Evaluation of Antibacterial, Antioxidant and Toxicological Activity of Crude Extracts of *Adiantum capillus-veneris*, *Blumea lacera*, *Cassia alata*, and *Cissus quadrangularis* from Faridpur, Bangladesh

A dissertation submitted to the faculty of
BRAC University
in partial fulfillment of the requirements for the degree of

Masters in Biotechnology

Submitted by:
Registration No. 09276001

Submitted to:
Department of Mathematics and Natural Sciences
BRAC University
June 2011

APPROVAL CERTIFICATE

This thesis entitled **"Evaluation of Antibacterial, Antioxidant and Toxicological Activity of Crude Extracts of *Adiantum capillus-veneris*, *Blumea lacera*, *Cassia alata*, and *Cissus quadrangularis* from Faridpur, Bangladesh"** is submitted by Luke Donald Halder in partial fulfillment of the requirements for the degree of Master of Science in Biotechnology, Department of Mathematics and Natural Sciences, BRAC University, Dhaka, the work was performed at Laboratories of BRAC University, Dhaka university and The University of Asia Pacific, Dhaka, Bangladesh.

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To my dear parents

ACKNOWLEDGEMENTS

The author obtained help from many individuals and institutions during conducting and completing this research work and as such he wishes to acknowledge them with gratitude and appreciation. I express my gratitude to the BRAC University, University of Dhaka and University of Asia Pacific, Dhaka, Bangladesh for funding this research project.

I would like to express my sincere thanks to my supervisor Dr. Naiyyum Choudhury for providing me with opportunity to work on this project. His support, guidance, encouragement, and patience throughout the project are very much appreciated. I would like to extend my appreciation to my supervisor Dr. Chowdhury Rafiqul Ahsan for helping me understand the logic behind the research project, providing me with the necessary resources, and helping to develop skills required to successful completion of the project.

I also appreciate Assistant Professor Mohammad Shahriar's help in the research and allowing me to use his laboratory facilities. Finally, I would like to express all my love and gratitude to my beloved family for their support, understanding and encouragement throughout my life, especially my dear parents.

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ABSTRACT

Antibacterial and antioxidant potentials of *Adiantum capillus-veneris*, *Blumea lacera*, *Cassia alata*, and *Cissus quadrangularis* from Faridpur, Bangladesh was investigated in this study along with their toxicological evaluation. Plant materials were extracted with organic solvents (chloroform and methanol) using Soxhlet extractor. Inhibitory action of methanol and chloroform extracts of selected plants against three Gram-positive clinical isolates — *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and three Gram-negative clinical isolates — *Escherichia coli, Vibrio cholerae, Salmonella typhi* was studied using agar disk diffusion, agar well diffusion methods. Broth microdilution method was utilized against *Escherichia coli 0157:H7* strain and clinical isolate of *Staphylococcus aureus* to define the minimum inhibitory concentration of methanol extracts of selected plants. Antioxidant potential of methanol extracts of selected plant was studied using DPPH free radical scavenging assay. Morphological study of methanol extract treated distal kidney (MDCK) cell line revealed the toxicological susceptibility. Investigated plants showed no antibacterial activity against the tested clinical isolates. Significant antioxidant activity was demonstrated in the methanol extracts of investigated plants. Only *Cissus quadrangularis* was devoid of toxic action in the cytotoxicity assay against MDCK cell line.

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
MHB	Mueller hinton broth
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 [1].

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-microbials, anti-inflammatories, immunostimulants, anti-diarrheals, digestive aids, and fertility regulators [2-4]. 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 [5].

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 [6-8]. In a recent review article Merlin [9] evaluates 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 [10] 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 [10, 11]. 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 [12]. Oakes and Gahlin [11] 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 [13, 14].

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 [12, 15]. About 1000 years ago healers in the Aztec and Maya Indian cultures of Mexico and Central America were experimenting with natural curing substances. Evans [16] noted that Post-Classic Mesoamericans developed a large and effective pharmacopoeia, formulae for medicines concocted from animals, minerals, and especially plants. According to Berdan [17], 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 Systema Naturae established the framework for modern biological taxonomy, and his famous works *Genera Botanica* and *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 [18-21].

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 [22]. 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 [23]. 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 [24]. 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 [25].

1.2.1 The Search of New Drugs through Ethnomedicine

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 [26] 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 [27]. 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 [28].

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 [29]. 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 [24].

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 [30, 31]. 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 [32].

1.2.3 On the Verge of Extinction

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 [33, 34]. 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 [35]. The scientific discipline known as ethnobotany (or ethnopharmacology), and animal products they have used to maintain health [36-39], whose goal is to utilize the impressive array of knowledge assembled by indigenous peoples about the plant [40].

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 [34] and as many as 15,000 medicinal plants under threat [41]. 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 [42].

1.2.4 Challenges in Drug Discovery from Medicinal Plants

Despite the evident successes of drug discovery from medicinal plants, future endeavors face 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 [43]. The process of drug discovery has been estimated to take an average of 10 years upwards [44] and cost more than 800 million dollars [45]. 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 [46].

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 [25]. 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 [47]: 50 kg of raw material are necessary to provide 500 mg of pure compound for bioassays, toxicology, and "*in vivo*" evaluation; full pre-clinical and clinical studies can require 2 kg of pure compounds obtained from 200 ton of raw material [32].

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 declining [46].

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 [48-50]. 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 [51]. 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 level. 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 [52].

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 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 exportation 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 tonnes [52].

1.2.6 Potentials of Medicinal Plants from 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 grow in its forests, jungles, wastelands and in the roadsides. It is not surprising therefore those 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 [53]. 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 to the scientific world many useful remedies 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 [54].

It is estimated that some 12,500 tonnes of dried medicinal plant material produced in Bangladesh is sold. 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 who are serviced by 6,000 to 10,000 local collectors, pikers and growers. In total there are said to be around 200 Unani and 200 Ayuverdic registered factories, plus some 70 homeopathic factories. Collectively they will employ 2,000 to 4,000 people. In addition, there are said to be 5,000 qualified and 80,000 unqualified herbal practitioners in the country [55].

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 [56]. That being said, 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 [57].

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 [58]. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total [59]. 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.

derived compounds which are in the highest oxidation state. The common herbs tarragon and thyme both contain caffeic acid, which is effective against viruses [60], bacteria [61, 62], and fungi [63]. Catechol and pyrogallol both are hydroxylated phenols, shown to be toxic to microorganisms. Catechol has two 2OH 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 [58]. In addition, some authors have found that more highly oxidized phenols are more inhibitory [64, 65].

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 [66], 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 [40].

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 [67]. 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 [68], 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 [40].

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 [69].

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 [66, 69].

1.3.1.5 Coumarins

Coumarins are phenolic substances made of fused benzene and a-pyrone rings [70]. Their fame has come mainly from their antithrombotic [71], anti-inflammatory [72], and vasodilatory [73] 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 [74], 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 [75-84], fungi [84-90], viruses [91-95], and protozoa [96, 97]. In 1977, it was reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria [98]. 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 [99], are commonly found to have antimicrobial properties [100]. Solamargine, a glycoalkaloid from the berries of *Solanum khasianum*, and other alkaloids may be useful against HIV infection [101, 102] as well as intestinal infections associated with AIDS [103].

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 [104]. Their mechanism of action may be the

formation of ion channels in the microbial membrane [104, 105] or competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors [106]. 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 [107].

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 [108]. 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 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 [109].

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 [110-112]. 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 [113]. 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 [114]. 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 Gram-negative pathogens [115].

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 [116]. 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 [117-121] and health problems [122, 123]. A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases [124], including cardiovascular diseases, cancers [125], neurodegenerative diseases, Alzheimer's disease [126] and inflammatory diseases [127]. One solution to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources [128]. 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 [129].

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 [130, 131]. 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 [132].

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 [133].

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 [134]. 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 [135]. 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 [136].

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 [137]. 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 takes their name from the most well-known member of this group, carotene, found in carrot and other orange colored fruits and vegetables [138]. 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 [139]. 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 [140]. 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 [141]. 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 [133].

1.5 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 [142, 143]. 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

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 [142-144]. 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 [145]. 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 [146, 147]. 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 [148].

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

1.5.1 *In vitro* Assessment of General Toxicity

Traditionally, animal testing has always played an important role in the safety evaluation of such agents. However, financial and ethical considerations, together with an increased

awareness of the limitations of animal models in relation to human metabolism, now warrant the development of alternative testing methods. Therefore, it is fitting that the potential of biotechnology should provide mammalian cell systems for *in vitro* testing. The ultimate aim of *in vitro* toxicity testing is the replacement of animals in testing protocols, but in the short term, procedures are refined to reduce the numbers of animals required. This "three Rs" philosophy of reduction, refinement, and replacement was first proposed by Russell and Burch as early as 1959 [149], and is now recognized in the UK Animals in Scientific Procedures Act, 1986 and EC Directive 86/609/ECC [150].

Cytotoxicity is considered primarily as the potential of a compound to induce cell death. Most *in vitro* cytotoxicity tests measure necrosis. Furthermore, detailed studies on dose and time dependence of toxic effects to cells, together with the observation of effects on the cell cycle and their reversibility, can provide valuable information about mechanisms and type of toxicity, including necrosis, apoptosis or other events. *In vitro* cytotoxicity tests are useful and necessary to define basal cytotoxicity, for example the intrinsic ability of a compound to cause cell death as a consequence of damage to basic cellular functions. Cytotoxicity tests are also necessary to define the concentration range for further and more detailed *in vitro* testing to provide meaningful information on parameters such as genotoxicity, induction of mutations or programmed cell death [151].

Over the last two decades there has been considerable interest in using basal cytotoxicity data to predict the acute effects of compounds *in vivo*. If a compound is acutely toxic, it is anticipated that, in most cases, this reflects an insult to the intrinsic functions of cells. This approach has been successfully applied in a validated *in vitro* method to assess phototoxicity [152], based on ATP-dependent neutral red uptake into lysosomes [153]. In a large study of a diverse range of chemicals, a reasonably good correlation was found between basal cytotoxicity and acute toxicity in animals and humans. Kinetic factors and target organ specificity of the toxic effect are major parameters compromising the correlation. For this reason basal cytotoxicity should be considered as a starting point in an integrated assessment of potential *in vivo* toxicity of food chemicals [151].

1.6 Profiles for Investigated Medicinal Plants

1.6.1 *Adiantum capillus-veneris*

Table 1.1 Taxonomic Hierarchy of *Adiantum capillus-veneris*

Kingdom	Plantae -- Planta, plantes, plants, Vegetal
Subkingdom	Tracheobionta -- vascular plants
Division	Pteridophyta
Class	Filicopsida
Order	Polypodiales
Family	Pteridaceae
Genus	*Adiantum* L. -- maidenhair fern
Species	*Adiantum capillus-veneris* L. -- common maidenhair, common maidenhair fern, venus hairfern

1.6.1.1 Description

A tufted fern. Stipes wiry, suberect, 10-23 cm long, polish, blackish; fronds bipinnate, with a short terminal pinna and numerous erect-patent lateral ones on each side, the lowest slightly branched again; segments 1.25-2.5 cm broad, the base cuneate, the outer edge rounded, deeply lobed; sori roundish or obreniform, placed in the roundish sinuses of the crenations [154].

1.6.1.2 Distribution

Adiantum capillus-veneris is native to the southern half of the United States from California to the Atlantic coast, through Mexico and Central America, to South America. It is also native to Eurasia, the Levant in Western Asia, and Australasia.

It is found in temperate climates from warm-temperate to tropical, where the moisture content is high but not saturating, in the moist, well-drained sand, loam or limestone many habitats, including rainforests, shrub and woodlands, broadleaf and coniferous forests, and desert cliff seeps, and springs. It will often be seen growing on sheltered and shaded south-facing limestone formations, kept moist by moving water [155].

