insecticidal activity of crude extracts of four different parts of the two plants used in traditional medicine - *Mikania cordata and Mucuna pruriens.*

The first specific objective was to assess the biological activity of the selected plant' extracts against clinical isolates of five Gram-positive — *Staphylococcus aureus*, *Bacillus subtilis, Bacillus cereus*, *Bacillus megaterium, Sarcina lutea* and eight Gram-negative — *Escherichia coli,Vibrio parahemolyticus*, Salmonella typhi, *Salmonella paratyphi, Pseudomonas aureus, Shigella boydii, Shigella dysenteriae*, *Vibrio mimicus* three fungus — *Candida albicans, Aspergillus niger, Saccharomyces cerevisiae* species using agar disk diffusion methods.

The second specific objective was to evaluate the antioxidant capability of methanol extracts of designated plants using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and Determination of Total Antioxidant Capacity.

The third specific objective was to investigate the potential toxicity of methanol and chloroform extracts of selected plants by assessing their insecticidal activity against *Sitophillus oryzae* insect by Surface Film Treatment method.

1.10 Hypothesis

The working hypothesis was that two medicinal plants for this study have some potential antioxidant, antibacterial commotion with the deficiency for potential toxicity and depending on the activities, will possibly lead to the development of alternate new therapeutic modalities.

1.11 Statistical analysis

The results are expressed as mean±SEM using Graph Pad Prism (version 5.0) computer program (Graph pad Software San Diego, CA, USA). We also use Microsoft Excell for statistical analysis of this thesis

Chapter 2

MATERIALS AND METHODS

2.1. METHODS OF PLANT MATERIAL

The chemical investigation of a plant material can be divided into following steps:

2.1.1 Collection and identification of the plant material

Two plants different parts were used for the current study — *Mikania cordata* (Leaves, flower, root, shoot) and *Mucuna pruriens* (shoot, root, seed, seed pulp) were collected in air tight polythene bag from two locations *Mikania cordata* from Dhaka and *Mucuna pruriens* from Bogra in January, 2011 and the incidence was recorded. The plant materials were identified by the Bangladesh National Herbarium, Dhaka, Bangladesh.

2.1.2 Preparation of Plant Materials

The whole plant parts were usually collected in fresh condition. Collected fresh plant materials were examined and old, insect and fungus infected leaves and shoots were removed. The roots part of these plants was washed through water for removing soil or dust. For effective drying the collected plant parts were kept in open mesh bag and kept apart from individual plants. Plant materials were placed in diffused sunlight for few days until they were thoroughly dried. Dried plant materials were minced to fine powder using laboratory blender with occasional shaking and kept in air tight bottle in the dark until extraction.

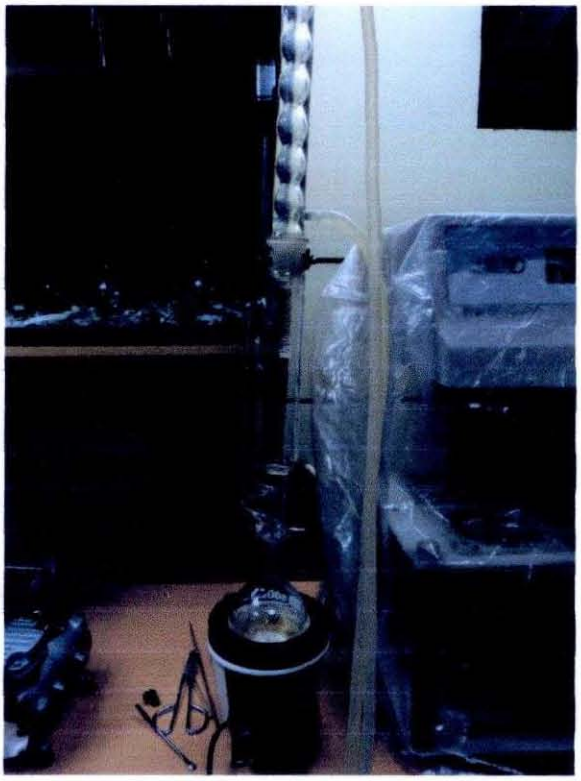
2.1.3 Extraction of Plant Materials with Chloroform

Air-dried and powdered plant materials were extracted with chloroform (CH_3Cl,) using Soxhlet extractor (Glasscolabs, UK). The Soxhlet extraction procedure is a semicontinuous process, which has been found to yield an optimal extraction of similar products [Schmitt, 2006]. The protocol followed was the standard method of extraction published by Current Protocols [Shahidi, 2001]. 30 g of each of powdered plant materials were weighed into extraction chamber. Plant material was extracted with 400 ml chloroform at the boiling point (51^0 C) using a heating mantle. The extraction was continued for 8 hours. After the extraction the extract was allowed to cool and filtered with Whatman grade 1: 11 μm cellulose filter paper. Filtered extract was

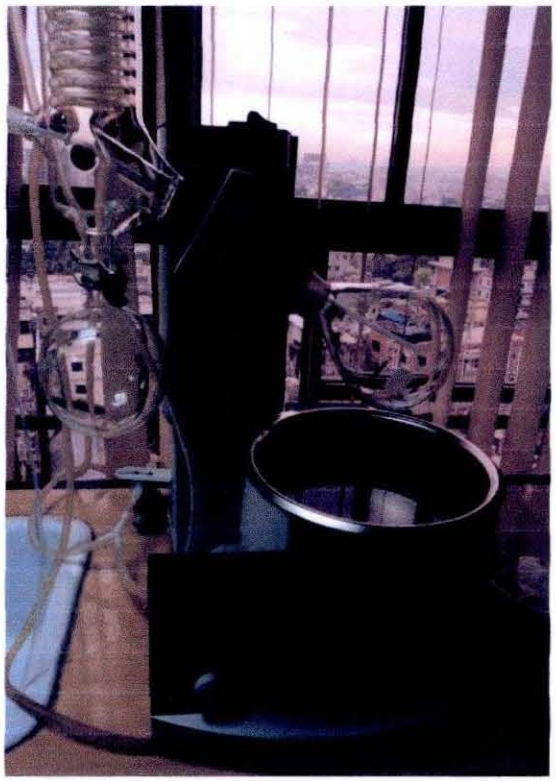
concentrated by using a rotary evaporator at low temperature (40° C). All extracts were stored at room temperature until used.

2.1.4 Extraction of Plant Materials with Methanol

The plant material residue after chloroform extraction was dried overnight and then extracted with methanol (CH_3OH,) using Soxhlet extractor. Dried plant material residues were weighed into extraction chamber. Plant material was extracted with 400 ml methanol at the boiling point (60^0 C) using a heating mantle. The extraction was continued for 8 hours. After the extraction the extract was allowed to cool and filtered with Whatman grade 1: 11 µm cellulose filter paper. Filtered extract was concentrated by using a rotary evaporator at low temperature (40^0 C). Dried extract was weighed and expressed in percentage of original sample. All extracts were stored at room temperature until used.



Soxhlet apparatus



Rotary evaporator

Fig 2.1 Soxhlet apparatus and Rotary evaporator

2.1.5 Chemical investigation of *Mikania cordata* and *Mucuna pruriens*

2.1.5.1 Total Phenolic Content Determination

Principle:

The content of total phenolic compounds in plant methanolic extracts was determined by Folin–Ciocalteu Reagent (FCR). The FCR actually measures a sample's reducing capacity. The exact chemical nature of the FC reagent is not known, but it is believed to contain heteropolyphosphotunstates - molybdates. Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}O_{40})^{4-}$. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo (VI):

$$Mo\ (VI) + e \rightarrow Mo\ (V)$$

Reagent:

- ✓ Folin – ciocalteu reagent
- ✓ Sodium carbonate (Na_2CO_3)
- ✓ Ethanol or Methanol
- ✓ Galic acid (Analytical or Reagent grade)

Preparation of 7.5% Sodium carbonate solution:

Accurately weigh 7.5 gm of Na_2CO_3 and place it into a 100 ml of a volumetric flask. Then the volume is adjusted by distilled water. Then the concentration of solution is 7.5% of Na_2CO_3.

