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Antimicrobial and Antioxidant Investigations of Methanolic Extract of *Dracaena spicata*

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACY, EAST WEST UNIVERSITY IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF PHARMACY

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Declaration by the research candidate

I, AYESHA SIDDIKA MONNI, hereby declare that the dissertation entitled "**Antimicrobial and antioxident investigation of methanolic extract of *Dracaena spicata***" submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a complete record of original research work carried out by me during 2015, under the supervision and guidance of Nazia Hoque, Senior Lecturer, Department of Pharmacy, East West University and the thesis has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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This is to certify that the thesis entitled "**Antimicrobial and antioxident investigations of Methanolic Extract of *Dracaena spicata***" submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of pharmacy was carried out by AYESHA SIDDIKA MONNI, ID# 2011-3-70-041 in 2015, under the supervision and guidance of me. The thesis has not formed the basis for the award of any other dgree/diploma/fellowship or other similar title to any candidate of any university.

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This is to certify that the thesis entitled "**Antimicrobial and antioxident investigations of methanolic extract of *Dracaena spicata***" submitted to the Department of Pharmacy, East West University, Dhaka, in the partial fulfillment of the requirement for the degree of Bachelor of pharmacy was carried out by AYESHA SIDDIKA MONNI, ID# 2011-3-70-041 in 2015 under the supervision and guidance of Nazia Hoque, senior lecturer, department of pharmacy, East West University.

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Dedication

Dedicated to my Parents & my Family Members

Abstract

Indigenous knowledge of herbal medicines for skin diseases like boils, eczema, scabies, septic abscess, itching and skin allergy, burns, chicken pox, warts and leucoderma, fungal and bacterial infections, including healing cuts and wounds has been documented by randomly interviewing Chakma, Marma and Tanchunga tribes of the hill tracts districts of Bangladesh since 1995.

The plant *Dracaena spicata* has been used for the general promotion of health and longevity by Asian tribal(specially Chakma, Marma and Tanchunga). It is used as a traditional medicine for the treatment of various diseases cough, syphilis , conjunctivitis, constipation, pills prepared from the leaves are taken with warm water twice daily for the treatment of measles by the Chakma etc.

The aim of the present study was to evaluate the antimicrobial activity and antioxidant activity of methanolic extract of *Dracaena spicata.*

The antimicrobial activities of methanolic solvent extract of *Dracaena spicata* plant were tested against the gram-positive and gram-negative bacterial strains by observing the zone of inhibition. The antimicrobial test was performed by disc diffusion method. The crude methanolic extract of *Dracaena spicata* plant showed poor antimicrobial activities against the microorganisms at concentrations of 600 μg/disc. However, no activity was found against *Bacillus megaterium, Salmonella paratyphi.*

The antioxidant effect of methanolic extract of *Dracaena spicata* was determined by calculating reducing power of the content. Reducing power was determined by using ascorbic acid as standard. By determining antioxidant property, the present result suggests that the tested plant extracts have potent antioxidant activity. In conclusion, further investigations are needed to identify the active constituents and the exact mechanism(s) of action responsible for the reported antimicrobial and antioxidant properties of *Dracaena spicata.*

Key Words: *Dracaena spicata,* Antimicrobial, Antioxidant.

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INTRODUCTION

1.1: General Introduction

Death is authentic but unavoidable. Nobody can desire to lose his short but sweet life. Man is therefore, being continued his struggle to achieve mastery over the forces of nature- Diseases Decay and Death. Human struggle against the misery of three D‟ s-Disease, Decay and Death is eternal. From the very inception of civilization, the inherent concern for getting as well as staying healthy has been instigating human venture for cure from his surroundings. Illness, physical discomforts, injuries, wounds & fear of death had forced prehistoric man to use any natural substances that he/she could lay his/her hands on- "the green friends" PLANTs (Ogdan,1981). Plants, besides providing nutrition, have always formed an important source of chemical compounds, which can be used for medicinal purposes. Human knowledge of the medicinal value of plants date back probably for more than five thousand years (Sofowora 1982). Medicinal properties of plants were known even to pre-historic men and many of these plants have been used in traitional medicine for hundreds of years with reputation as efficacious remedies (Ghani, 1998).). According to the WHO, about 80% of the world's population relies on traditional medicine for their primary health care (Behera, 2006). Over the last century, ethnobotany has evolved into a specific discipline that looks at the people–plant relationship in a multidisciplinary way such as ecology,economic botany, pharmacology, public health and other disciplines as needed (Balick, 1996). A large number of plants are being used as medicinal agents all over the world. 1500 species in India (Handa, 1998), 5000 species in China (WHO, 2003) and 1600 species in north-west Amazonia (Schultes and Raffauf, 1990) have been reported to possess medicinal uses. Limitations o diseases and the potential of plant based medicine as a more effective and cheaper alternative was probably responsible for the fast growing industry of herbal medicine (Rojas et al., 1992). Many drugs that are currently in the market have come from folk medicine and traditional use of plants by indigenous communities (Prance, 1994). Discovering the cardiac effect of the leaves of *Digitalis purpurea* that were useful for treating dropsy is the best example of folk use based herbal medicine (Cox, 1994). About 25% of the prescription drugs issued in the USA and Canada contain bioactive compounds that are derived from or modeled after plant natural products (Farnsworth, 1984).