1.6.1.3 Uses

In the Peruvian Amazon, local people prepare the fronds of the plant as an infusion or syrup and use it as a diuretic, as an expectorant and to calm coughs, to promote perspiration and

menstruation, and to treat urinary disorders, colds, rheumatism, heartburn, gallstones, alopecia (hair loss), and sour stomach. In the highlands of the Peruvian Andes, local shamans and healers decoct the rhizome and use it for alopecia, gallstones, and jaundice. In the Brazilian Amazon, it is recommended as a good expectorant and used for bronchitis, coughs, and other respiratory problems.

Adiantum capillus-veneris has long held a place in herbal medicine systems worldwide. In European herbal medicine, its documented use predates the era of Dioscorides and Pliny (23-79 A.D.). Culpepper (1787 ed.) said, "This and all other Maiden Hair Ferns is a good remedy for coughs, asthmas, pleurisy, etc., and on account of its being a gentle diuretic also in jaundice, gravel and other impurities of the kidneys." In France, the fronds and rhizomes were once made into a syrup called "Sirop de Capillaire," which was a favorite medicine for upper respiratory problems such as coughs and excessive mucus. The plant is also used widely throughout the world for dandruff, hair loss, and menstrual difficulties.

In Brazilian herbal medicine today, the frond and leaf are employed for hair loss, coughs, bronchitis, laryngitis and throat dryness, and to improve appetite and digestion, stimulate renal function, regulate menstruation, and facilitate childbirth. In Peruvian herbal medicine, the frond and rhizome are used for hair loss, gallstones, hepatic calculi, hydrophobia, asthma, coughs, catarrh, and to regulate menstruation. In India, the entire plant is used for its cooling effects, for diabetes, colds, bronchial disease, and for its menstrual promoting properties. Externally it is used for boils, eczema, and wounds [155].

1.6.1.4 Chemical Constituents

Chemical analysis of *Adiantum capillus-veneris* reveals an array of compounds including triterpenes, flavonoids, phenylpropanoids, and carotenoids. Interestingly, despite its ancient use, there has been no specific research on *Adiantum capillus-veneris* to isolate and test its chemicals for biological activities.

Adiantone, adiantoxide, astragalin, beta-sitosterol, caffeic acids, caffeylgalactose, caffeylglucose, campesterol, carotenes, coumaric acids, coumarylglucoses, diplopterol, epoxyfilicane, fernadiene, fernene, filicanes, hopanone, hydroxy-adiantone, hydroxy-cinnamic acid, isoadiantone, isoquercetin, kaempferols, lutein, mutatoxanthin, naringin, neoxanthin, nicotiflorin, oleananes, populnin, procyanidin, prodelphinidin, quercetins, querciturone, quinic acid, rhodoxanthin, rutin, shikimic acid, violaxanthin, and zeaxanthin are chemicals found in *Adiantum capillus-veneris* [155].

1.6.2 *Blumea lacera*

Table 1.2 Taxonomic Hierarchy of *Blumea lacera*

Kingdom	Plantae -- Planta, plantes, plants, Vegetal
Subkingdom	Tracheobionta -- vascular plants
Division	Magnoliophyta -- angiospermes, angiosperms, flowering plant
Class	Magnoliopsida -- dicots, dicotylédones, dicotyledons
Subclass	Asteridae
Order	Asterales
Family	Asteraceae -- sunflowers, tournesols
Genus	*Blumea* DC. -- false oxtongue
Species	*Blumea lacera* (Burm. f.) DC. -- blumea

1.6.2.1 Description

An erect, villous, foetid herb. Lower leaves petioled, often incised or lyrate, the upper subsessile elliptic oblong or obovate, finely silky pubescut. Heads 8 mm across, numerous in short axillary cymes and terminal spiciform panicles; flowers yellow [156].

1.6.2.2 Distribution

The plant occurs throughout the plains of India from the north-west ascending to 2,000 ft in the Himalayas. It is a common roadside weed in Ceylon and Malaya. It is distributed to the Malay Islands, Australia, China and Tropical Africa. *Blumea* consists of about 80 species. *Blumea lacera* competes with rabi crops such as linseed, chickpea, and wheat for light, food and moisture and harbors diseases and insects such as Euplexia dolorosa, Eublemma trifasiciata [157]. Grow as a weed in fallow lands all over Bangladesh [156].

1.6.2.3 Uses

Leaf juice is used as astringent, febrifuge, anthelmintic and diuretic; is also used to prepare an astringent eye lotion. The juice mixed with black pepper is given in piles. The plant also acts as a good stomachic, antispasmodic and diuretic. Alcoholic extract of the herb exhibited anti-inflammatory activity against carrageenan and bradykinin-induced inflammation in rats. Essential oils from leaves possess antimicrobial properties, R: astringent, febrifuge and it is used in cholera when mixed with pepper [156].

1.6.2.4 Chemical Constituents

Various parts of the plant yield an essential oil containing cineol, fenchone and camphor. Leaves also contain coniferyl alcohol derivatives, camp sterol and flavonoid. Ethanol extract of aerial parts contains hentriacontane, hentriacontanol, lupeol and its acetate and beta sitosterol. Root and root bark contain triterpenes, phenolic glycosides and sterols [54].

1.6.3 *Cassia alata*

Table 1.3 Taxonomic Hierarchy of *Cassia alata*

Kingdom	Plantae -- Planta, plantes, plants, Vegetal
Subkingdom	Tracheobionta -- vascular plants
Division	Magnoliophyta -- angiospermes, angiosperms, flowering plants,
Class	Magnoliopsida -- dicots, dicotylédones, dicotyledons
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Cassia* P. Mill. -- cassia
Species	*Cassia alata* (L.) Roxb. -- emperor's candlesticks

1.6.3.1 Description

An erect glabrous or subglabrous shrub of 3-8 ft.; stem terete, smooth, glabrous, or minutely puberulous. Leaves from 1-2 or 3 ft. in length, rachis acutely margined above when dry, glandular with a prominent transverse ridge connecting the opposite leaflets; leaflets in 9-12 pairs, very broadly oblong elliptic-oblong or the upper larger leaflets obovate-elliptical, rounded above and very obtuse or retuse, mucronate, base oblique truncate or subcordate at least as to the lower margin, glabrous, firmly membranous; the larger leaflets varying from 3-7 in. in length, subsessile or petiolules 1 line. Stipules obliquely triangular, acute, broad-based, persistent. Flowers in long erect axillary or apparently terminal stoutly pedunculate racemes. Bracts coloured, ovate or elliptical, obtuse or broadly pointed, imbricating at first, early deciduous, 1/2-1 in. long. Pedicels 1/4-1/2 in. Sepals coloured membranous, nearly equal in length. Two anterior stamens with enlarged strongly curved anthers about 5 lines long. Legume 2-valved, thinly but firmly coriaceous, linear apiculate, 5-6 in. long, 1/2- 3/4 in. broad, each valve with a very prominent crenate longitudinal wing extending the entire length of the valve and incurved towards the ventral suture. Seeds rhomboid-cuneate,

compressed; cotyledons sigmoid, in transverse section occupying the median third or half of the seed [158].

1.6.3.2 Distribution

It is native to the Amazon Rainforest and can be found in Peru, Brazil, French Guiana, Guyana, Suriname, Venezuela and Colombia. Due to its beauty, it has been cultivated around the world as an ornamental plant and has naturalized in many tropical regions in the world including tropical Africa, tropical Asia, Australia, Mexico, the Caribbean, Melanesia, Polynesia, & Hawaii [159]. In Bangladesh, it grows wild in all districts of the country [54].

1.6.3.3 Uses

The Tikuna Indians of the Amazon prepare a decocotion of the flowers as a purgative and one cup is taken each morning. In Cuba, the plant is named guacamaya francesa and it is used for herpes ulcers and other skin conditions, as a diuretic and as a laxative. In Peruvian herbal medicine systems the plant is called retama and the flowers are prepared in an infusion to treat urinary infections and used to increase urination; the leaves and stems are prepared in a decoction for acaries, herpes ulcers, ringworm, and other skin conditions; and, the root, leaves, wood and flowers are decocted for a remedy against intestinal parasites and hepatitis. Interestingly, the flowers are used as a diuretic (to increase urination), while the leaves are believe to be anti-diuretic. In Brazil, the plant is called guajava or mata-pasto. An infusion of the bark and roots is used for hydropsy, skin erruptions and fever. The leaves are considered an ememmagogue and diuretic and are prepared in extracts or capsules for liver problems, anemia, dyspepsia, menstrual problems, and high fevers. The leaves are juiced and mixed with lemon juice and applied to the skin for dermatitis and taken internally for syphilis [159].

1.6.3.4 Chemical Constituents

Cassia plant contains a group of chemicals called anthraquinones. These chemicals are well known for their laxative effect. Guajava leaves also contain a chemical called adenine which has been documented as an effective platelet aggregating inhibitor (reduces sticky blood and arterial plaque).

Other chemicals in guajava include chrysoeriol-7-O-(2"-O-beta-D-mannopyranosyl)-beta-D-allopyranoside, kaempferol, kaempferol 3-O-gentiobioside, naringenin, quercetin, and rhamnetin-3-O-(2"-O-beta-D-mannopyranosyl)-beta-D-allopyranoside [159].

1.6.4 *Cissus quadrangularis*

Table 1.4 Taxonomic Hierarchy of *Cissus quadrangularis*

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Rosidae
Order	Rhamnales
Family	Vitaceae – Grape family
Genus	*Cissus* L. – treebine
Species	*Cissus quadrangularis* L. – veldt-grape

1.6.4.1 Description

Cissus quadrangularis reaches a height of 1.5 m and has quadrangular-sectioned branches with internodes 8 to 10 cm long and 1.2 to 1.5 cm wide. Along each angle is a leathery edge. Toothed trilobe leaves 2 to 5 cm wide appear at the nodes. Each has a tendril emerging from the opposite side of the node. Racemes of small white, yellowish, or greenish flowers; globular berries are red when ripe [160].

1.6.4.2 Distribution

It is probably native to India or Sri Lanka, but is also found in Africa, Arabia, and Southeast Asia. It has been imported to Brazil and the southern United States [160]. It grows in the Sundarbans of Bangladesh and occasionally planted in the gardens in others areas.

1.6.4.3 Uses

Has been used as a medicinal plant since antiquity. The siddha medicine mentions it as a tonic and analgesic, and prescribes its use to help heal broken bones, thus its name asthisamharaka (that which prevents the destruction of bones). Has also been used to treat osteoporosis, asthma, cough, hemorrhoids, and gonorrhea. In the traditional medicinal systems of India it has been reported to possess not only bone fracture healing, but also It is said to have antibacterial, antifungal, antioxidant, anthelmintic, antihemorrhoidal and analgesic activities [161]. It contains a rich source of carotenoids, triterpenoids and ascorbic acid. Compounds that act as receptor antagonists of glucocorticoids have reduced the healing time of broken bones 30 to 50 percent in clinical trials. It has also been used to treat obesity and associated oxidative stress [162]. Its bactericidal effects on Helicobacter pylori hold

promise as an effective treatment of gastric ulcers and preventative of stomach cancer in conjunction with NSAID therapy [163]. A weight loss supplement containing *Cissus quadrangularis* with several dietary supplements (green tea, soy, selenium, chromium,and B vitamins) was evaluated in an 8-week clinical trial. The supplement helped reduce body weight by 4-8% (placebo 2.4%) a clinically significant weight loss [164].

1.6.4.4 Chemical Constituents

The herb contains an oxo-steroid, having fracture healing action similar to that of durabalin, 3-ketsteroid and other principles and also contains carotene, beta sitosterol, ascorbic acid and calcium oxalate [54].

1.7 Study Rationale

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 [165]. 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 [166]. 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 [167].

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.

1.8 Major Aim and Objectives

The major aim of the proposed study was to investigate the antibacterial, antioxidant potential and to execute toxicological evaluation of crude extracts of the four plants used in traditional

medicine — *Adiantum capillus-veneris, Blumea lacera, Cassia alata,* and *Cissus quadrangularis*

The first specific objective was to assess the biological activity of the selected plants' extracts against clinical isolates of three Gram-positive — *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and three Gram-negative — *Escherichia coli, Vibrio cholerae, Salmonella typhi* species using agar disk diffusion, agar well diffusion and broth microdilution methods.