Preparation of Standard solution:

Take 0.025 gm galic acid and dissolved it into 5 ml of Ethanol. The concentration of this solution is 5µg/µl of galic acid. This solution is called stock solution. Then prepared the experimental concentration from this stock solution by the following manner:

Concentration (µg/ml)	Solution taken from stock solution	Solution taken from others	Adjust the volume by distilled Ethanol	Final volume
250	250 µl	-	4.75 ml	5 ml
200	200 µl	-	4.80 ml	5 ml
150	150 µl	-	4.85 ml	5 ml
100	100 µl	-	4.90 ml	5 ml
50	50 µl	-	4.95 ml	5 ml

Preparation of Extract solution:

Take 0.050 gm of plant extract and dissolved it into 5 ml of distilled to methanol. The concentration of this solution was 10µg/µl of plant extract.

Experimental procedure (Folin, O., & Ciocalteu, V. (1927*)*:

1. 1.0 ml of plant extract or standard of different concentration solution was taken in a test tube.
2. 5 ml of Folin – ciocalteu (Diluted 10 fold) reagent solution was added into the test tube.
3. 4 ml of Sodium carbonate solution was added into the test tube.
4. The test tubes were incubeted for 30 minutes at 20^0C to complete the reaction. (only applicable for standard). Incubate the test tube for 1 hour at 20^0C to complete the reaction (Applicable for extract).
5. Then the absorbance of the solution was measured at 765 nm using a spectrophotometer against blank.
6. A typical blank solution contained ethanol or methanol.
7. The Total content of phenolic compounds in plant methanol extracts in gallic acid equivalents (GAE) was calculated by the following formula equation

$C = (c \times V)/m$

where:

C = total content of phenolic compounds, mg/g plant extract, in GAE;

c = the concentration of gallic acid established from the calibration curve, mg/ml;

V = the volume of extract, ml;

m = the weight of pure plant methanolic extract, g.

2.1.5.2 Total Flavonoid Content Determination (Chang *et al.*, 2002)

Principle:

The total flavonoid content was determined with the aluminum chloride ($AlCl_3$) method using quercetin as standard.

Reagent:

- ✓ Aluminium Chloride ($AlCl_3$)
- ✓ Potassium Acetate
- ✓ Ethanol or Methanol
- ✓ Quercetin (Analytical or Reagent grade)

Preparation of 10% Aluminium chloride ($AlCl_3$) solution:

Accurately weigh 10 gm of $AlCl_3$ and place it into a 100 ml of a volumetric flask. Then the volume is adjusted by distilled water. Then the concentration of solution is 10% of $AlCl_3$.

Preparation of 1M Potassium acetate solution:

Accurately weigh 9.815 gm of potassium acetate and place it into a 100 ml of a volumetric flask. Then the volume is adjusted by distilled water. Then the concentration of solution is 1M of potassium acetate.

Preparation of Standard solution:

Take 0.025 gm quercetin and dissolved it into 5 ml of methanol. The concentration of this solution is 5µg/µl of quercetin. This solution is called stock solution. Then prepared the experimental concentration from this stock solution by the following manner:

Concentration (µg/ml)	Solution taken from stock solution	Solution taken from others	Adjust the volume by distilled methanol	Final volume
100	100 µl	-	4.90 ml	5 ml
50	-	2 ml (100µg/ml)	2 ml	4 ml
25	-	2 ml (50µg/ml)	2 ml	4 ml
12.5	-	2 ml (25µg/ml)	2 ml	4 ml

Preparation of Extract solution:

Take 0.050 gm of plant extract and dissolved it into 5 ml of distilled to methanol. The concentration of this solution is 10µg/µl of plant extract.

Experimental procedure:

1. 1.0 ml of plant extract or standard of different concentration solution was taken in a test tube.
2. 3 ml of methanol was added into the test tube.
3. 200µl of 10% aluminium chloride solution was added into the test tube.
4. 200µl of 1 M potassium acetate solution was taken into the test tube.
5. 5.6 ml of distilled water was added into the test tube.

6. The test tubes were incubated for 30 minutes at room temperature to complete the reaction.
7. Then the absorbance of the solution was measured at 415 nm using a spectrophotometer against blank.
8. A typical blank solution contained methanol.
9. The Total content of flavonoid compounds in plant methanol extracts in quercetin equivalents was calculated by the following formula equation

$C = (c \times V)/m$

where:

C = total content of flavonoid compounds, mg/g plant extract, in quercetin;

c = the concentration of quercetin established from the calibration curve, mg/ml;

V = the volume of extract, ml;

m = the weight of pure plant methanolic extract, g.

2.2 Assessment of Antibacterial Activity

The antimicrobial screening, which is the first stage of antimicrobial drug research, is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the in vitro fungal and bacterial growth. This ability may be estimated by any of the following three methods.

Disc diffusion method

Serial diffusion method

Bioautographic method

Among the above mentioned techniques the disc diffusion is a widely accepted in vitro investigation for preliminary screening of test agents, which may possess antimicrobial activity. Therefore in this investigation, only disc diffusion method was used.

2.2.1 Principle of Disc Diffusion Method (KIRBY BAUER METHOD)

The disc diffusion method for antibiotic susceptibility testing is the Kirby-Bauer method. Kirby-Bauer antibiotic testing (KB testing or disk diffusion antibiotic sensitivity testing) is a test which uses antibiotic-impregnated disc to test whether particular bacteria are susceptible to specific antibiotics.

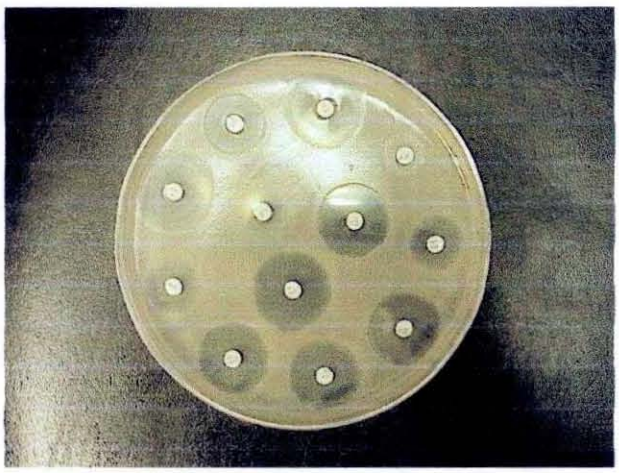


Figure 2.2: In Kirby-Bauer testing, white disc containing antibiotics are placed on a plate of bacteria. Circles of poor bacterial growth surround some discs indicating susceptibility to the antibiotic.

In the disc-diffusion susceptibility test, solution of known concentration (µg/ml) of the test samples are made by dissolving measured amount of the samples in definite volume of solvents. Dried and sterilized whatman paper discs (6 mm diameter) are then impregnated with known amounts of the test substances using micropipette. Discs containing known amounts of an antimicrobial agent are placed on the surface of an agar plate containing a Muller Hinton agar medium. The antimicrobial agent diffuses into the medium, causing a zone of inhibition of growth of the strain around the discs corresponding to the susceptibility of the strain to the agent. The antimicrobial activity of the test agent is determined by measuring the diameter of zone of inhibition expressed in millimeter.

2.2.2 Experimental work

2.2.2.1 Apparatus and Reagents

Whatman paper discs

Sterile cotton

Micropipette

Laminar air flow hood

Refrigerator

Petridishes

Sterile forceps

Screw cap test tubes

Autoclave

Nutrient Agar Medium

Inoculating Loop

Spirit burner

Nose mask and Hand gloves

Incubator

Ethanol

2.2.2.2 Test Materials

Table 2.2.1 List of test materials

Plant	Plant parts	Test materials
Mikania cordata	Leaves	**Methanol soluble fraction**
		Cloroform soluble fraction
	Flower	**Methanol soluble fraction**
		Cloroform soluble fraction
	Shoot	**Methanol soluble fraction**
		Cloroform soluble fraction
	Root	**Methanol soluble fraction**
		Cloroform soluble fraction
Mucania pruriens	Shoot	**Methanol soluble fraction**
		Cloroform soluble fraction
	Root	**Methanol soluble fraction**
		Cloroform soluble fraction
	Seed	**Methanol soluble fraction**
		Cloroform soluble fraction
	Seed pulp	**Methanol soluble fraction**
		Cloroform soluble fraction

2.2.2.3 Test Organisms

The bacterial and fungal strains used for the experiments were collected as pure cultures from the Clinical pharmacy lab, University of Dhaka. All gram positive, gram negative and fungal organisms were taken for the test and they listed in the Table 2.2.2

Table 2.2.2 List of test organism

Gram positive Bacteria	**Gram negative Bacteria**	**Fungi**
1.*Bacillus cereus*	1.*Escherichia coli*	1.*Candida albicans*
2.*Becillus Megaterium*	2.*Shigella dysenteriae*	2.*Aspergillus niger*
3.*Bacillus subtilis*	3.*Salmonella paratyphi*	3.*Sacharomyces cerevacea*
4.*Staphylococcus aureus*	4.*Salmonella typhy*	
5.*Sarcina lutea*	5.*Shigella boydii*	
6.*Psedomonas aureus*	6.*Vibrio mimicus*	
	7.*Vibrio parahemolyticus*	

2.2.2.4 Culture Medium

Muller Hinton agar medium was used for making plates on which antimicrobial sensitivity tests were carried out. And nutrient agar medium was also used to prepare fresh cultures.