1.1: The Medicinal Plants contribution in the New World

Just Before Modern Medicine: At the early of modern medicine the Muslim physicians were done a great job. The Arabian Muslim physicians, like Al-Razi and IbnSina (9th to 12th century AD), brought about a revolution in the history of medicine by bringing new drugs of plant and mineral origin into general use. Al Razi‟ s important books are: Qitab-al-Mansuri, Al-Hawai, Qitab-al-Muluki, Qitab-al-Judari-wal-Hasabah, Maan La YahoduruhoTibb etc.The famous medical book, Al-Kanun, of IbnSina was the prescribed book of medicine in the schools of western medicine for several centuries (Mian&Ghani., 1990).

The use of medicinal plants in Europe in the 13th and 14th centuries was based on the Doctrine of Signatures or Similar developed by Paracelsus (1490-1541 AD), aswiss alchemist and physician (Murray, 1995). The South American countries have provided the world with many useful medicinal plants, grown naturally in their forests and planted in the medicinal plant gardens. Use of medicinal plants like coca (Erythroxylum species) and tobacco (Nicotianatabacum) was common in these countries in the 14th and 15th centuries. The earliest mention of the medicinal use of plants in the Indian subcontinent is found in the Rig Veda (4500-1600 BC). It supplies various information of the medicinal use of plants in the Indian subcontinent (Hill, 1972). Medicinal plants used by the Australian aborigines many centuries ago tremendously enriched the stock of medicinal plants of the world. The current list of the medicinal plants growing around the world includes more than a thousand items (Sofowora, A., 1982).

1.1.1: Medicinal plants

Any plant whose roots, leaves, seeds, bark, or plant part is used for therapeutic, tonic, purgative, or other health-promoting purposes. plants used as natural medicines.

Accordingly, the WHO consultative group on medicinal plants has formulated a definition of medicinal plants in the following way: " A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which is a precursor for synthesizing of useful drugs" (Sofowara, 1982).

Modern approaches to determining the medicinal properties of plants involve collaborative efforts that can include ethnobotanists, anthropologists, pharmaceutical chemists, and physicians. Many modern medicines had their origin in medicinal plants. Examples include aspirin from willow bark *(Salix spp*.), digitalis from foxglove (*Digitalis purpurea*), and vinblastine from Madagascar periwinkle (*Vinca rosea*) for the treatment of childhood leukemia .

1.1.2: Importance of medicinal plants

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world.

There are three ways in which plants have been found useful in medicine.

First, they may be used directly as teas or in other extracted forms for their natural chemical constituents.

Second, they may be used as agents in the synthesis of drugs.

Finally, the organic molecules found in plants may be used as models for synthetic drugs. Historically, the medicinal value of plants was tested by trial and error, as in the Doctrine of Signatures.

Others: Importance of medicinal plants

- Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin.
- Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.
- Many food crops have medicinal effects, for example garlic.
- Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species.

- Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.
- Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants (Britannica 2013).

1.1.3: Classification of medicinal plants

Of the 2,50,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value. They are classified according to the part used, habit, habitat, therapeutic value etc, besides the usual botanical classification (Joy et al. 1998).

Table - 1: Classification of medicinal plants (Joy et al. 1998)

Based on part used	**1. Whole plant: diffusa, *Phyllanthus neruri*** **2. Root: Dasamula** **3. Stem:*Tinospora cordifolia, Acorus calamus*** **4. Bark: *Saraca asoca*** **5. Leaf: *Indigofera tinctoria, Lawsonia inermis*, Aloe vera** **6. Flower:*Biophytum sensityvum, Mimusops elenji*** **7. Fruit: Solanum species** **8. Seed: *Datura stramonium***