The second specific objective was to evaluate the antioxidant capability of methanol extracts of designated plants using DPPH free radical scavenging assay.

The third specific objective was to investigate the potential toxicity of methanol extracts of selected plants by assessing their cytotoxicity against *in vitro* cultures of MDCK cell line.

1.9 Hypothesis

The working hypothesis was that four 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.

CHAPTER 2

MATERIALS and METHODS

2.1 Plant Extract Preparation

2.1.1 Collection and Identification of Plant Materials

Four plants were used for the current study — *Adiantum capillus-veneris*, *Blumea lacera*, *Cassia alata* and *Cissus quadrangularis*. Leaves of *Adiantum capillus-veneris*, *Blumea lacera*, *Cassia alata* and whole plant of *Cissus quadrangularis* were collected in air tight polythene bag from different locations of Faridpur in January, 2010 and the incidence was recorded. The plant materials were identified by the Bangladesh National Herbarium, Dhaka, Bangladesh.

2.1.2 Preparation of Plant Materials

Collected fresh plant materials were examined and old, insect and fungus infected leaves were removed. For effective drying the collected leaves were kept in open mesh bag and kept apart from individual plants. Plant materials were placed in diffused sunlight for 60 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 polyethylene bags 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 [168]. The protocol followed was the standard method of extraction published by Current Protocols [169]. 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 (61^0 C) using a heating mantle. The condensation rate for the chloroform was set at about 100-120 drops per minute and the extraction was continued for 6 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 hot plate at low temperature (40-50^0 C). Dried extract was weighed and expressed in percentage of original sample. All extracts were stored at 4^0 C 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 (65^0 C) using a heating mantle. The condensation rate for the chloroform was set at about 60-80 drops per minute and 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 hot plate at low temperature (40-50^0 C). Dried extract was weighed and expressed in percentage of original sample. All extracts were stored at 4^0 C until used.

2.2 Assessment of Antibacterial Activity

2.2.1 Disk Diffusion Assay for Determination of Antibacterial Activity

The antibacterial activity test of each extract was carried out by the Kirby-Bauer disk diffusion method [170]. For the antibacterial assessment, conventional methods of testing antibiotic abilities are usually applied. There are two basic techniques used for the assessment of antibacterial activity

1. The agar diffusion method (paper disc or well),
2. The dilution method (agar or liquid broth).

The agar diffusion method is the most widespread technique of antibacterial activity assessment. The method is recognized as precise and reliable, even though it produces semi-quantitative results, and according to some authors, only qualitative [171] and not always repeatable [172]. Nonetheless, it makes it possible to estimate the degree of microorganism's growth inhibition and their morphological changes in a simple way.

2.2.1.1 Microorganisms

Three Gram-positive clinical isolates — *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and three Gram-negative clinical isolates — *Escherichia coli, Vibrio cholerae,*

Salmonella typhi were obtained for agar disk diffusion assay from the Microbiology Department of Dhaka University.

2.2.1.2 Inoculum Preparation

Stock cultures of bacterial isolates were streaked onto nutrient agar plates and incubated at 37^0C overnight. From overnight subcultures of each isolates, three to five morphologically similar colonies were transferred to 5 ml of normal saline, mixed using a vortex mixer. The suspension's turbidity was adjusted at 0.08-0.13 measured by a spectrophotometer (Shimadzu, Japan) at λ = 625 nm to obtain a bacterial cell concentration of 1×10^8 cfu/ml. Turbidity was adjusted by adding sterile normal saline, if the turbidity is too high, or by adding more bacterial material if is too low. The inoculum suspension was used within 15 min of preparation.

2.2.1.3 Agar Disk Diffusion

Methanol and chloroform extracts were redissolved into the respective solvents and mixed using a vortex mixer. The plant extract solutions were filtered using a 0.22µm Millex Millipore filters (Carrigtwohill, Ireland). Sterile filter paper disks of 6 mm diameter were impregnated with 25 µl, 50 µl and 75 µl of extract solution to produce three disks for each plant extracts equivalent to 0.5, 1 and 1.5 mg of the dried extract respectively. Disks were dried for 6 hours to allow complete evaporation of solvent.

Suspension of each bacterial isolate was inoculated onto the MHA plate by dipping a cotton swab into the suspension and streaking over the surface of the plates to create a confluent lawn of bacterial growth. The inoculated plate is allowed to dry with the lid left ajar for no more than 15 min. Using sterile forceps the discs were placed onto the agar surface gently pressed down to ensure contact. Plates were kept for 2 hours in refrigerator to enable prediffusion of the extracts into the agar. Then the plates were incubated overnight at 37°C. Kanamycin (30 µg) was used as positive control. Negative controls were performed with paper discs loaded with organic solvents (chloroform and methanol) and dried. At the end of the incubation period the antibacterial activity was evaluated by measuring the inhibition zones.

2.2.2 Agar Well Diffusion for Determination of Antibacterial Activity

In the burn arena, prior to the advent of topical antibacterial agents, the overall mortality rate in a typical burn population would be reported a 38% to 45%. However after the use of

topical antibacterial therapy the overall mortality was reduced to 14% to 24%. [173] This enhanced survival was probably due to a susceptibility assay developed at the Cincinnati Shriners Burns Hospital in 1978 by Nathan and his colleagues [174] called Nathan's Agar Well Diffusion Assay. This assay has become the "gold standard" among many burn centers throughout the world [175].

2.2.2.1 Microorganisms

Three Gram-positive clinical isolates — *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and three Gram-negative clinical isolates — *Escherichia coli, Vibrio cholerae, Salmonella typhi* were obtained for agar well diffusion assay from the Microbiology Department of Dhaka University.

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2.2.2.3 Agar Well Diffusion

Methanol and chloroform extracts were dissolved into the respective solvents and mixed using a vortex mixer. The plant extract solutions were filtered using a 0.22µm Millex Millipore filters (Carrigtwohill, Ireland).

By sterile technique, 6 mm wells were made on MHA plates. Suspension of each bacterial isolate was inoculated onto the MHA plate by dipping a cotton swab into the suspension and streaking over the surface of the plates to create a confluent lawn of bacterial growth. The inoculated plate is allowed to dry with the lid left ajar for no more than 15 min. Using sterile micropipette, wells were loaded with 25 µl, 50 µl and 75 µl of extract solution to produce three well for each plant extracts equivalent to 0.5 mg, 1 mg and 1.5 mg of the dried extract respectively. Plates were kept for 2 hours in refrigerator to enable prediffusion of the extracts into the agar. Then the plates were incubated overnight at 37°C. Kanamycin was used as

positive control. Negative controls were performed with wells loaded with organic solvents (chloroform and methanol). At the end of the incubation period the antibacterial activity was evaluated by measuring the inhibition zones.

2.2.3 MIC Determination by Broth Microdilution

2.2.3.1 Microorganisms

The following microorganisms were used in this study: *Escherichia coli 0157:H7* and clinical isolate of *Staphylococcus aureus* were obtained from Microbiology Department of The University of Dhaka.

2.2.3.2 Inoculum preparation

Stock cultures of bacterial isolates were streaked onto nutrient agar plates and incubated at 37^0C overnight. From overnight subcultures of each isolates, three to five morphologically similar colonies were transferred to 5 ml of Muller-Hinton broth, mixed using a vortex mixer. The suspension's turbidity was adjusted at 0.08-0.13 measured by a spectrophotometer (Shimadzu, Japan) at λ = 625 nm to obtain a bacterial cell concentration of 1×10^8 cfu/ml. Turbidity was adjusted by adding sterile Muller-Hinton broth, if the turbidity is too high, or by adding more bacterial material if is too low. The turbidity adjusted solution was diluted by a factor of 1:100 by adding 500 µl bacterial suspension to 49.5 ml sterile MHB to achieve a concentration of 1×10^6 cfu/ml.

2.2.3.3 Microtiter plate assay

Methanol extracts of the plants were dissolved in MHB containing 5% DMSO to obtain a concentration of 2mg/ml and sterilized using a 0.22µm Millex Millipore filters (Carrigtwohill, Ireland). Sterilized methanol extract solutions were stored at -20^0 C until used. Ampicillin solution of 2mg/ml was prepared in the similar manner.

Row A, B, C and D of a sterile 96-well microtiter plate was used to determine MIC of *Adiantum capillus-veneris*, *Blumea lacera*, *Cassia alata* and *Cissus quadrangulari* respectively against one bacterial isolates. Row E is used for the positive control Ampicillin. Column 11 & 12 were used as growth control well & sterility control well respectively.

200 µl of MHB containing 5% DMSO was pipetted into sterility control wells (column 12) and 100 µl into growth control wells. 100 µl of 5% DMSO in MHB was added to each well of 1 to 10 columns. 100 µl sterilized methanol extract solutions were pipetted into wells of

column 1 for respective plants. After proper mixing 100 µl of the mixture was transferred from wells of column 1 to wells of column 2. The process was repeated with a multichannel pipette until column 10 was reached from where 100 µl of the mixture was discarded to achieve a final volume of 100 µl in each wells of column 1 to 10 of row A to D. Wells of column 1 to 10 of row E was similarly filled with ampicillin solution. 100 µl of 1×10^6 cfu/ml bacterial suspension was pipetted into each well of column 1 to 11 of row A to E, making the final volume of each well 200 µl. The final concentration of bacteria in each well was 5×10^5 cfu/ml and the concentration of extracts ranged between 1000 to 1.953 µg/ml. Plates were then incubated at 37°C for overnight. After incubation, the MIC of each extract was determined as the lowest concentration at which no growth was observed.

2.3 Determination of Antioxidant Activity of Methanol Extracts

The DPPH free radical scavenging assay was carried out for the evaluation of the antioxidant activity. This assay measures the free radical scavenging capacity of the investigated extracts. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant, which can donate an electron to DPPH, the purple color typical for free DPPH radical decays, and the absorbance change at λ = 517 nm is measured. This test provides information on the ability of a compound to donate a hydrogen atom, on the number of electrons a given molecule can donate, and on the mechanism of antioxidant action. The method was carried out as previously described by Brand *et al.* [176]. The methanol extracts of four plants (*Adiantum capillus-veneris*, *Blumea lacera*, *Cassia alata* and *Cissus quadrangularis*) were redissolved in methanol and various concentrations (10, 50, 100, 500 and 1000 µg/ml) of each extract were used. Similar concentrations of ascorbic acid, propyl galate and BHT were used as positive control. The assay mixture contained in a total volume of 1 ml, 500 µl of the extract, 125 µl prepared DPPH (1 mM in methanol) and 375 µl solvent (methanol). After 30 min incubation at 25°C, the decrease in absorbance was measured at λ = 517 nm.

The radical scavenging activity was calculated from the equation:

$$\% \text{ of radical scavenging activity} = Abs_{control} - Abs_{sample}/Abs_{control} \times 100$$

2.4 Toxicological evaluation

Application of *in vitro* techniques to the assessment of cytotoxic potential in drug development continues to be a rapidly growing area. The use of continuous cell lines is an important component in this discovery process and may provide fundamental information about *in vivo* xenobiotic toxicity [177].

2.4.1 Cell Culture

The toxicity of methanol plant extracts was examined on distal kidney (MDCK) cell line. MDCK cell line, provided by the Microbiology Laboratory of University of Dhaka, was cultured in DMEM (Sigma Aldrich) supplemented with 200 mM of glutamine, 10% of fetal bovine serum (Sigma), 3.7g/L $NaHCO_3$ (Merck), 20mM HEPES, 50 µg/ml Penicillin, 50 µg/ml Streptomycin and 50 µg/ml Gentamycin. The cultures were maintained in 37°C in 5% of humidity CO_2 atmosphere. After 60% - 80% confluence, the cells were trypsinized with 0.25% Trypsin, counted and passaged.