Table 2.2.3: Composition of Nutrient agar Medium

Ingredients	Amount (gms/lit)
Peptic digest of animal tissue	5.00
Beef extract	1.50
Yeast extract	1.50
Sodium chloride	5.00
Agar	15.00
Final pH (at 25°C)	7.4±0.2

Table 2.2.4: Composition of Muller Hinton agar Medium

Ingredients	Amount (gms/lit)
Beef, infusion form	300.00
Casein acid hydrolysate	17.5
Starch	1.50
Agar	17.00
Final pH (at 25°C)	7.3±0.2

2.2.2.5 Preparation of Medium

To prepare required volume of this medium, calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a gas burner to make a clear solution. The pH (at 25° C) was adjusted at 7.2-7.6 by NaOH or HCl. 8 ml of the Nitruent agar medium was then transferred in screw cap test tubes to prepare slants. And 20 ml Muler hinton agar medium was then transferred in Petri dishes.The slants were used for making fresh culture of bacteria and fungi that were in turn used for sensitivity study.

2.2.2.6 Sterilization Procedures

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precaution were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petridishes and other glassware were sterilized by autoclaving at a temperature of 121° C and a pressure of 15-lbs / sq inch for 20 min. Micropipette tips, cotton buds, forceps, blank discs were sterilized.

2.2.2.7 Preparation of Fresh Subculture

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37° C for their optimum growth. These fresh cultures were used for the sensitivity test.

2.2.2.8 Preparation of the Test Plates

The test organisms were transferred from the subculture to the screw test tubes containing about 10 ml of physiological saline with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by vortex machine to get a uniform suspension of the organisms. The bacterial and fungal suspension was immediately transferred to the sterilized petridishes (plates) by a sterilized cotton-wool swab. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media. Then the plates were allowed to dry before applying discs.

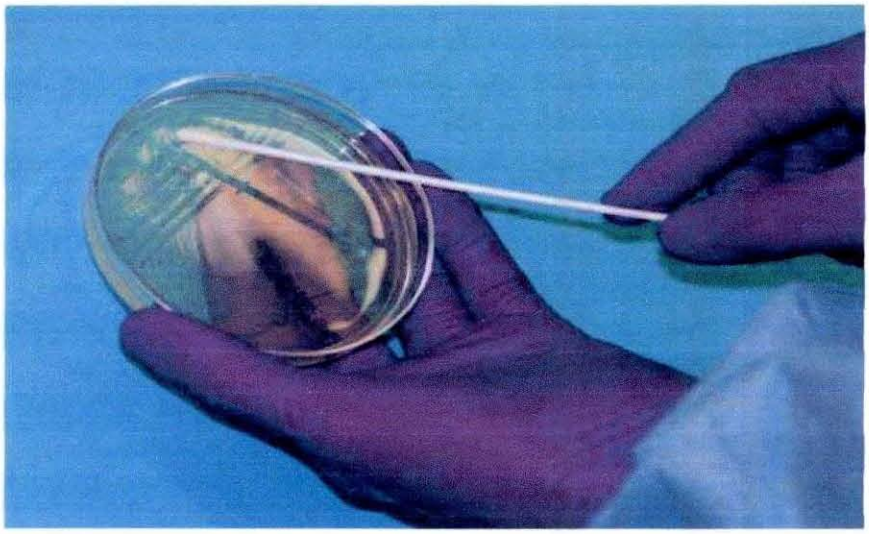


Fig 2.3: Spreading of test plate

2.2.2.9 Preparation of Discs

Three types of discs were used for antimicrobial screening.

Standard discs

These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, Kanamycin (30 µg/disc) standard disc was used as the reference.

Blank discs

These were used as negative control to ensure that the residual solvents (Left over the discs even after air-drying) and the whatman paper did not have antimicrobial activity.

Sample discs

Discs containing an antimicrobial agent are called sample discs. They are placed on the surface of an agar plate containing a medium that has been inoculated with the disease agent being tested, which will grow and fill the disc. These were used to ensure that the antimicrobial agent diffuses into the medium, killing some of the disease agent around where the antimicrobial agent was inoculated, depending on the how susceptible the disease agent is to the antimicrobial agent.

2.2.2.10 Preparation and Application of Sample Discs with Test Samples

Measured amount of each test sample was dissolved in specific volume of solvent (methanol and chloroform) to obtain the desired concentrations in an aseptic condition. Then discs were soaked with solutions of test samples and dried.

Table 2.2.5: Preparation of sample discs

Plant name	Plant part use	Sample	Dose (µg/disc)
Mikania cordata	Leaf	1.Methanol soluble fraction 2.Cloroform soluble fraction	**400**
	Flower	3. Methanol soluble fraction 4.Cloroform soluble fraction	
	Shoot	5.Methanol soluble fraction 6.Cloroform soluble fraction	
	Root	7.Methanol soluble fraction 8.Cloroform soluble fraction	
Mucuna pruriens	Shoot	9.Methanol soluble fraction 10.Cloroform soluble fraction	
	Root	11.Methanol soluble fraction 12.Cloroform soluble fraction	
	Seed	13.Methanol soluble fraction 14.Cloroform soluble fraction	
	Seed pulp	15.Methanol soluble fraction 16.Cloroform soluble fraction	

The test samples were weighed accurately and calculated amounts of the solvents were added accordingly using micropipette to the dried samples to get desired the concentrations. The test samples were applied to previously sterilized discs using adjustable micropipette under aseptic conditions.

2.2.2.11 Diffusion and Incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi. The plates were then kept in a refrigerator at 4° C for about 12 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24 hours. If plates are left at room temperature, after discs have been applied, larger zones of inhibition may be obtained compared with zones produced when plates are incubated immediately. Plates therefore should be incubated within 15 min of disc application.

2.2.2.12 Determination of the Zone of Inhibition

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

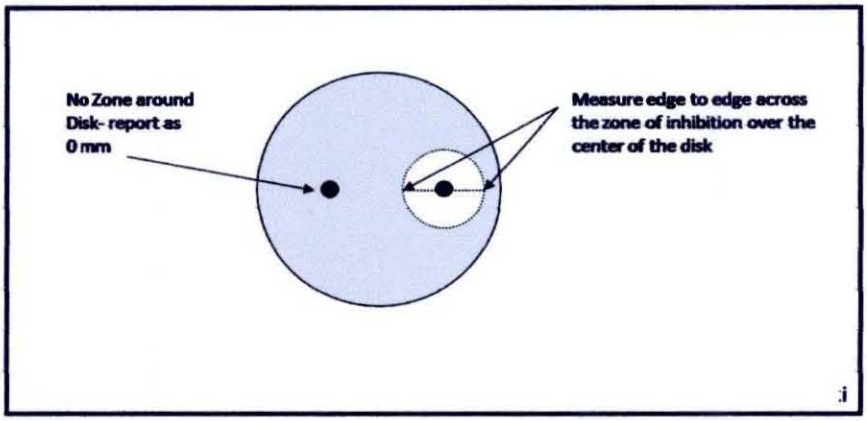
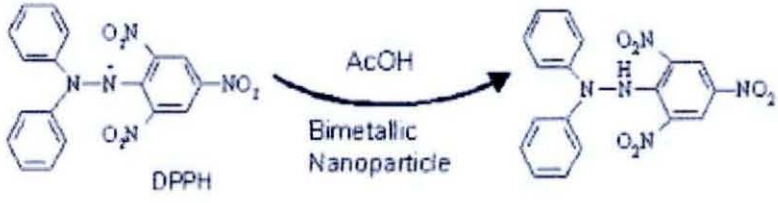


Figure 2.4: Determination of the zone of inhibition

2.3 Determination of Antioxidant Activity of Methanol Extracts

The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. With this method it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH T 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule.



2.3.1 Materials

DPPH(1,1-diphenyl-2-picrylhydrazyl)
Methanol
Ascorbic acid (Analytical or Reagent grade)
Distilled water

UV-spectrophotometer
Beaker (100 & 200 ml)
Test tube
Light-proof box
Pipette (5 ml)
Micropipette (20-200 µl)
Amber reagent bottle

2.3.2 Preparation of Reagent solution

Accurately weigh 0.004 gm of DPPH and place it into a 100 ml of a volumetric flask. Then the volume is adjusted by methanol. Then the concentration of solution is 0.004% of DPPH. Take the absorbance of this solution at 517 nm against methanol as a blank and record it as a control solution absorbance.

2.3.3 Preparation of Standard solution

Take 0.025 gm ascorbic acid and dissolved it into 5 ml of distilled water. The concentration of this solution is 5 µg/µl of ascorbic acid. This solution is called stock solution. Then prepared the experimental concentration from the stock solution by the following manner:

Concentration (µg/ml)	Solution taken from stock solution	Solution taken From others	Adjust the volume by distilled water	Final volume
500	200µl	-	1.8 ml	2.0 ml
200	80µl	-	1.92 ml	2.0 ml
100	-	1 ml(200µg/ml)	1 ml	2.0 ml
	-	1 ml(100µg/ml)	1 ml	2.0 ml
	-	1 ml(50µg/ml)	1 ml	2.0 ml
	-	200(25 µg/ml)	800 µl	1.0 ml

2.3.4 Preparation of Extract solution

Take o.o25 gm of plant extract and dissolved it into 5 ml of distilled water or methanol. The concentration of this solution is 5µg/µl of plant extract. This solution is called stock solution. Then prepared the experimental concentration from this stock solution by the following manner:

Concentration (µg/ml)	Solution taken from stock solution	Solution taken from others	Adjust the volume by distilled water	Final volume
500	200µl	-	1.8 ml	2.0 ml
200	80 µl	-	1.92ml	2.0 ml
100	-	1 ml (200µg/ml)	1 ml	2.0 ml
50	-	1 ml (100µg/ml)	1 ml	2.0 ml
25	-	1 ml (50µg/ml)	1 ml	2.0 ml
5	-	200µl (25µg/ml)	800µl	1.0 ml

2.3.5 Experimental procedure (Manzocco et al., 1998)

1. 200 µl of plant extract or standard of different concentration solution was taken in a test tube.
2. 2 ml of reagent solution was added into the test tube.
3. The test tubes were incubated for 30 minutes to complete the reaction.
4. The absorbance of the solution was measured at 515 nm using a spectrophotometer against blank.
5. A typical blank solution contained methanol.
6. The percentage (%) of inhibition activity was calculated from the following equation.

$\{(A_c - A_1)/A_c\} * 100$

Where,

A_c is the absorbance of the control, and

A_1 is the absorbance of the extract/standard.

7. Then % inhibitions were plotted against log concentration and from the graph lC50 were calculated.

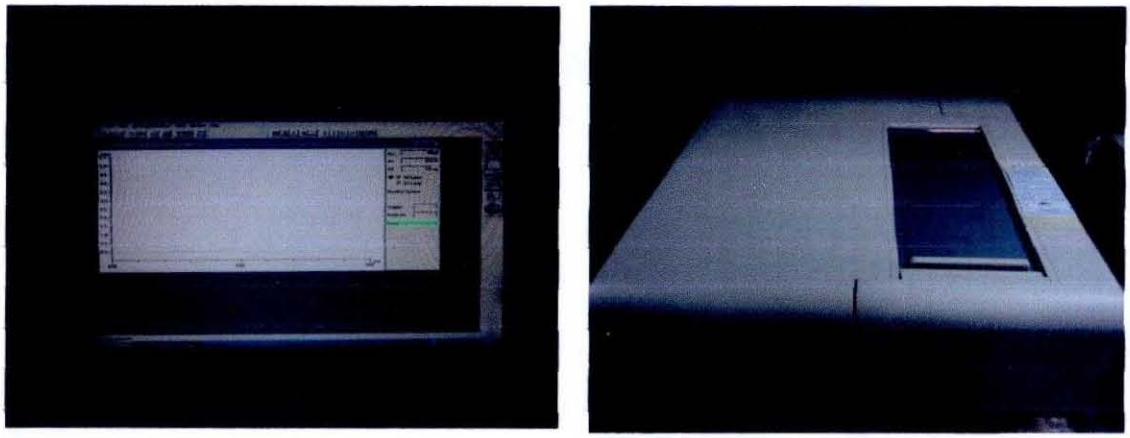


Fig 2.5 UV spectrophotometer

2.4 Assessment of Insecticidal Activity

2.4.1 Background information on the test organism (*Sitophillus oryzae*)

Sitophilus oryzae is a cosmopolitan genus of weevils found on rice. It is commonly known as rice weevil. It is a serious stored product pest which attacks several economically important crops, including wheat, rice, and maize. It has also been found on Chickpea. Notable species, the Rice weevil, *S. oryzae* and the Maize weevil (*S. zeamais*) both damage a variety of standing crops, and other stored cereals.

Identical to *S. oryzae* is preferably taking place in rice. Eggs are white and oval. The female lays the eggs inside the grain by chewing a minute hole in which each egg is deposited, followed by the sealing of the hole with a secretion. These eggs hatch into tiny grubs which stay and feed inside the grain and are responsible for most of the damage. Mature larvae are plump, legless and white, about 4 mm long. Pupation takes place inside the grain. The adult beetle emerges by biting a circular hole through outer layers of the grain. They are small brown weevils, virtually indistinguishable from each other, about 3.5-4.0 mm long with rostrum and thorax large and conspicuous. The elytra are uniformly dark brown. Each female is capable of laying 300-400 eggs, and the adults live for five to eight months and are capable fliers. The life-cycle is about five weeks at 30°C and 70%

RH; optimum conditions for development are 27-31°C and more than 60% RH; below 17°C development ceases. The developing larva lives and feeds inside the grain hollowing it out in the process. In rice (the preferred host) the entire grain is usually destroyed by the time the adult emerges. Pest status: A very serious major (primary) pest of stored rice and other cereals in the warmer parts of the world. ["*Sitophilus oryzae*". Integrated Taxonomic Information System]

2.4.2 Effect of different crude extracts on *Sitophilus oryzae*

2.4.2.1 Collection of test insects

Source of test insect's Sitophillus oryzae used in the present experiment were taken from the stock cultures of the Crop Protection and Toxicology Laboratory, University of Rajshahi, Bangladesh reared as subcultures to be used in the experimentation.

2.4.2.2 Culture of test insect *Sitophillus oryzae*

Mass cultures were maintained in plastic containers (1200 ml) and sub cultures in beakers (1000 ml) with the food medium. The beaker was kept in the food medium. The beaker was kept in an incubator at 30°C ± 0.5°C without light and contained 250 g and 150 g of food respectively. About 200 adults in each container and 100 adults in each beaker were introduced. The culture was checked in regular intervals and eggs and larvae were separate to increase properly. The container and beakers were covered with pieces of muslin cloth tightly fixed with the help of rubber bands to avoid possible escape of the beetles.

2.4.2.3 Collection of adults

A huge number of beetles were thus reared to get a regular supply of the newly formed adults. When sufficient adults produced in the subcultures, they were collected from the food medium. For this purpose some pieces of filter paper were kept inside the beaker on the food. Adults crawled upon the paper and then the paper was taken out with a forceps. Beetles were then collected in a small beaker (100 ml) with the fine Camel-hairbrush.

Fig 2.6 Sitophillus oryzae

2.4.2.4 Application of crude extracts in the surface film test

To conduct surface film activity test 60 mm petridishes were taken for all extracts and their replication. 50 mg of each extract was dissolved into 1 ml respective solvent. Then they were poured into the lower part of the petridish and allowed them to dry out. Being volatile the solvent was evaporated out within a few minutes. Then 10 insects were released in each of the treated petridish. A control experiment by applying the only solvent into the petridish was also set at the same time under the same condition.