2.4.2 Morphological Study

Methanol extracts were dissolved in 1% DMSO and sterilized using 0.22µm Millex Millipore filter. Sterilized methanol extract solutions were stored at -20^0 C until used. MDCK cells suspended in DMEM (Sigma) containing 10% heat-inactivated fetal calf serum were cultured in 96-Well flat bottom Microtiter plate (Iwaki, SciTech) at a density of 10^5 cell/well in the presence of 5% CO_2 at 37°C.

The cells are then treated with 37.5, 75, 150 µg/ml of methanol extracts dissolved in DMSO and incubated for 24 h. Untreated cells receiving only media were used as control. After the treatment period, morphological observations were made with an inverted phase contrast microscope using standard criteria for non-specific cytopathological effects (i.e. membrane bleb formation, cell detachment, cellular crenation and cell lysis). Viable MDCK cells retained normal shape, size and attachment as a complete monolayer toxic effect of methanol extract and was characterized by cell detachment with the appearance of floating, rounded cells and debris [178].

CHAPTER 3

RESULTS and DISCUSSION

3.1 Results

3.1.1 Collection and Extraction of Plant Material

Records of the collected plants are adapted in table 3.1 presenting the habit and habitat of the plants. Plant materials extracted with Soxhlet extractor are presented in table 3.2 showing % yield of the different plant extract.

3.1.2 Antibacterial Activity

Antibacterial activity of the plant extracts was screened using the Kirby-Bauer disk diffusion method and Nathan's agar well diffusion assay. In addition to that, MIC of plant methanol extracts was determined by broth microdilution assay [179] using 96-well microtiter plate. The results of disk diffusion assay, agar well diffusion assay, and broth microdilution assay are recorded in table 3.3, 3.4 and 3.5.

No zone of inhibition was observed for any extracts in disk diffusion assay using different concentrations of disks against either Gram-positive or Gram-negative isolates. No inhibition was also observed for disks loaded with the solvent (negative control). However, kanamycin disks of 30 µg (positive control) showed strong inhibition against every Gram-positive or Gram-negative isolates.

No zone of inhibition was observed for any extracts in agar well diffusion assay using different concentrations of extract loaded in each well against either Gram-positive or Gram-negative isolates. No inhibition was also observed for wells loaded with the solvent (negative control). However, wells laden with kanamycin solution (30 µg/well) (positive control) exhibited strong inhibition against every Gram-positive or Gram-negative isolates.

No inhibition was seen for any methanol extracts of different concentrations used against *Staphylococcus aureus* isolate and *Escherichia coli 0157:H7* strain for the determination of

MIC. However, no growth was noted in the wells loaded with Ampicillin (positive control). No growth was observed for the wells of column 12 (sterility control) whereas distinct growth was observed in the wells of column 11 (growth control).

3.1.3 Radical scavenging activity

The methanol extracts of *Adiantum capillus-veneris*, *Blumea lacera,* and *Cassia alata* showed a highly effective free radical scavenging activity in the DPPH assay. These extracts exhibited a noticeable antioxidant effect at low concentrations compared with the standards (Table 3.6). The methanol extracts of *Cissus quadrangularis* demonstrated moderate free radical scavenging activity with diminutive antioxidant activity at lower concentration and high antioxidant activity at increasing concentration.

3.1.4 Cytotoxic activity

Toxicological evolution of methanol extracts was performed by *in vitro* cytoxicity assay against MDCK cell line. Morphological study of methanol extract treated MDCK cell under inverted phase contrast microscope revealed the viability. MDCK cultures treated with different concentrations of *Adiantum capillus-veneris*, *Blumea lacera,* and *Cassia alata* methanol extract demonstrated the characteristics of dead cells with disappearance of monolayer and normal shape and size, cell detachment, presence of floating, rounded cells and debris. This designates the toxic effect of *Adiantum capillus-veneris*, *Blumea lacera,* and *Cassia alata* methanol extract on MDCK cells at the investigated concentration. The methanol extract of *Cissus quadrangularis*, on the other hand, provided viable condition for the growth of MDCK cell as it demonstrates similar appearances to that of untreated MDCK control.

Table 3.1 Plant Collection Records

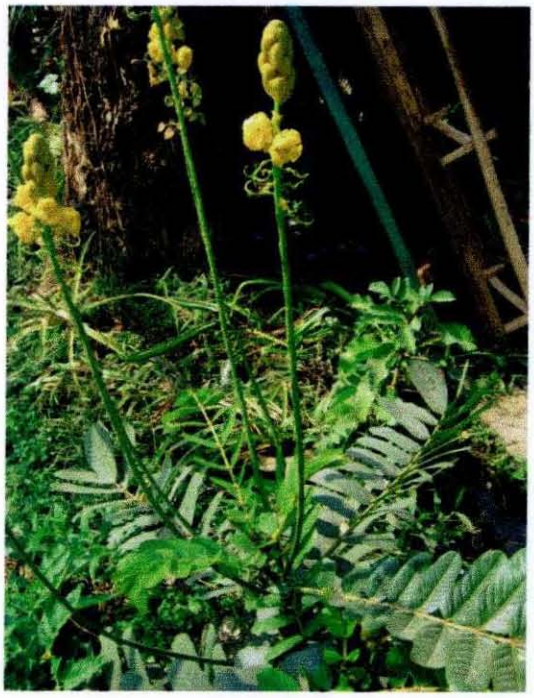
Plant No.	Plant's Local Name	Scientific Name	Location of Collection	Tropology	Abundance	Plant Type	Leaf type	Date of Collection	Time of Collection
1.	Bidhayapata	*Adiantum capillus-veneris*	Faridpur, Bangladesh	Plains	Occasional	Herb	Light green fronds much subdivided into pinnae	15/01/2010	Morning
2.	Kukursunga	*Blumea lacera*	Faridpur, Bangladesh	Plains	Abundant	Herb	Lower leaves incised or lyrate, the upper oblong or obovate, finely silky pubescut	16/01/2010	Morning
3.	Dadmadan	*Cassia alata*	Faridpur, Bangladesh	Plains	Very few	Shrub	Leaves glandular with a prominent transverse ridge connecting the opposite leaflets	16/01/2010	Morning
4.	Harjoda	*Cissus quadrangularis*	Faridpur, Bangladesh	Plains	Occasional	Herb	Toothed trilobe leaves appear at the nodes, has a tendril emerging	15/01/2010	After noon

Table 3.2 Percent yield of different plant material extraction

Plant	Family	Parts used	Extract	% yield
Adiantum capillus-veneris	Pteridaceae	Whole plant	Chloroform	2.8
			Methanol	13.3
Blumea lacera	Compositae	Leaves	Chloroform	4.4
			Methanol	13.6
Cassia alata	Caesalpiniaceae	Leaves	Chloroform	5.6
			Methanol	14.8
Cissus quadrangularis	Vitaceae	Whole plant	Chloroform	2.1
			Methanol	6.3

Adiantum capillus-veneris (Bidhayapata)

Blumea lacera (Kukursunga)



Cassia alata (Dadmadan)

Cissus quadrangularis (Harjoda)

Figure 3.1 Pictures of the collected pants

Table 3.3 Antibacterial activity of plant extracts observed in disk diffusion assay expressed in terms of zone of inhibition (mm)

Evaluated Item	Extract	Concentration per disk	Zone of Inhibition Against Different Microorganisms in mm					
			SA	BS	BC	EC	VC	ST
Adiantum capillus-veneris	Chloroform	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
	Methanol	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
Blumea lacera	Chloroform	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
	Methanol	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
Cassia alata	Chloroform	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
	Methanol	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
Cissus quadrangularis	Chloroform	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
	Methanol	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
Kanamycin (+ Control)	n/a	30 µg	25	28	24	22	23	25
Chloroform (- Control)	n/a	5 ml	-	-	-	-	-	-
Methanol (- Control)	n/a	5 ml	-	-	-	-	-	-

SA= *Staphylococcus aureus*, BC= *Bacillus cereus*, BS= *Bacillus subtilis*, EC= *Escherichia coli*, VC = *Vibrio cholerae*, ST= *Salmonella typhi*

Table 3.4 Antibacterial activity of plant extracts observed in agar well diffusion assay expressed in terms of zone of inhibition (mm)

Investigated element	Extract	Concentration per well	Zone of inhibition against different microorganisms in mm					
			SA	BS	BC	EC	VC	ST
Adiantum capillus-veneris	Chloroform	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
	Methanol	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
Blumea lacera	Chloroform	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
	Methanol	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
Cassia alata	Chloroform	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
	Methanol	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
Cissus quadrangularis	Chloroform	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
	Methanol	0.5 mg	-	-	-	-	-	-
		1.0 mg	-	-	-	-	-	-
		1.5 mg	-	-	-	-	-	-
Kanamycin (+ Control)	n/a	30 µg	23	21	21	23	25	21
Chloroform (- Control)	n/a	5 ml	-	-	-	-	-	-
Methanol (- Control)	n/a	5 ml	-	-	-	-	-	-

SA= *Staphylococcus aureus*, BC= *Bacillus cereus*, BS= *Bacillus subtilis*, EC= *Escherichia coli*, VC = *Vibrio cholerae*, ST= *Salmonella typhi*

Table 3.5 MIC of methanol extracts of investigated plants

Investigated Element	MIC against different microorganisms (µg/ml)	
	Escherichia coli 0157:H7	*Staphylococcus aureus*
Adiantum capillus-veneris	-	-
Blumea lacera	-	-
Cassia alata	-	-
Cissus quadrangularis	-	-
Ampicillin	1.953 µg/ml	1.953 µg/ml

Table 3.6 Free radical scavenging activity of methanol extracts of investigated plants

Investigated Element	% Radical scavenging activity at different concentrations				
	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml	1000 µg/ml
Adiantum capillus-veneris	83.87	79.21	97.13	96.06	88.89
Blumea lacera	17.20	52.69	88.53	94.62	94.98
Cassia alata	68.46	76.70	92.11	96.42	84.59
Cissus quadrangularis	16.13	35.13	43.37	79.93	85.30
Ascorbic Acid (Standard)	95.34	97.13	97.49	98.57	99.28
BHT (Standard)	83.87	94.98	97.13	98.92	99.28
Propyl Gallate (Standard)	96.77	98.92	99.28	99.64	100.00