2.4.2.5 Observation of mortality in surface film test

After completing the arrangements treated petridishes were placed in a secured place at room temperature. The whole experiment was observed from time to time and mortality was observed first 30 minutes (after starting the experiment) then 12h, 24h and 36h. The data was recorded. A simple microscope was used to check each and every beetle by tracing natural movement of its organs. In some cases hot needle was taken closer to the bodies (without movement) to conform death. Attention was also paid to recovery of the insects if occurred. The mortality records of the *Sitophillus oryzae* adults were corrected by the Abbott's formula.

P = (P – P \ 100 - P) x 100

Where

P = Corrected mortality%; P = Observed mortality%;

P = Control mortality%, sometimes called natural mortality%

CHAPTER 3

RESSULT and DISCUSSION

3.1 Results

3.1.1 Extraction and Chemical Investigation of plant material

Plant materials extracted with Soxhlet extractor are presented in table 3.1 showing % yield of the different plant extract. In this study we only investigate the phenol and flavonoid content of different parts of *Mikania cordata* and *Mucuna pruriens* in both methanol and chloroform extracts. Phenolic compounds may contribute directly to the antioxidative action. The total phenol content of different plant parts are shown Table 3.2 and total flavonoid content of the different plant extracts are showing in the table 3.3.

3.1.2 Antimicrobial Activity

For antimicrobial investigation, both methanol and chloroform extracts of leaves, flower, shoot and root of *Mikania cordata* and shoot, root, seed and Seed pulp of *Mucuna pruriens* were used. Antibacterial activity of the plant extracts was screened using the Kirby-Bauer disk diffusion method. The results of disk diffusion assay are recorded in table 3.3 and 3.4.

No zone of inhibition was observed for methanolic extracts in disk diffusion assay using different concentrations of disks against either Gram-positive or Gram-negative bacteria or fungal isolates. No inhibition was also observed for disks loaded with the solvent (negative control). However Chloroform extract of leaves and flowers of *Mikania cordata* in 400 µg/disk concentration showed strong inhibitory activity against every gram positive, gram negative and fungal isolates. The chloroform extract of shoot and roots of *Mikania cordata* showed lowest inhibitory activity against maximum Gram positive, and Gram negative and fungal isolates. kanamycin disks of 30 µg (positive control) showed strong inhibition against every Gram-positive or Gram-negative isolates. Chloroform extract of leaves of *Mikania cordata* showed highest inhibition zone against *Bacillus megaterium.*

3.1.3 Radical scavenging activity

There is a strong need for development of effective antioxidants from natural sources as alternatives to synthetic antioxidants in order to prevent the free radicals implicated

diseases which can have serious effects on the cardiovascular system, either through lipid peroxidation or vasoconstriction (Ames *et al.*, 1993)

In the present study, the antioxidant activity of the methanol extracts of leaves, flower, shoot and root of *Mikania cordata* and shoot, root, seed and seed pulp of *Mucuna pruriens* was investigated using the DPPH scavenging assay. All these have proven the effectiveness of the methanol extract of *Mikania cordata* and *Mucuna pruriens* compared with the reference standard antioxidant ascorbic acid. The methanol extracts of roots and shoots of *Mikania cordata* showed a highly effective free radical scavenging activity with IC50 value 28.18 and 19.95 in the DPPH assay. These extracts exhibited a noticeable antioxidant effect at the concentrations of 200 µg/ml (89.8, 85 and 79.8% respectively) (Table 3.6). Comparisons of different extracts with the standards (Ascorbic Acid) have been shown in the fig 3.2 and fig 3.3. The other methanol extracts of these two medicinal plants demonstrated moderate free radical scavenging activity with diminutive antioxidant activity at lower concentration and high antioxidant activity at increasing concentration.

3.1.4 Insecticidal activity

Insecticidal activity of methanol and chloroform extracts was performed by in vitro surface film test assay against the insect, *Sitophillus oryzae* (Rice weevil). In this study we found shoot and root methanol extracts of *Mikania cordata*, and shoot and seed pulp methanol extracts of *Mucuna pruriens* and chloroform extract of seed of *Mucuna pruriens* have shown 100% mortality rate of *Sitophillus oryzae* at the dose of 50 mg/ml in 12 hours. On the other hand chloroform extracts of seed *Mucuna pruriens* have shown 50% mortality rate of *Sitophillus oryzae* at the dose of 50 mg/ml in 30 min. Since 6 extracts have strong insecticidal activity, dose dependent activity was done, where 4 graded doses (viz, 40, 20, 10 and 5µg/ml) were used .The percentage of mortality of all extract were shown in the table 3.6. At the same dose with no activity for chloroform extract of *M. cordata* flower and *M. pruriens* shoot, seed pulp. We use methanol and chloroform solvent as control and no mortality happened in control.

Table 3.1 Percent yield of extracts of different plant parts in methanol and chloroform

Mikania cordata	Asteraceae	Leaves	Methanol	**16.7**
			Choloroform	**6.8**
		Flower	Methanol	**12.6**
			Choloroform	**3.8**
		Shoot	Methanol	**14.6**
			Choloroform	**6.4**
		Root	Methanol	**8.8**
			Choloroform	**3.5**
Mucuna pruriens	Fabaceae	Shoot	Methanol	**8.3**
			Choloroform	**2.6**
		Root	Methanol	**7.8**
			Choloroform	**2**
		Seed	Methanol	**10.6**
			Choloroform	**5.7**
		Seed pulp	Methanol	**13.9**
			Choloroform	**5.4**

Table 3.2 Total phenol and flavonoid content of investigated plants' extracts

Investigated Element	**Extract**	**Concentration**	**Phenol content mg/g Gallic acid equivalent**	**Flavonoid content mg/g Quarcetin equivalent**
M. cordata (Leaf)	methanol	200µg/ml	872.94 ± 4.85	692.40 ± 6.40
	choloroform		74.918 ± 4.64	688.00 ± 14.78
M. cordata (Flower)	methanol		628.41 ± 4.43	893.39 ± 10.36
	choloroform		468.41 ± 4.08	600.66 ± 11.89
M. cordata (Shoot)	methanol		438.22 ± 9.98	471.20 ± 7.49
	choloroform		111.81 ± 2.97	913.39 ± 12.38
M. cordata (Root)	methanol		274.07 ± 8.20	422.48 ± 4.81
	choloroform		170.05 ± 5.94	824.84 ± 10.85
M. pruriens (Shoot)	methanol		172.18 ± 10.24	460.66 ± 4.36
	choloroform		326.27 ± 12.58	1644.90 ± 11.63
M. pruriens (Root)	methanol		398.98 ± 10.20	598.24 ± 5.74
	choloroform		335.00 ± 10.99	686.72± 12.61
M. pruriens (Seed)	methanol		133.320 ± 20.35	515.52 ± 5.96
	choloroform		42.00± 1.64	698.84 ± 7.10
M. pruriens (Seed pulp)	methanol		462.75± 19.96	543.09 ± 7.32
	choloroform		157.93 ± 8.50	539.454 ± 14.86

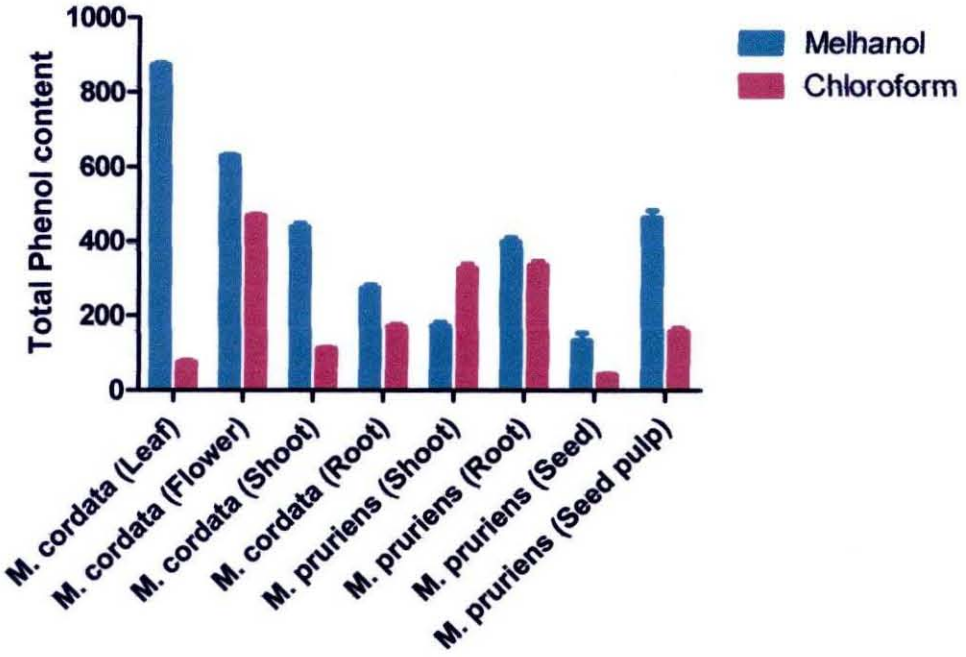


Fig 3.1 Total Phenol content of investigated plants

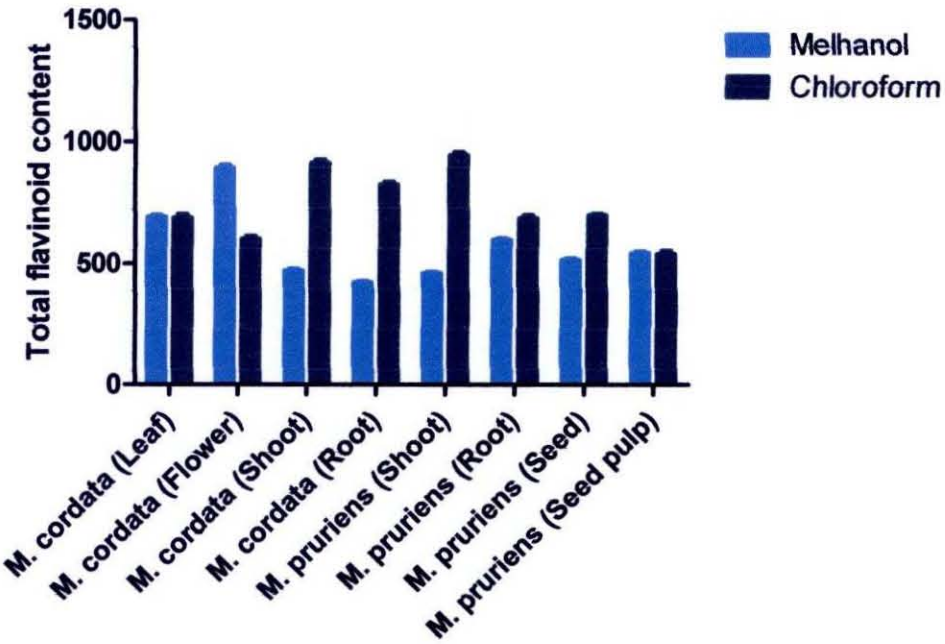


Fig 3.2 Total Flavonoid content of investigated plants

Mikania cordata (Flower)

Mikania cordata (Leaves and shoot)

Mucuna pruriens (fruites)

Mucuna pruriens (Shoot)

Figure 3.3 Pictures of the collected plants part

Table3.3: Antimicrobial activity of the crude extracts of *Micania Cordata*

Test microorganisms	Diameter of the zone of inhibition (mm)								Kanamycin (30µg/disc) mm
	Methanol extract (400µg/disc)mm				Chloroform extract (400 µg/disc)mm				
	leaf	flower	Shoot	root	leaf	flower	Shoot	root	
Gram Positive									
Bacillus cereus	--	--	--	--	17	14	07	07	24
Bacillus megaterium	--	--	--	--	18	12	08	--	24
Bacillus subtilis	--	--	--	--	17	15	08	07	22
Staphylococcus aureus	--	--	--	--	16	13	08	--	24
Sarcina lutea	--	--	--	--	17	12	07	--	24
Gram Negative									
Pseudomonas aureus	--	--	--	--	17	13	08	07	
Escherichia coli	--	--	--	--	16	12	07	07	25
Salmonella paratyphi	--	--	--	--	15	14	09	--	24
Salmonella typhi	--	--	--	--	14	13	08	07	22
Shigella boydii	--	--	--	--	16	14	08	--	23
Shigelladysenteriae	--	--	--	--	15	7	--	--	24
Vibrio mimicus	--	--	--	--	15	13	08	07	21
Vibrio parahemolyticus	--	--	--	--	15	13	--	--	20
Fungi									
Candida albicans	--	--	--	--	12	10	--	--	25
Aspergillus niger	--	--	--	--	16	14	--	--	23
Saccharomyces cerevacae	--	--	--	--	14	12	07	--	25

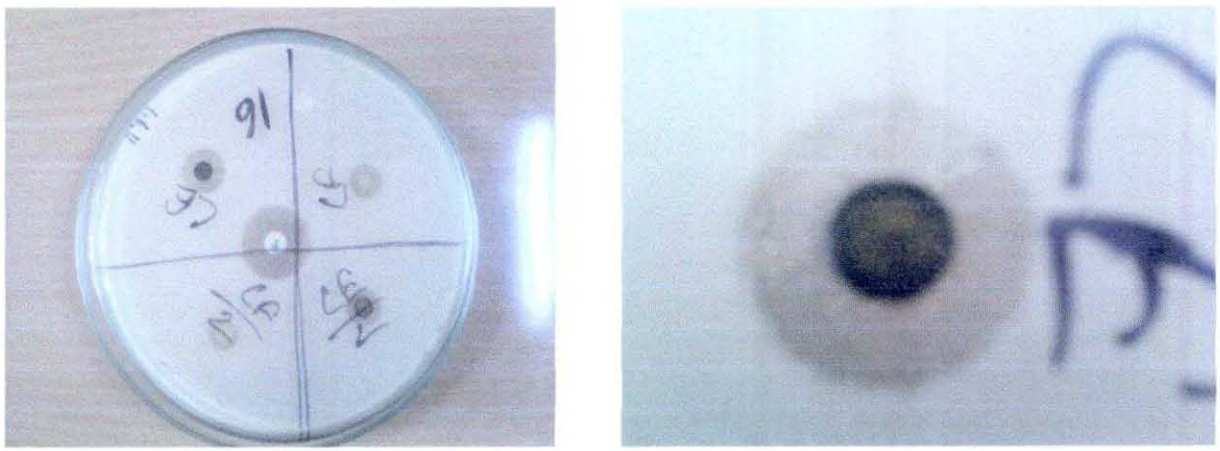


Fig 3.3.1 Zone of inhibition produced by chloroform extract of Mikania cordata

Fig 3.3.2 Zone of inhibition produced by chloroform extract of Mikania cordata on 16 clinical isolets.

Table 3.4: Antimicrobial activity of the crude extracts of *Mucuna pruriens*

Test microorganisms	Diameter of the zone of inhibition (mm)								Kanamycin (30µg/disc) mm
	Methanolicextract(400µg/disc)mm				Cloroform extract (400 µg/disc)mm				
	Shoot	Root	Seed	Seed pulp	Shoot	Root	Seed	Seed pulp	
Gram Positive									
Bacillus cereus	--	--	--	--	--	--	--	--	34
Bacillus megaterium	--	--	--	--	--	--	--	--	35
Bacillus subtilis	--	--	--	--	--	--	--	--	26
Staphylococcus aureus	--	--	--	--	--	--	--	--	35
Sarcina lutea	--	--	--	--	--	--	--	--	34
Gram Negative									
Pseudomonas aureus	--	--	--	--	--	--	--	--	33
Escherichia coli	--	--	--	--	--	--	--	--	33
Salmonella paratyphi	--	--	--	--	--	--	--	--	30
Salmonella typhi	--	--	--	--	--	--	--	--	27
Shigella boydii	--	--	--	--	--	--	--	--	33
Shigelladysenteriae	--	--	--	--	--	--	--	--	28
Vibrio mimicus	--	--	--	--	--	--	--	--	27
Vibrio parahemolyticus	--	--	--	--	--	--	--	--	33
Fungi									
Candida albicans	--	--	--	--	--	--	--	--	33
Aspergillus niger	--	--	--	--	--	--	--	--	33
Saccharomyces cerevacae	--	--	--	--	--	--	--	--	33

Table 3.5. Free radical scavenging activity of methanol extracts of investigated plants

Investigated Element	%Redical scavenging activity at different concentrations				IC50 µg/ml
	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	
***M. cordata* (Leafe)**	41.7	45.8	54	64.96	**63.09**
***M.cordata* (Flower)**	31.8	35.2	42.6	47	**251.18**
***M. cordata* (Shoot)**	54	67	80	85	**19.95**
***M. cordata* (Root)**	35	54.8	78.9	89.8	**28.18**
***M. pruriens* (Shoot)**	24	54.8	44	79.2	**39.81**
***M. pruriens* (Root)**	44	46.7	54	55	**79.43**
***M. pruriens* (Seed)**	40	42	43	54	**58.48**
***M. pruriens* (Seed pulp)**	11	13	33	56.8	**199.95**
Ascorbic acid (standard)	**88.4**	**89**	**89.2**	**89.6**	**12.58**