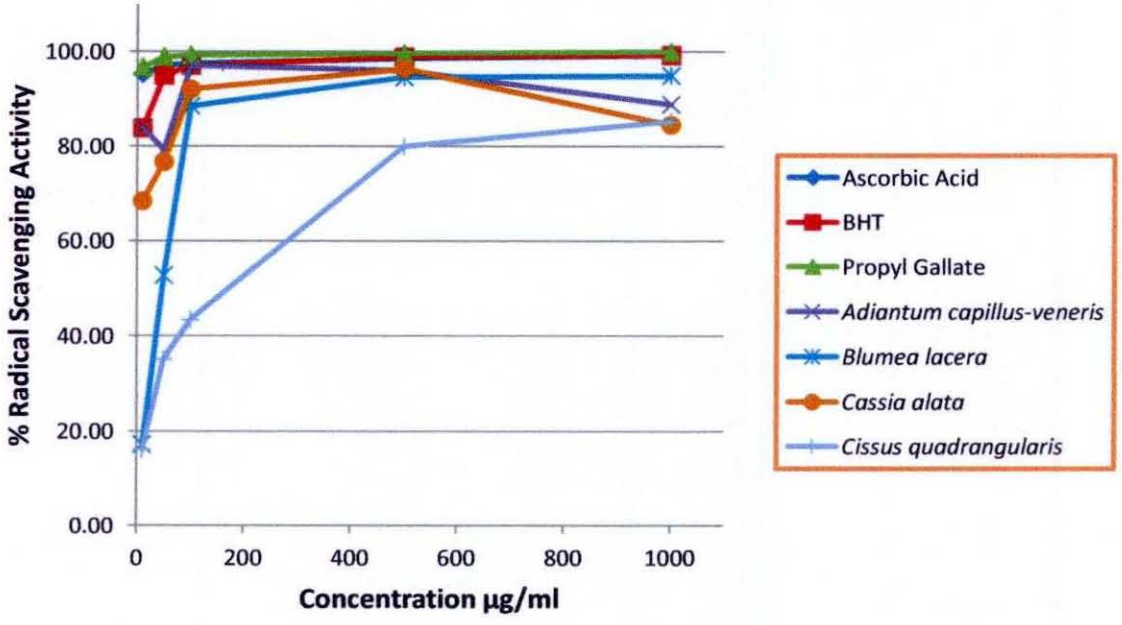
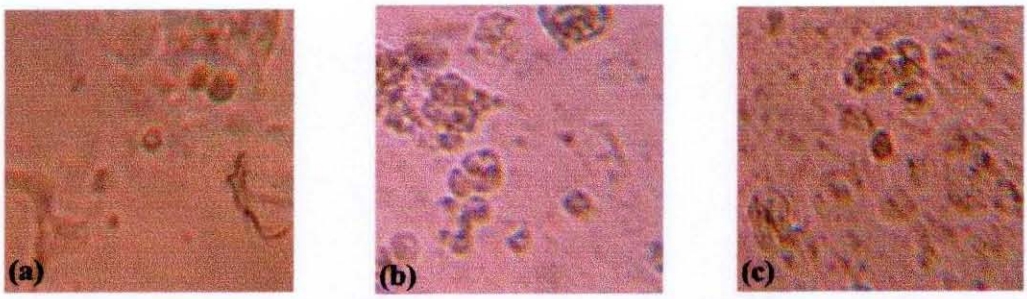
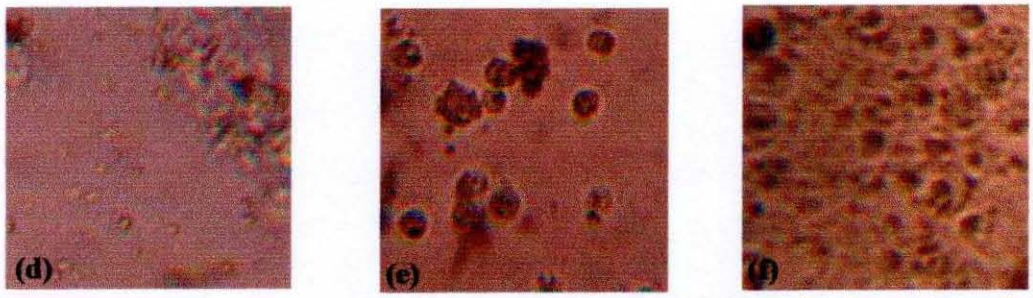


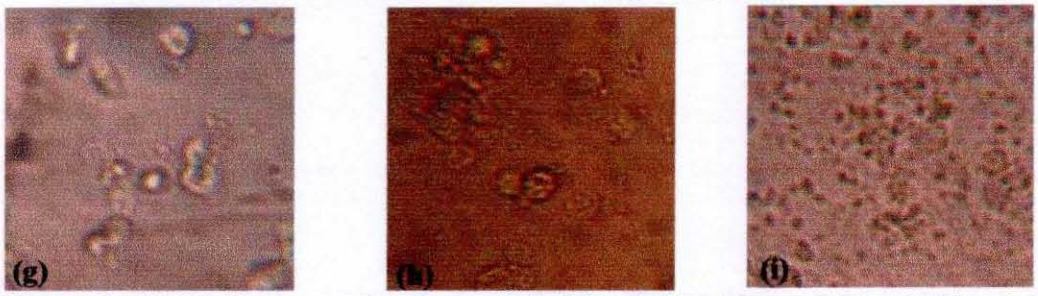
Figure 3.2 Free radical scavenging activity of methanol extracts of investigated plants



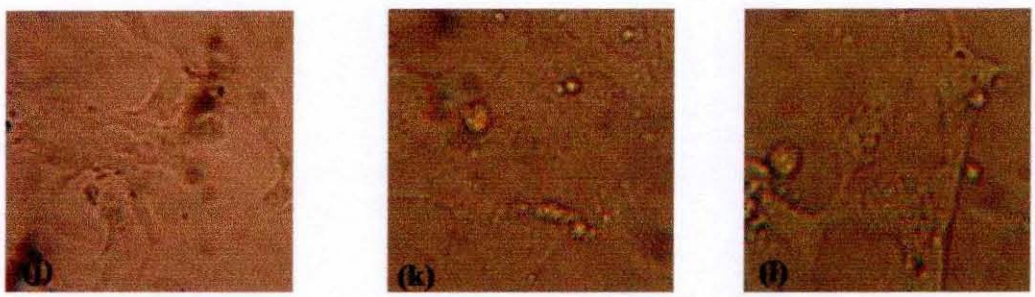
MDCK cells treated with *Adiantum capillus-veneris* methanol extract of 37.5 µg/ml (a), 75 µg/ml (b) and150 µg/ml (c)



MDCK cells treated with *Blumea lacera* methanol extract of 37.5 µg/ml (d), 75 µg/ml (e) and 150 µg/ml (f)



MDCK cells treated with *Cassia alata* methanol extract of 37.5 µg/ml (g), 75 µg/ml (h) and 150 µg/ml (i)



MDCK cells treated with *Cissus quadrangularis* methanol extract of 37.5 µg/ml (j), 75 µg/ml (k) and150 µg/ml (k)

Untreated MDCK control (m)

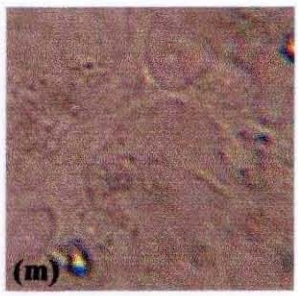


Figure 3.3 Micrographs of MDCK cultures treated with methanol extracts of different concentrations

3.2 Discussion

Finding healing powers in plants is an ancient idea. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory [40]. A survey by UNCTAD has shown that 33% of total drugs produced by the industrialized nations are plant derived and microbes are considered, 60% of medicinal products are of natural origin [180]. With the estimated 10–100 million species or 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, it looks increasingly like we have only scratched the surface of this world's wonderful resource [181]. 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 [182, 183]. 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 [184].

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

Investigated plant parts were extracted with Soxhlet extraction procedure, provided productive yield with the highest yield for methanol extract was 14.8% and the highest yield for chloroform extract was 5.6%.

Adiantum Linn. of Adiantaceae family is one of the most common and widely distributed species. Ethnomedicinally, the genus is important and popularly known as "Hansraj" in Ayurvedic System of Medicine. It has been used in cold, tumors of spleen, liver and other viscera, skin diseases, bronchitis and inflammatory diseases. Its leaves are also considered as expectorant tonic [185]. In current study, no antibacterial activity of *Adiantum capillus-veneris* was demonstrated against any of the Gram positive or Gram negative isolates used for the evaluation. However, methanol extract of *Adiantum capillus-veneris* has previously

demonstrated inhibition against five Gram positive and Gram negative *E. coli* strains and the activity was concluded due to the presence of flavonoid and tannin along with reducing sugar [185].

Adiantum capillus-veneris showed antioxidant activity at a very low concentration (10µg/ml) as it was also previously reported [186, 187]. However, previous report required high concentration (4mg/ml) of *Adiantum capillus-veneris* extract to demonstrate obtrusive activity and recognized due to the presence of flavonoid and phenol. Our study also confirms cytotoxic activity of *Adiantum capillus-veneris* against noncancerous MDCK cell line. No previous record on toxicological evaluation of *Adiantum capillus-veneris* was found.

Blumea lacera is a common weed found throughout the Bangladesh. The leaves of the plant are anthelmintic, febrifuge, and astringent. The plant is a diuretic, antiscorbutic, and useful in catarrhal affections [188]. Our study was unable to demonstrate the presence of any antibacterial activity in *Blumea lacera* activity against the screened organism. Although, previous records shows antibacterial activity of *Blumea lacera* extracts against Gram positive or Gram negative microorganisms accounted to the existence of monoterpene glycoside and flavonoids [188, 189]. *Blumea lacera* displayed antioxidant function at low concentration (50 µg/ml) as it was previously reported. *Blumea lacera* also expressed toxic activity against MDCK cell line. Similar cytotoxicity of *Blumea lacera* against noncancerous mouse fibroblasts cell line was previously observed [190].

Cassia alata is often called the Ringworm Bush because of its very effective fungicidal properties, for treating ringworm and other fungal infections of the skin. Its active ingredients include the yellow chrysophanic acid. Its laxative effect, due to its anthraquinone content, is also well proven. Extracts of *Cassia alata* were also incapable to express any antibacterial activity in our study. Similar observation was previously reported, where Gram positive and Gram negative bacterial species showed resistance against ethanolic extract of *Cassia alata* in disk diffusion assay using 500 mg extract-impregnated disks [191]. Nevertheless, other study reported antibacterial activity of methanol and ethanol extract of *Cassia alata* against Gram positive and Gram negative bacterial species when extracts were used at a much lower concentrations [192, 193]. Methanol extracts of *Cassia alata* showed free radical scavenging activity at minute concentration (10µg/ml). Comparable observation was also previously reported [194, 195]. *Cassia alata* expressed toxic activity against MDCK cell line activity in

our study. In other report, however, *Cassia alata* isolate showed no cytotoxicity to the normal cell line, CHO-AA8 [196].

Cissus quadrangularis grows in the Sundarbans and occasionally planted in the gardens in others areas [54]. The plant is mentioned in the ancient systems of medicine such as Ayurveda, and is useful for treatment of bloody diarrhoea, skin disorders, earache, haemorrhoids, irregular menstruation, and accelerates healing of bone fracture [197]. *Cissus quadrangularis* extracts conferred no antibacterial activity towards the examined Gram positive and Gram negative bacterial species, although earlier report indicates the presence of antibacterial activity in ethyl acetate, acetone, and methanol extract of *Cissus quadrangularis.* Methanol extracts of *Cissus quadrangularis* also showed progressive antioxidant activity. In earlier report, less significant antioxidant activity was recorded in methanol extracts of *Cissus quadrangularis* [198]. Toxicological evaluation demonstrates no cytotoxic action of methanol extract of *Cissus quadrangularis* against noncancerous MDCK cell line. At our best notice no such previous records of cytotoxicity for *Cissus quadrangularis* was reported. However, in one subchronic toxicity investigation *Cissus quadrangularis* did not produce any toxicity in the rats [199].

In summary, antibacterial and antioxidant potential of four selected medicinal plant was investigated in the present study. To assist the potential development of the medicinal plants as dietary supplement or therapeutic agent, toxicological evaluation of the plants was also apprehended. Although, disparate to previous investigations, no antibacterial activity was observed in the methanol and chloroform extracts of the investigated plants. This variation in the results may indicate a number of dissimilarities in the apprehended study from the previous studies including environmental effects on the studied plant, age of the plant therefore presence of active substance, season for plant collection, method of extraction used, time of extraction, method of antibacterial susceptibility assay, different concentrations of extracts used during the assay, and other reasons associated to medicinal plant research. In contrast, 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. Methanol extracts of *Adiantum capillus-veneris*, *Blumea lacera*, and *Cassia alata* exhibited cytotoxic activity against distal kidney (MDCK) cell line indicating their potential harmful effect on mammalian kidney. No such cytotoxic effect was observed for *Cissus quadrangularis* referring to their relative nontoxic aspect towards mammalian

kidney, although more toxicological study is required to declare them safe for potential development.

REFERENCES

1. Halberstein, R.A., *Medicinal plants: historical and cross-cultural usage patterns.* Ann Epidemiol, 2005. 15(9): 686-99.

2. Baker, M., *Fur rubbing use of medicinal plants by capuchin monkeys (Cebus capucinus).* American Journal of Primatology, 1996. 38(3): 263-270.

3. Etkin, N.L., *Eating on the wild side : the pharmacologic, ecologic, and social implications of using noncultigens*. 1994, Tucson: University of Arizona Press.

4. Plotkin, M.J., *Medicine quest : in search of nature's healing secrets*. 2000, New York: Viking.

5. Huffman, M.A., *Current evidence for self-medication in primates: A multidisciplinary perspective.* American Journal of Physical Anthropology, 1997. 104(S25): 171-200.

6. Adovasio, J.M. and G.F. Fry, *Prehistoric Psychotropic Drug Use in Northeastern Mexico and Trans-Pecos Texas.* Economic Botany, 1976. 30(1): 94-96.

7. de Montellano, B.O., *Empirical Aztec medicine.* Science, 1975. 188(4185): 215-20.

8. D.G. Sharon and C.B. Donnan, *The Magic Cactus: Ethnoarchaeological Continuity in Peru.* Archaeology, 1977. 30: 374–381.

9. Merlin, M., *Archaeological evidence for the tradition of psychoactive plant use In the old world.* Economic Botany, 2003. 57(3): 295-323.

10. Nunn, J.F., *Ancient Egyptian medicine.* Trans Med Soc Lond, 1996. 113: 57-68.

11. Oakes, L. and L. Gahlin, *Ancient Egypt : an illustrated reference to the myths, religions, pyramids and temples of the land of the pharaohs*. 2003, New York: Barnes & Noble.

12. Selin, H. and H. Shapiro, *Medicine across cultures : the history of non-Western medicine*. 2003, Dordrecht; London: Kluwer Academic.

13. Von Staden, H., *Herophilus : the art of medicine in early Alexandria : edition, translation, and essays*. 1989, Cambridge; New York: Cambridge University Press.

14. Sigerist, H.E., *A history of medicine, I. Primitive and archaic medicine*. 1967, New York: Oxford University Press.

15. Jonas, W.B., J.S. Levin, and B. Berman, *Essentials of complementary and alternative medicine*. 1999, Philadelphia: Williams & Wilkins.

16. Evans, S.T., *Ancient Mexico and Central America*. 2004, London: Thames & Hudson.

17. Berdan, F., *The Aztecs of central Mexico : an imperial society*. 2005, Belmont, CA: Thomson Wadsworth.

18. Anderson, F.J., *An illustrated history of the herbals*. 1977, New York: Columbia University Press.

19. Grossinger, R., *Planet medicine : from Stone Age shamanism to post-industrial healing*. 1980, Garden City, N.Y.: Anchor Press.

20. Tierra, M. and D. Frawley, *Planetary herbology : an integration of Western herbs into the traditional Chinese and Ayurvedic systems*. 1988, Twin Lakes, Wis.: Lotus Press.

21. Wheelwright, E.G., *Medicinal plants and their history*. 1974, New York: Dover Publications.

22. de Pasquale, A., *Pharmacognosy: the oldest modern science.* J Ethnopharmacol, 1984. 11(1): 1-16.

23. Shu, Y.Z., *Recent natural products based drug development: a pharmaceutical industry perspective.* J Nat Prod, 1998. 61(8): 1053-71.

24. Hamburger, M., *Bioactivity in plants: the link between phytochemistry and medicine.* Phytochemistry, 1991. 30(12): 3864-3874.

25. Williamson, E.M., et al., *Selection, Preparation and Pharmacological Evaluation of Plant Material.* Journal of medicinal chemistry., 1997. 40(10): 1559.

26. Elisabetsky, E., *Pesquisas em plantas medicinais.* Ciência e cultura, 1987. 39(8): 697-702.

27. Rouhi, A.M., *SEEKING DRUGS IN NATURAL PRODUCTS.* Chem. Eng. News Chemical & Engineering News, 1997. 75(14): 14-29.

28. Reid, W.V., et al., *Biodiversity prospecting : using genetic resources for sustainable development*. 1993, [Washington, D.C.]; [Santo Domingo, Heredia]; [New York, N.Y.]; [Nairobi]: World Resources Institute (WRI), USA ; Instituto Nacional de Biodiversidad (INBio), Costa Rica ; Rainforest Alliance, USA ; African Centre for Technology Studies (ACTS), Kenya.

29. Payne, G., *Plant cell and tissue culture in liquid systems*. 1992, Munich; New York; New York: Hanser Publishers ; Distributed in the U.S.A. and Canada by Oxford University Press.

30. Goldfrank, L., et al., *The pernicious panacea: herbal medicine.* Hosp Physician, 1982. 18(10): 64-9, 73-8 passim.

31. Dukes, M.N.G., *Meyler's side effects of drugs : an encyclopedia og adverse reactions and interactions*. 1988, Amsterdam: Elsevier.

32. Rates, S.M., *Plants as source of drugs.* Toxicon 2001. 39(5): 603-13.

33. Lewis, W.H. and M.P. Elvin-Lewis, *Medicinal Plants as Sources of New Therapeutics.* Annals of the Missouri Botanical Garden., 1995. 82(1): 16.

34. IUCN. *Release of the 2006 IUCN Red List of Threatened Species reveals ongoing decline of the status of plants and animals*. 2006 [cited 2011 31st May]; Available from: http://www.natureserve.org/aboutUs/PressReleases/IUCN_Red_List_release.pdf.

35. Borris, R.P., *Natural products research: perspectives from a major pharmaceutical company.* J Ethnopharmacol, 1996. 51(1-3): 29-38.

36. Georges, M. and K.M. Pandelai, *Investigations on plant antibiotics. IV. Further search for antibiotic substances in Indian medicinal plants.* J. Med. Res., 1949. 37: 169–181.

37. Rojas, A., et al., *Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants.* J Ethnopharmacol, 1992. 35(3): 275-83.

38. Silva, O., et al., *Antimicrobial activity of Guinea-Bissau traditional remedies.* J Ethnopharmacol, 1996. 50(1): 55-9.

39. Vanden Berghe, D.A., *PLANT PRODUCTS AS POTENTIAL ANTIVIRAL AGENTS.* The Pediatric Infectious Disease Journal The Pediatric Infectious Disease Journal, 1987. 6(2).

40. Cowan, M.M., *Plant products as antimicrobial agents.* Clinical microbiology reviews, 1999. 12(4): 564-82.

41. International Union for Conservation of, N. and G. Natural Resources. Medicinal Plant Specialist, *International standard for sustainable wild collection of medicinal and aromatic plants (ISSC-MAP)*. 2007, Bonn, Germany: Bundesamt für Naturschutz.

42. Hawkins, B. and I. Botanic Gardens Conservation, *Plants for life : medicinal plant conservation and botanic gardens*. 2008, Richmond, Surrey: Botanic Gardens Conservation International.

43. Butler, M.S., *The role of natural product chemistry in drug discovery.* J Nat Prod, 2004. 67(12): 2141-53.

44. Reichert, J.M., *Trends in development and approval times for new therapeutics in the United States.* Nat Rev Drug Discov, 2003. 2(9): 695-702.

45. Dickson, M. and J.P. Gagnon, *Key factors in the rising cost of new drug discovery and development.* Nat Rev Drug Discov, 2004. 3(5): 417-29.

46. Balunas, M.J. and A.D. Kinghorn, *Drug discovery from medicinal plants.* Life Sci, 2005. 78(5): 431-41.

47. McChesney, J.D., *The Promise of Plant-Derived Natural Products for the Development of New Pharmaceuticals and Agrochemicals.* ACS symposium series., 1995. 588: 66.

48. Ohrmann, R., *Pflanzenextrakte in Haushaltsprodukten.* Dragoco Report (Holzminden), 1991. 3: 67-76.

49. Gorecki, P., *Vitafoods und Kosmetika: Arzneipflanzen erobern sich neue Wirkungsbereiche.* Drogenreport, 2002. 28: 9-15.

50. Lange, D., *Untersuchungen zum Heilpflanzenhandel in Deutschland : ein Beitrag zum internationalen Artenschutz* 1996, Münster: Landwirtschaftsverl.

51. Farnsworth, N.R. and D.D. Soejarto, *Global importance of medicinal plants*, in *The conservation of medicinal plants: proceedings of an International consultation 21-27 March 1988, Chiang Mai, Thailand*, O. Akerele, V. Heywood, and H. Synge, Editors. 1991, Cambridge University Press: Cambridge. 25-51.

52. Lange, D., *International trade in medicinal and aromatic plants*, in *Medicinal and aromatic plants : agricultural, commercial, ecological, legal, pharmacological, and social aspects*, R.J. Bogers, L.E. Craker, and D. Lange, Editors. 2006, Springer: Dordrecht.

53. Yusuf, M., et al., *Medicinal plants of Bangladesh.* 1994, Dhaka: BCSIR Laboratories, Bangladesh.

54. Gani, A., *Medicinal plants of Bangladesh: Chemical Constituents and Uses*. 1998, Dhaka: Asiatic Society of Bangladesh.

55. Dixie, G., et al., *Medicinal plant marketing in Bangladesh.* 2005, Dhaka: Intercooperation.

56. Heinrich, M., *Fundamentals of pharmacognosy and phytotherapy*. 2004, Edinburgh; New York: Churchill Livingstone.

57. Vanaclocha, B. and S. Cañigueral Folcara, *Fitoterapia : vademécum de prescripción.* 2003, Barcelona; México: Masson.

58. Geissman, T.A., *Flavonoid compounds, tannins, lignins and related compounds*, in *Pyrrole pigments, isoprenoid compounds and phenolic plant constituents*, M. Florkin and E.H. Stotz, Editors. 1963, Elsevier: New York. 265.

59. Schultes, R.E., *The kingdom of plants*, in *Medicines from the Earth*, W.A.R. Thomson, Editor. 1978, McGraw-Hill Book Co.: New York. 208.

60. Warren, R., *The Complete book of natural & medicinal cures : how to choose the most potent healing agents for over 300 conditions and diseases*. 1994, Emmaus, Pa.; [New York]: Rodale Press

61. Brantner, A., et al., *Antimicrobial activity of Paliurus spina-christi Mill. (Christ's thorn).* J Ethnopharmacol, 1996. 52(2): 119-22.

62. Thomson, W.A.R., *Medicines from the earth : a guide to healing plants*. 1978, New York: McGraw-Hill.

63. Duke, J.A., *Handbook of medicinal herbs*. 1985, Boca Raton: CRC Press.

64. Scalbert, A., *Antimicrobial properties of tannins.* Phytochemistry, 1991. 30(12): 3875-3883.

65. Rama Raje Urs, N.V., *Enhancement of the Bactericidal Activity of a Peroxidase System by Phenolic Compounds.* Phytopathology Phytopathology, 1975. 65(6).

66. Stern, J.L., et al., *Phlorotannin-Protein Interactions.* Journal of chemical ecology., 1996. 22(10): 1877.

67. Fessenden, R.J. and J.S. Fessenden, *Organic chemistry*. 1982, Boston, Mass.: W. Grant Press.

68. Dixon, R.A., P.M. Dey, and C.J. Lamb, *Phytoalexins: enzymology and molecular biology.* Adv Enzymol Relat Areas Mol Biol, 1983. 55: 1-136.

69. Haslam, E., *Natural polyphenols (vegetable tannins) as drugs: possible modes of action.* Journal of natural products, 1996. 59(2): 205-15.

70. O'Kennedy, R. and R.D. Thornes, *Coumarins : biology, applications, and mode of action*. 1997, Chichester; New York: Wiley.

71. Thastrup, O., et al., *Inhibitions of human platelet aggregation by dihydropyrano- and dihydrofuranocoumarins, a new class of cAMP phosphodiesterase inhibitors.* Biochem. Pharmacol., 1985. 34: 2137–2140.

72. Piller, N.B., *A comparison of the effectiveness of some anti-inflammatory drugs on thermal oedema.* British journal of experimental pathology, 1975. 56(6): 554-60.