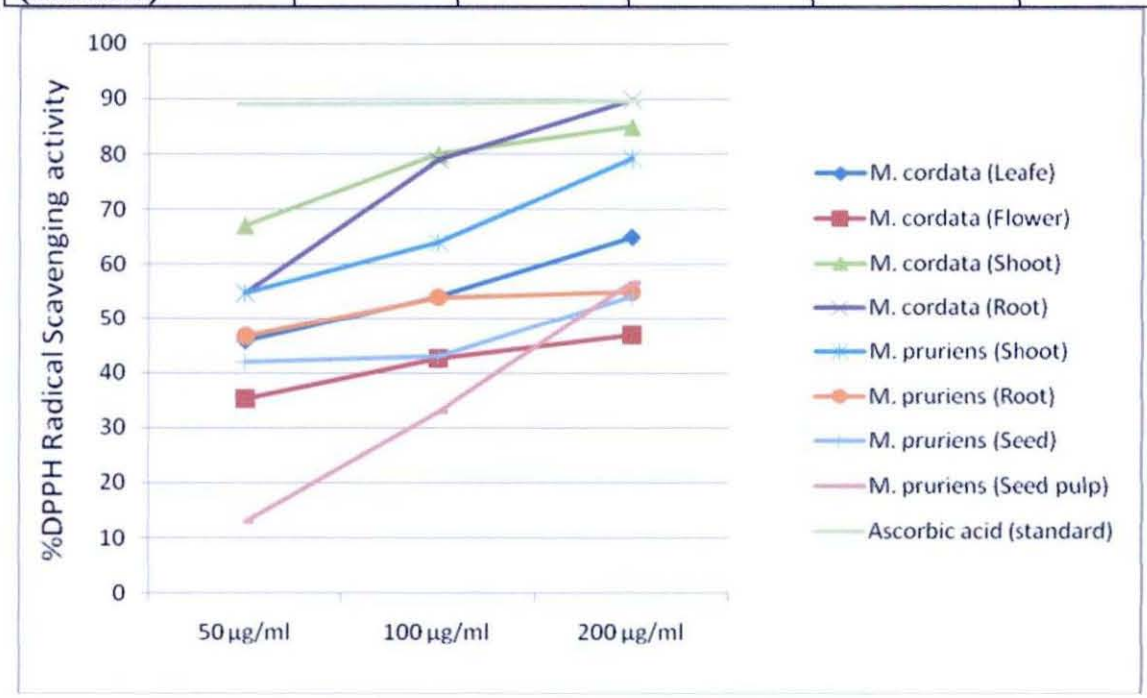
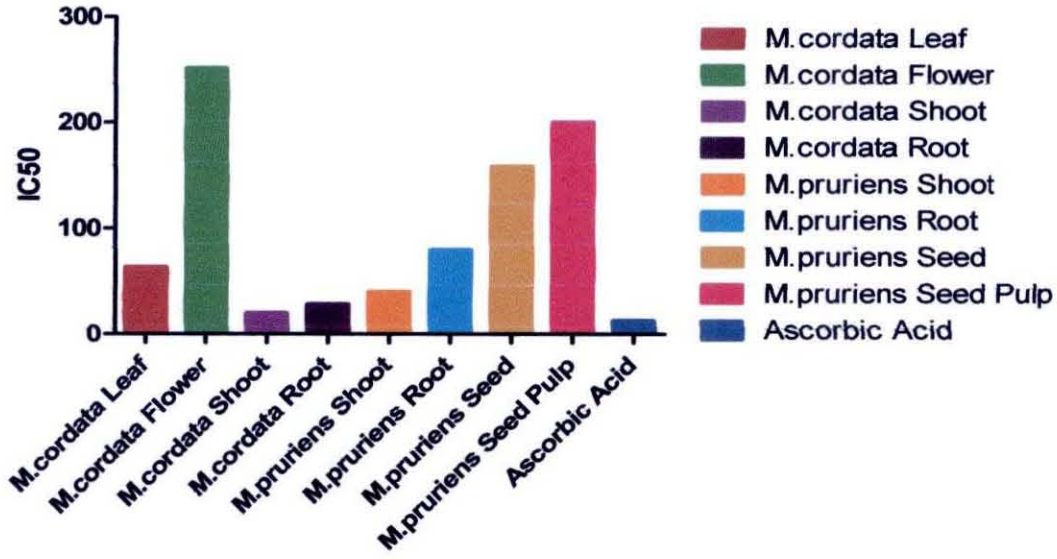


Figure 3.4.1 Free radical scavenging activity of methanol extracts of investigated plants



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ig 3.4.2 IC50 value of methanolic extract of investigated plants

Table 3.6 Insecticidal activity of both methanol and choloroform extracts of investigated plants

Investigated Element	Solvent	Concentration	No.of insect used	No. of insect killed				Mortality (%)
				30 min	12 hour	24 hour	36 hour	
M. cordata (Leaf)	Methanol	50 mg	10	1	3	6	7	70%
	Choloroform		10	1	-	-	-	10%
M. cordata (Flower)	Methanol		10	3	5	10	-	100%
	Choloroform		10	-	-	-	-	No activity
M. cordata (Shoot)	Methanol		10	3	10	-	-	100%
	Choloroform		10	2	2	2	3	30%
M. cordata (Root)	Methanol		10	2	9	10	-	100%
	Choloroform		10	-	-	-	-	No activity
M. pruriens (Shoot)	Methanol		10	1	10	-	-	100%
	Choloroform		10	-	-	-	-	No activity
M. pruriens (Root)	Methanol		10	-	1	1	3	30%
	Choloroform		10	-	-	1	2	20%
M. pruriens (Seed)	Methanol		10	-	3	5	5	50%
	Choloroform		10	5	10	-	-	100%
M. pruriens (Seed pulp)	Methanol		10	4	10	-	-	100%
	Choloroform		10	-	-	-	-	No activity
Control	Methanol		10	-	-	-	-	No activity
	Choloroform		10	-	-	-	-	No activity