73. Namba, T., et al., *Studies on cardio-active crude drugs; I. Effect of coumarins on cultured myocardial cells.* Planta Med, 1988. 54(4): 277-82.

74. Casley-Smith, J.R., *Coumarin in the treatment of lymphoedema and other high-protein oedemas*, in *Coumarins: biology, applications and mode of action*, R. O'Kennedy and R.D. Thornes, Editors. 1997, John Wiley & Sons, Inc.: New York. 348.

75. Ahmed, A.A., et al., *New Sesquiterpene -Methylene Lactones from the Egyptian Plant Jasonia candicans.* Journal of natural products, 1993. 56(8): 1276.

76. Amaral, J.A., et al., *Effect of selected monoterpenes on methane oxidation, denitrification, and aerobic metabolism by bacteria in pure culture.* Appl Environ Microbiol, 1998. 64(2): 520-5.

77. Barre, J.T., et al., *A bioactive triterpene from Lantana camara.* Phytochemistry, 1997. 45(2): 321-4.

78. Habtemariam, S., A.I. Gray, and P.G. Waterman, *A new antibacterial sesquiterpene from Premna oligotricha.* J Nat Prod, 1993. 56(1): 140-3.

79. Himejima, M., et al., *Antimicrobial terpenes from oleoresin of ponderosa pine tree Pinus ponderosa: A defense mechanism against microbial invasion.* Journal of Chemical Ecology, 1992. 18:10: Pages: 1809-1818.

80. Kubo, I., H. Muroi, and M. Himejima, *Antibacterial activity of totarol and its potentiation.* J Nat Prod, 1992. 55(10): 1436-40.

81. Mendoza, L., M. Wilkens, and A. Urzúa, *Antimicrobial study of the resinous exudates and of diterpenoids and flavonoids isolated from some Chilean Pseudognaphalium (Asteraceae).* Journal of ethnopharmacology, 1997. 58(2): 85-8.

82. Scortichini, M. and M.P. Rossi, *Preliminary in vitro evaluation of the antimicrobial activity of terpenes and terpenoids towards Erwinia amylovora (Burrill) Winslow et al.* J Appl Microbiol Journal of Applied Microbiology, 1991. 71(2): 109-112.

83. Tassou, C.C., E.H. Drosinos, and G.J. Nychas, *Effects of essential oil from mint (Mentha piperita) on Salmonella enteritidis and Listeria monocytogenes in model food systems at 4 degrees and 10 degrees C.* The Journal of applied bacteriology, 1995. 78(6): 593-600.

84. Taylor, R.S., et al., *Antimicrobial activities of southern Nepalese medicinal plants.* J Ethnopharmacol, 1996. 50(2): 97-102.

85. Ayafor, J.F., et al., *Novel bioactive diterpenoids from Aframomum aulacocarpos.* J Nat Prod, 1994. 57(7): 917-23.

86. Harrigan, G.G., et al., *Bioactive and other sesquiterpenoids from Porella cordeana.* J Nat Prod, 1993. 56(6): 921-5.

87. Kubo, I., H. Muroi, and M. Himejima, *Combination effects of antifungal nagilactones against Candida albicans and two other fungi with phenylpropanoids.* J Nat Prod, 1993. 56(2): 220-6.

88. Rana, B.K., U.P. Singh, and V. Taneja, *Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of Aegle marmelos.* J Ethnopharmacol, 1997. 57(1): 29-34.

89. Rao, K.V., et al., *Two new sesquiterpene lactones from Ceiba pentandra.* J Nat Prod, 1993. 56(12): 2041-5.

90. Suresh, B., et al., *Anticandidal activity of Santolina chamaecyparissus volatile oil.* J Ethnopharmacol, 1997. 55(2): 151-9.

91. Fujioka, T., et al., *Anti-AIDS agents, 11. Betulinic acid and platanic acid as anti-HIV principles from Syzigium claviflorum, and the anti-HIV activity of structurally related triterpenoids.* Journal of natural products, 1994. 57(2): 243-7.

92. Hasegawa, H., et al., *Inhibitory effect of some triterpenoid saponins on glucose transport in tumor cells and its application to in vitro cytotoxic and antiviral activities.* Planta medica, 1994. 60(3): 240-3.

93. Pengsuparp, T., et al., *Pentacyclic triterpenes derived from Maprounea africana are potent inhibitors of HIV-1 reverse transcriptase.* J Nat Prod, 1994. 57(3): 415-8.

94. Sun, H.D., et al., *Nigranoic acid, a triterpenoid from Schisandra sphaerandra that inhibits HIV-1 reverse transcriptase.* Journal of natural products, 1996. 59(5): 525-7.

95. Xu, H.X., et al., *Anti-HIV triterpene acids from Geum japonicum.* J Nat Prod, 1996. 59(7): 643-5.

96. Ghoshal, S., B.N. Prasad, and V. Lakshmi, *Antiamoebic activity of Piper longum fruits against Entamoeba histolytica in vitro and in vivo.* J Ethnopharmacol, 1996. 50(3): 167-70.

97. Vishwakarma, R.A., *Stereoselective Synthesis of a-Arteether from Artemisinin.* J. Nat. Prod. Journal of Natural Products, 1990. 53(1): 216-217.

98. Chaurasia, S.C. and K.K. Vyas., *In vitro effect of some volatile oil against Phytophthora parasitica var. piperina.* J. Res. Indian Med., 1977. 1977: 24–26.

99. Atta ur, R. and M.I. Choudhary, *Diterpenoid and steroidal alkaloids.* Nat Prod Rep, 1995. 12(4): 361-79.

100. Omulokoli, E., B. Khan, and S.C. Chhabra, *Antiplasmodial activity of four Kenyan medicinal plants.* J Ethnopharmacol, 1997. 56(2): 133-7.

101. McMahon, J.B., et al., *Michellamine B, a novel plant alkaloid, inhibits human immunodeficiency virus-induced cell killing by at least two distinct mechanisms.* Antimicrobial agents and chemotherapy, 1995. 39(2): 484-8.

102. Sethi, M.L., *Inhibition of reverse transcriptase activity by benzophenanthridine alkaloids.* J Nat Prod, 1979. 42(2): 187-96.

103. McDevitt, J.T., et al. *Berberine: a candidate for the treatment of diarrhea in AIDS patients, abstr.* in *Program and Abstracts of the 36th Interscience Conference on*

Antimicrobial Agents and Chemotherapy. 1996. Washington, D.C.: American Society for Microbiology.

104. Zhang, Y. and K. Lewis, *Fabatins: new antimicrobial plant peptides.* FEMS Microbiol Lett, 1997. 149(1): 59-64.

105. Terras, F., et al., *Synergistic Enhancement of the Antifungal Activity of Wheat and Barley Thionins by Radish and Oilseed Rape 2S Albumins and by Barley Trypsin Inhibitors.* Plant Physiol, 1993. 103(4): 1311-1319.

106. Sharon, N. and I. Ofek, *Mannose specific bacterial surface lectins*, in *Microbial lectins and agglutinins*, D. Mirelman, Editor. 1986, John Wiley & Sons, Inc.: New York, N.Y. 55–82.

107. De Bolle, M.F., et al., *Antimicrobial peptides from Mirabilis jalapa and Amaranthus caudatus: expression, processing, localization and biological activity in transgenic tobacco.* Plant Mol Biol, 1996. 31(5): 993-1008.

108. Livermore, D.M., *Bacterial resistance: origins, epidemiology, and impact.* Clin Infect Dis, 2003. 36(Suppl 1): S11-23.

109. Coates, A., et al., *The future challenges facing the development of new antimicrobial drugs.* Nat Rev Drug Discov, 2002. 1(11): 895-910.

110. McDevitt, D. and M. Rosenberg, *Exploiting genomics to discover new antibiotics.* Trends Microbiol, 2001. 9(12): 611-7.

111. Spellberg, B., et al., *Trends in antimicrobial drug development: implications for the future.* Clin Infect Dis, 2004. 38(9): 1279-86.

112. Labischinski, H., *New antibiotics.* Int J Med Microbiol, 2001. 291(5): 317-8.

113. Abbanat, D., M. Macielag, and K. Bush, *Novel antibacterial agents for the treatment of serious Gram-positive infections.* Expert Opin Investig Drugs, 2003. 12(3): 379-99.

114. DiMasi, J.A., R.W. Hansen, and H.G. Grabowski, *The price of innovation: new estimates of drug development costs.* J Health Econ, 2003. 22(2): 151-85.

115. Poole, K., *Overcoming multidrug resistance in gram-negative bacteria.* Curr Opin Investig Drugs, 2003. 4(2): 128-39.

116. Craig, W.J., *Health-promoting properties of common herbs.* Am J Clin Nutr, 1999. 70(3 Suppl): 491S-499S.

117. Aruoma, O.I., *Free Radicals, Oxidative Stress, and Antioxidants in Human Health and Disease.* The journal of the American Oil Chemists' Society., 1998. 75(2): 199.

118. Lefer, D.J. and D.N. Granger, *Oxidative stress and cardiac disease.* Am J Med, 2000. 109(4): 315-23.

119. Bhatia, S., et al., *Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with nephropathy.* Clin Biochem, 2003. 36(7): 557-62.

120. Peuchant, E., et al., *Oxidative and antioxidative status in pregnant women with either gestational or type 1 diabetes.* Clinical biochemistry, 2004. 37(4): 293-8.

121. Smith, M.A., et al., *Oxidative stress in Alzheimer's disease.* Biochim Biophys Acta, 2000. 1502(1): 139-44.

122. Steer, P., et al., *Cardiac and vascular structure and function are related to lipid peroxidation and metabolism.* Lipids, 2002. 37(3): 231-6.

123. Uchida, K., *Role of reactive aldehyde in cardiovascular diseases.* Free Radic Biol Med, 2000. 28(12): 1685-96.

124. Shahidi, F. and P.K. Wanasundara, *Phenolic antioxidants.* Crit Rev Food Sci Nutr, 1992. 32(1): 67-103.

125. Gerber, M., et al., *Food and Cancer: state of the art about the protective effect of fruits and vegetables.* Bull Cancer, 2002. 89: 293–312.

126. Di Matteo, V. and E. Esposito, *Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis.* Curr Drug Targets CNS Neurol Disord, 2003. 2(2): 95-107.

127. Sreejayan, N. and M.N. Rao, *Free radical scavenging activity of curcuminoids.* Arzneimittelforschung, 1996. 46(2): 169-71.

128. Knekt, P., et al., *Flavonoid intake and coronary mortality in Finland: a cohort study.* BMJ, 1996. 312(7029): 478-81.

129. Sies, H., *Strategies of antioxidant defense.* Eur J Biochem, 1993. 215(2): 213-9.

130. Sherwin, E.R., *Antioxidants*, in *Food antioxidants*, A. L. Branen, P. M. Davidson, and S. Salminen, Editors. 1990, Marcel Dekker Inc.: New York.

131. Wanasundara, U.N. and F. Shahidi, *Antioxidant and pro-oxidant activity of green tea extracts in marine oils.* Food chemistry., 1998. 63(3): 335.

132. Moure, A., et al., *Natural antioxidants from residual sources.* Food chemistry., 2001. 72(2): 145-171.

133. Dugo, L., Y. Negis, and A. Azzi, *Antioxidants*, in *Encyclopedia of Life Sciences.* 2001, John Wiley & Sons, Ltd.: Chichester.

134. Harborne, J.B., *Flavonoids in the environment: structure-activity relationships.* Prog Clin Biol Res, 1988. 280: 17-27.

135. Lotito, S.B. and B. Frei, *The increase in human plasma antioxidant capacity after apple consumption is due to the metabolic effect of fructose on urate, not apple-derived antioxidant flavonoids.* Free radical biology & medicine, 2004. 37(2): 251-8.

136. Williamson, G. and C. Manach, *Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies.* Am J Clin Nutr, 2005. 81(1 Suppl): 243S-255S.

137. Krinsky, N.I., *The antioxidant and biological properties of the carotenoids.* Ann N Y Acad Sci, 1998. 854: 443-7.

138. Britton, G., *Structure and properties of carotenoids in relation to function.* FASEB J, 1995. 9(15): 1551-8.

139. Tanvetyanon, T. and G. Bepler, *Beta-carotene in multivitamins and the possible risk of lung cancer among smokers versus former smokers: A meta-analysis and evaluation of national brands.* Cancer Cancer, 2008. 113(1): 150-157.

140. Rigotti, A., *Absorption, transport, and tissue delivery of vitamin E.* Mol Aspects Med, 2007. 28(5-6): 423-36.

141. Iuliano, L., et al., *Bioavailability of vitamin E as function of food intake in healthy subjects: effects on plasma peroxide-scavenging activity and cholesterol-oxidation products.* Arteriosclerosis, thrombosis, and vascular biology, 2001. 21(10): 34-7.

142. De Smet, P.A., *Health risks of herbal remedies: an update.* Clin Pharmacol Ther, 2004. 76(1): 1-17.

143. De Smet, P.A., *Health risks of herbal remedies.* Drug Saf, 1995. 13(2): 81-93.

144. De Smet, P.A.G.M., *Adverse effects of herbal remedies.* Adverse Drug Reaction Bulletin Adverse Drug Reaction Bulletin, 1997. 183(1): 695-698.

145. Shaw, D., et al., *Traditional remedies and food supplements. A 5-year toxicological study (1991-1995).* Drug Saf, 1997. 17(5): 342-56.

146. Chitturi, S. and G.C. Farrell, *Herbal hepatotoxicity: an expanding but poorly defined problem.* J Gastroenterol Hepatol, 2000. 15(10): 1093-9.

147. Pittler, M.H. and E. Ernst, *Systematic review: hepatotoxic events associated with herbal medicinal products.* Aliment Pharmacol Ther, 2003. 18(5): 451-71.

148. *The Medicines for Human Use (Kava-kava) (Prohibition) Order, 2002*, S. Instrument, Editor. 2002.

149. Russell, W.M.S. and R.L. Burch, *The principles of humane experimental technique.* 1959, London: Methuen.

150. Buckberry, L.D., *Cytotoxicity Testing Using Cell Lines*, in *Animal Cell Biotechnology : Methods and Protocols*, N. Jenkins, Editor. 1999, Humana Press: Totowa. 239-252

151. Eisenbrand, G., et al., *Methods of in vitro toxicology.* Food Chem Toxicol, 2002. 40(2-3): 193-236.

152. Spielmann, H., *Reproduction and development.* Environ Health Perspect, 1998. 106 Suppl 2: 571-6.

153. Borenfreund, E., H. Babich, and N. Martin-Alguacil, *Comparisons of two in vitro cytotoxicity assays-The neutral red (NR) and tetrazolium MTT tests.* Toxicol In Vitro, 1988. 2(1): 1-6.

154. *ADIANTUM CAPILLUS -VENERIS L.* Medicinal Plants of Bangladesh [cited 2011 1st June]; Available from: http://www.mpbd.info/plants/adiantum-capillus-veneris.php.

155. *Database File for: AVENCA (Adiantum capillus-veneris).* Tropical Plant Database [cited 2011 1st June]; Available from: http://www.rain-tree.com/avenca.htm.

156. *BLUMEA LACERA (Burn.f.) DC.* Medicinal Plants of Bangladesh [cited 2011 1st June]; Available from: http://www.mpbd.info/plants/blumea-lacera.php.

157. Oudhia, P. *Blumea lacera: Useful Plant.* New Crop Resource Online Program 2001 [cited 2011 1st June]; Available from: http://www.hort.purdue.edu/newcrop/CropFactSheets/blumea.html.

158. Baker, J.G. *Entry for CASSIA alata Linn, [family LEGUMINOSAE-CAESALPINIOIDEAE].* JSTOR Plant Science [cited 2011 1st June]; Available from: http://plants.jstor.org/flora/flota002408.

159. *Database File for: GUAJAVA (Cassia alata).* Tropical Plant Database [cited 2011 1st June]; Available from: http://www.rain-tree.com/guajava.htm.

160. *Cissus quadrangularis.* Wikipedia [cited 2011 1st June]; Available from: http://en.wikipedia.org/wiki/Cissus_quadrangularis.

161. Mishra, G., S. Srivastava, and B.P. Nagori, *Pharmacological and therapeutic activity of Cissus quadrangularis: An overview.* Int. J. Pharm. Res. International Journal of PharmTech Research, 2010. 2(2): 1298-1310.

162. Oben, J.E., et al., *The effect of Cissus quadrangularis (CQR-300) and a Cissus formulation (CORE) on obesity and obesity-induced oxidative stress.* Lipids Health Dis, 2007. 6: 4.

163. Jainu, M., K.V. Mohan, and C.S. Devi, *Protective effect of Cissus quadrangularis on neutrophil mediated tissue injury induced by aspirin in rats.* J Ethnopharmacol, 2006. 104(3): 302-5.

164. Greenway, F.L. and G.A. Bray, *Combination drugs for treating obesity.* Curr Diab Rep, 2010. 10(2): 108-15.

165. Rios, J.L. and M.C. Recio, *Medicinal plants and antimicrobial activity.* J Ethnopharmacol, 2005. 100(1-2): 80-4.

166. Eloff, J.N., *Which extractant should be used for the screening and isolation of antimicrobial components from plants?* Journal of ethnopharmacology, 1998. 60(1): 1-8.

167. Yanishlieva, N.V., E. Marinova, and J. Pokorný, *Natural antioxidants from herbs and spices.* Eur. J. Lipid Sci. Technol. European Journal of Lipid Science and Technology, 2006. 108(9): 776-793.

168. Schmitt, L.A., et al., *Evaluation of the light-sensitive cytotoxicity of Hypericum perforatum extracts, fractions, and pure compounds.* J Agric Food Chem, 2006. 54(8): 2881-90.

169. Shahidi, F., *Extraction and Measurement of Total Lipids*, in *Current Protocols in Food Analytical Chemistry*, R.E. Wrolstad, Editor. 2001, John Wiley & Sons, Inc.

170. Bauer A. W., et al., *Antibiotic susceptibility testing by a standardized single disk method.* American Journal of Clinical Pathology, 1966. 45(4): 493-496.

171. Janssen, A.M., J.J. Scheffer, and A. Baerheim Svendsen, *Antimicrobial activity of essential oils: a 1976-1986 literature review. Aspects of the test methods.* Planta Med, 1987. 53(5): 395-8.

172. Kalodera, Z., et al., *Chemical composition and antimicrobial activity of Tanacetum parthenium essential oil.* Die Pharmazie, 1997. 52(11): 885-6.

173. Mason, A.D., Jr., A.T. McManus, and B.A. Pruitt, Jr., *Association of burn mortality and bacteremia. A 25-year review.* Arch Surg, 1986. 121(9): 1027-31.

174. Nathan, P., et al., *A laboratory method for selection of topical antimicrobial agents to treat infected burn wounds.* Burns Burns, 1978. 4(3): 177-187.

175. Hangnga Vu, et al., *Burn Wound Infection Susceptibilities To Topical Agents: The Nathan's Agar Well Diffusion Technique.* P&T, 2002. 27(8): 390-396.

176. Brand-Williams, W., M.E. Cuvelier, and C. Berset, *Use of a free radical method to evaluate antioxidant activity.* LWT - Food Science and Technology, 1995. 28(1): 25-30.

177. Bacon, J.A., D.A. Linseman, and T.J. Raczniak, *In vitro cytotoxicity of tetracyclines and aminoglycosides in LLC-PK(1), MDCK and Chang continuous cell lines.* Toxicology in vitro : an international journal published in association with BIBRA, 1990. 4(4-5): 4-5.

178. Halliday, D.C.T., et al., *Cane toad toxicity: An assessment of extracts from early developmental stages and adult tissues using MDCK cell culture.* Toxicon Toxicon, 2009. 53(4): 385-391.

179. Wiegand, I., K. Hilpert, and R.E. Hancock, *Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances.* Nat Protoc, 2008. 3(2): 163-75.

180. International Trade Centre, U.G., *Markets for selected medicinal plants and their derivatives*. 1974, Geneva: International Trade Centre.

181. Gurib-Fakim, A., *Medicinal plants: traditions of yesterday and drugs of tomorrow.* Mol Aspects Med, 2006. 27(1): 1-93.

182. Galal, M., et al., *Activity of water extracts of Albizzia anthelmintica and A. lebbek barks against experimental Hymenolepis diminuta infection in rats.* J Ethnopharmacol, 1991. 31(3): 333-7.

183. Hoffmann, J.J., et al., *Potential antimicrobial activity of plants from the south western United states.* International Journal of Pharmacology, 1993. 31: 101–115.

184. Sati, S.C., et al., *Medicinal plants as a source of antioxidants.* Res. J. Phytochem. Research Journal of Phytochemistry, 2010. 4(4): 213-224.

185. Singh, M., et al., *Antimicrobial activity of some important Adiantum species used traditionally in indigenous systems of medicine.* J. Ethnopharmacol. Journal of Ethnopharmacology, 2008. 115(2): 327-329.

186. ANILKUMAR, *ANTIOXIDANT EFFECT OF ADIANTUM CAPILLUS VENERIS LINN. ON HUMAN LYMPHOCYTE : AN IN VITRO STUDY.* Journal of Cell and Tissue Research, 2009. 9(2): 1899-1902.

187. F. Pourmorad, S.J.H.N.S., *Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants.* African Journal of Biotechnology, 2006. 5(11): 1142-1145.

188. B Singh, et al., *Ethnobotanical Significance and Antimicrobial Activity of Blumea lacera (Roxb.) DC.* International Journal of Pharmaceutical & Biological Archives, 2010. 1(3): 314 - 316.

189. Ragasa, C., J. Wong, and J. Rideout, *Monoterpene glycoside and flavonoids from Blumea lacera.* Journal of Natural Medicines, 2007. 61(4): 474-475.

190. Uddin, S.J., I.D. Grice, and E. Tiralongo, *Cytotoxic Effects of Bangladeshi Medicinal Plant Extracts.* Evid Based Complement Alternat Med, 2009.

191. Ibrahim, D. and H. Osman, *Antimicrobial activity of Cassia alata from Malaysia.* J Ethnopharmacol, 1995. 45(3): 151-6.

192. Khan, M.R., M. Kihara, and A.D. Omoloso, *Antimicrobial activity of Cassia alata.* Fitoterapia, 2001. 72(5): 561-4.

193. Somchit, M.N., et al., *In vitro antimicrobial activity of ethanol and water extracts of Cassia alata.* J Ethnopharmacol, 2003. 84(1): 1-4.

194. Olarte, E.I., et al., *Antioxidant Activity of a New Indole Alkaloid from Cassia alata L.* The Philippine Agricultural Scientist, 2010. 93(3): 250-254.

195. Phansawan, B. and S. Poungbangpho, *Antioxidant Capacities of Pueraria mirifica, Stevia rebaudiana Bertoni, Curcuma longa Linn., Andrographis paniculata (Burm.f.) Nees. and Cassia alata Linn. for the Development of Dietary Supplement.* Kasetsart J. (Nat. Sci.), 2007. 41: 548 - 554.

196. Olarte, E., et al., *Selective cytotoxicity of an isolate from Cassia alata L. leaves.* European Journal of Cancer Supplements European Journal of Cancer Supplements, 2006. 4(1): 59-60.

197. Kashikar, N.D. and I. George, *Antibacterial Activity of Cissus quadrangularis Linn.* Indian journal of pharmaceutical sciences., 2006. 68(2): 245-247.

198. Chidambara Murthy, K.N., et al., *Antioxidant and antimicrobial activity of Cissus quadrangularis L.* J Med Food, 2003. 6(2): 99-105.

199. Attawish, A., et al., *Subchronic toxicity of Cissus quadrangularis Linn.* Songklanakarin J. Sci. Technol., 2002. 24(1): 39-51.