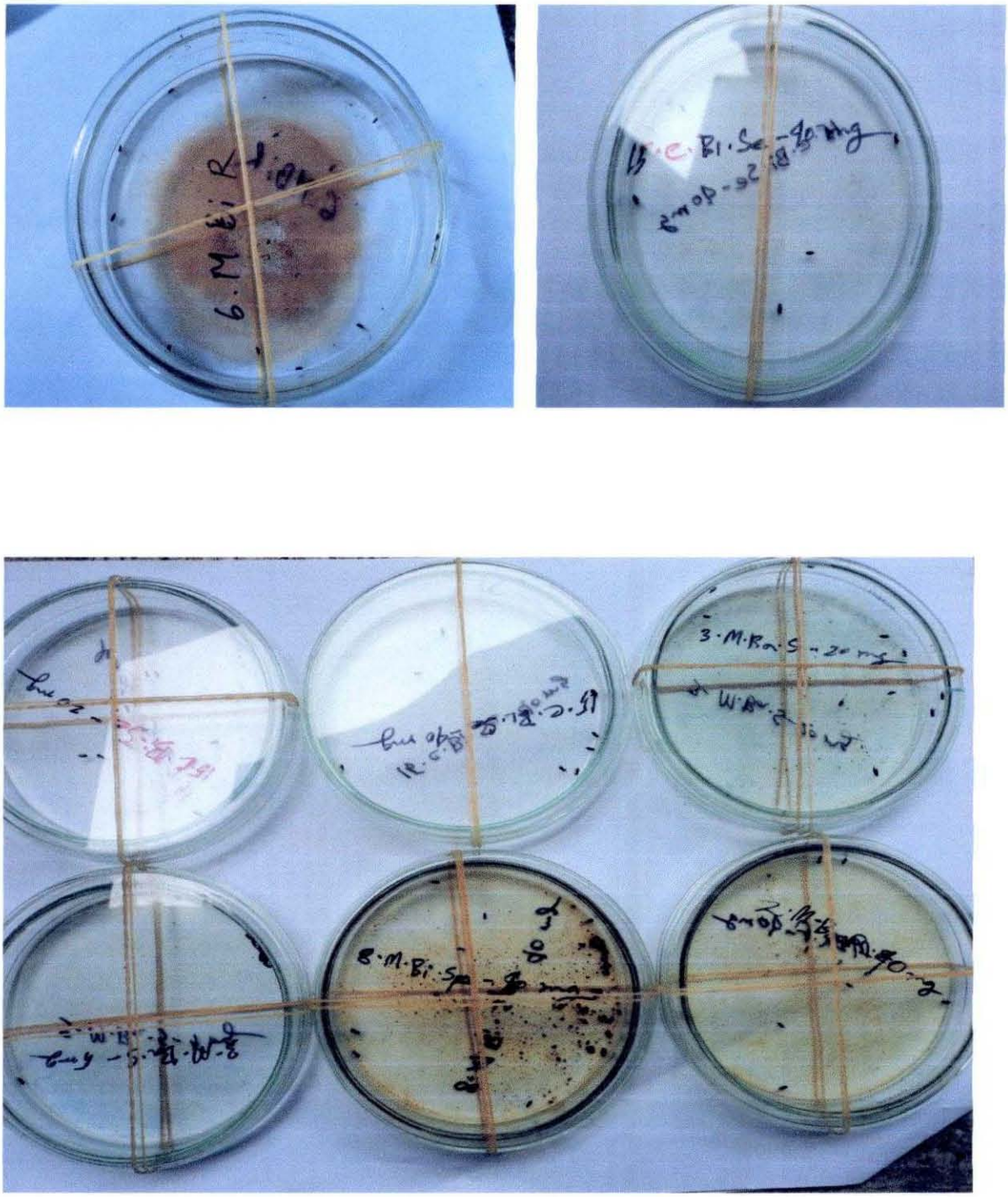

Fig 3.5 Insecticidal activity of both methanol and choloroform extract of investigated plants

3.2 Discussion

Finding healing powers in plants is an ancient phenomenon. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory [Cowan, 1999]. A survey by UNCTAD has shown that 33% of total drugs produced by the industrialized nations are plant and microbes derived and 60% of medicinal products are of natural origin [International Trade Centre, 1974]. It is estimated that are 10-100 million species of organisms living on earth and higher plants forming a group of some 250,000 species, out of which only 6% has been investigated for biological activities and 15% for their chemical constituents. Thus it appears that we have only scratched the surface of this world's wonderful resource. Researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against cancer, as well as viral and microbial infections. Presently, there has been an amplified interest worldwide to identify antioxidant compounds which are pharmacologically effective and have low or no side effects for use in preventive medicine and the food industry [Sati et *al.*, 2010].

In continuance of the search for plants with pharmacological effects, this study has screened four plants collected from Bogra and Dhaka, Bangladesh for antibacterial and antioxidant activities as well as their potential insecticidal activity. Identification of the investigated plants was done at the Bangladesh National Herbarium, Dhaka, Bangladesh (Bushra Khan and Ahsan Habib 2011, personal communication). The existing knowledge about the investigated plants is in many cases is very limited.

Selected plant parts were extracted with methanol and chloroform in Soxhlet apparatus. The highest yield for methanol extract was 14.8% and the highest yield for chloroform extract was 5.6%.

Mikania cordata of Asteraceae family is one of the most common and widely distributed species. Ethnomedicinally, the genus is important and popularly known as Asamlata, Bonnolatain, It is widely used as traditional medicine against gastric ulcer and a variety of other diseases. In southern Nigeria a decoction is given for coughs, and the leaf-juice is

a remedy for sore eyes. In Portuguese East Africa, the Tongas use the plant as a remedy for snake and scorpion bite. An infusion of the plant is given in infection of the stomach and intestines. In Malaysia the leaves are used for rubbing on the body against itches. In Java they are used for poulticing the wound of circumcision and other wounds. In India, leaves are used for itchiness and as wound plaster. In Bangladesh, decoction is used for treatment of gastric ulcer. In southern Africa, leaves are applied to wounds. In current study, the antibacterial activity of choloroform extract of *Mikania cordata* was demonstrated against selected Gram positive, Gram negative bacteria and fungal isolates used for the evaluation. No antimicrobial activity of methanol extract of *Mikania cordata* was observed against any of the Gram positive, Gram negative bacteria or fungal isolates. The leaves and flower extract of *Mikania cordata* showed large inhibitory zone against all microorganisms However, methanol extract of *Mikania cordata* has previously demonstrated inhibition against four Gram positive and six Gram negative bacteria and one fungal isolate. The effect was claimed to be due to the presence of flavonoid. In this study we also determined the flavonoid content of this plant extract. In the fig 3.2 we see flavonoid content of choloroform extract of Mikania cordata is higher than methanol extract of this plant. The flavonoid content of this choloroform leaves extract is 688 mg/g more than 60%.

All parts of *Mikania cordata* methanolic extract showed antioxidant activity even at a very low concentration (25µg/ml). The methanolic extracts of shoot and root of *Mikania cordata* showed strong radicals scavenging activity (85 and 89.8% respectively) at the concentration of 200 µg/ml. The activity was concluded due to the presence of phenol. The phenol content of this plant extract is shown in table 3.2 and fig 3.1. Our study also confirms Insecticidal activity of *Mikania cordata* against *Sitophilus* oryzae insect. The insecticidal effect observed in the present study is interesting as there is no report in literature about insecticidal evaluation of *Mikania cordata.*

The plant *Mucuna pruriens* an annual, climbing shrub with long vines that can reach over 15 m in length, is a common shrub found throughout the Bangladesh. When the plant is young, it is almost completely covered with fuzzy hairs, but when older, it is almost

completely free of hairs. The leaves are tripinnate, ovate, reverse ovate, rhombus shaped or widely ovate. The sides of the leaves are often heavily grooved and the tips are pointy. In young *M. pruriens* plants, both sides of the leaves have hairs. The stems of the leaflets are two to three millimeters long. Additional adjacent leaves are present and are about 5 mm long. Traditionally, *M. pruriens* has been used as an effective aphrodisiac [4] It is still used to increase libido in both men and women due to its dopamine inducing properties and in Ayurvedic medicine it is said to increase sperm count. Dopamine has a profound influence on sexual function. Use of *Mucuna pruriens* is well documented in Siddha medicine for a host of uses [Yerra Rajeshwar *et al.*, 2005]. *M.pruriens* seeds have also been found to have antidepressant properties in cases of depressive neurosis when consumed. Our study was unable to demonstrate the presence of any antibacterial activity in *Mucuna pruriens* against the screened organism. Although, previous records showed antibacterial activity of *Mucuna pruriens* extracts against Gram positive or Gram negative microorganism [Yerra Rajeshwar *et al.*, 2005]. *Mucuna pruriens* shoot methanolic extract displayed progressive antioxidant function as it was previously reported. But other parts root, seed, seed pulp of this plant show moderate antioxidant function. *Mucuna pruriens* also expressed insecticidal activity against *Sitophilus* oryzae insect. The strongest insecticidal activity (50% mortality after 30 min) was found in choloroform extract of seed of *Mucuna pruriens*. Also there is no previous record on insecticidal evaluation of *Mucuna pruriens* was found.

In summary, antibacterial and antioxidant potentials of two selected medicinal plants were investigated in the present study. To explore potential use of the medicinal plants as insecticide, insecticidal activity of the plants was also investigated. Although, contrary to previous investigations, no antibacterial activity was observed in the methanol extract of the investigated plants but the chloroform extracts of *Mikania cordata* showed significant zone of inhibition on the selected experimented isolates. On the other hand, methanol extracts of investigated plants demonstrate effective antioxidant action and warrant for further study to identify and isolate the compounds responsible for free radical scavenging activity.

An interesting observation made in the present study was the insecticidal activities of the methanol extracts of the two selected plants. Thus the methanol extracts of different parts of *M. cordata* showed 70-100% mortality of the insect, *Sitophilus oryzae,* whereas no significant mortality was observed with chloroform extract of this plant. In case of the other plant i.e. *M. pruriens, methanol extracts showed 30-100% mortality of the insect Sitphil;us oryzae* depending on the different parts of the plant while chloroform extract of the seed of this plant showed 100% mortality of the same insect. The observation is interesting as it indicates the potential of the plant extracts to act as insectides and possibility of developing appropriate insect control strategy using the indigenous plant extracts.

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