Based on habitat	1. Tropical: *Andrographis paniculata* 2. Sub-tropical: *Mentha arvensis* 3. Temperate: *Atropa belladonna*
Based on therapeutic value	1. Antimalarial: *Cinchona officinalis, Artemisia annua* 2. Anticancer: *Catharanthus roseus, Taxus baccata* 3. Antiulcer: *Azadirachta indica, Glycyrrhiza glabra* 4. Antidiabetic: *Catharanthus roseus, Momordica charantia* 5. Anticholesterol: *Allium sativum* 6. Antiinflammatory: *Curcuma domestica, Desmodium gangeticum* 7. Antiviral: *Acacia catechu* 8. Antibacterial: *Plumbago indica* 9. Antifungal: *Allium sativum* 10. Antiprotozoal: *Ailanthus sp., Cephaelis ipecacuanha* 11. Antidiarrhoeal: *Psidium gujava, Curcuma domestica* 12. Hypotensive: *Coleus forskohlii, Alium sativum* 13. Tranquilizing: *Rauvolfia serpentina* 14. Anaesthetic: *Erythroxylum coca* 15. Spasmolytic: *Atropa belladona, Hyoscyamus niger* 16. Diuretic: *Phyllanthus niruri, Centella asiatica* 17. Astringent: *Piper betle, Abrus precatorius* 18. Anthelmentic: *Quisqualis indica, Punica granatum* 19. Cardiotonic: *Digitalis sp., Thevetia sp.* 20. Antiallergic: *Nandina domestica, Scutellaria baicalensis* 21. Hepatoprotective: *Silybum marianum, Andrographis paniculata*

1.1.4: Traditional medicine

Bangladesh possesses a rich flora of medicinal plants. Out of the estimated 5000 species of different plants growing in this country more than a thousand are regarded as having medicinal properties. Use of these plants for therapeutic purposes has been in practice in this country since time immemorial.

Because of their potentialities and close association with the culture and tradition of the people, traditional systems of medicine have assumed a unique position in the health care of the people living in even the remotest areas of the country. Although the use of traditional medicine is so deeply rooted in the cultural heritage of Bangladesh the concept, practice, type and method of application of traditional medicine vary widely among the different ethnic groups. Traditional medical practice among the tribal people is guided by their culture and life style and is mainly based on the use of plant and animal parts (Samy, Pushparaj & Gopalakrishankone 2008). Among the largest ethnic group, the bangles on the main land, there are two distinct forms of Traditional medicine practice:

1. **One is the old and original form based on old knowledge, experience and belief of the**

older generations. This includes:

- **Folk medicine,** which uses mainly plant and animal parts and their products as medicines for treating different diseases and also includes treatments like blood-letting , bone-setting, , hot and cold baths, therapeutic fasting and cauterization.

- **Religious medicine**, which includes use of verses from religious books written on papers and given as amulets, religious verses recited and blown on the face or on water to drink or on food to eat, sacrifices and offerings in the name of God and gods, etc.
- **Spiritual medicine,** which utilizes methods like communicating with the supernatural beings, spirits or ancestors through human media, torturous treatment of the patient along

with incantations to drive away the imaginary evil spirits and other similar methods.

1. The other is the improved and modified form based on the following two main traditional systems:

- **Unani-Tibb or Graeco-Arab system,** which has been developed by the Arab and Muslim scholars from the ancient Greek system, and
- **Ayurvedic system,** which is the old Indian system, based on the Vedas the oldest scriptures of the Hindu saints of the Aryan age (Ghani 1998).

Both the Unani and Ayurvedic systems of traditional medicine have firm roots in Bangladesh and are widely practiced all over the country. Apparently the recipients of these systems of medicine appear to be the rural people, but practically a good proportion of the urban population still continues to use these traditional medicines, although organized modern health care facilities are available to them.

As only a certain percentage of plants are used in traditional medicines, it is roughly estimated that of the discovered 17,000 species, nearly 3,000 species are used in medicinal field. Some crude drugs used as medicine in Bangladesh are reported in following table.

Table - 2: Some crude drugs used as medicine in Bangladesh (Samy, Pushparaj)

Common name	Botanical name	Uses
Amla	*Emblica officinalis*	Vitamin - C, Cough, Diabetes, Cold, Laxative, hyper acidity.
Ashok	*Saraca asoca*	Menstrual Pain, Uterine disorder, Diabetes.
Bael / Bilva	*Aegle marmelous*	Diarrhea, Dysentery, Constipation.
Chiraita	*Swertia chiraita*	Skin disease, Burning sensation, Fever.
Kalmegh/ Bhui neem	*Andrographis paniculata*	Fever, Weakness, Release of gas.
Long peeper / Pippali	*Peeper longum*	Appetizer, enlarged spleen, Bronchitis, Cold, antidote.
Pashan Bheda / Pathar Chur	*Coleus barbatus*	Kidney stone, Calculus.
Sandal Wood	*Santalum album*	Skin disorder, Burning sensation, Jaundice, Cough.
Satavari	*Asparagus racemosus*	Enhance lactation, Weakness, Fatigue and cough.

Senna	*Casia augustifolia*	General debility tonic, Aphrodisiac.
Tulsi	*Ocimum sanclum*	Cough, Cold, Bronchitis, Expectorant
Pippermint	*Mentha pipertia*	Digestive, Pain killer
Henna/Mehd	*Lawsennia iermis*	Burning, Steam, Anti Inflammatory
Gritkumari	*Aloe verra*	Laxative, Wound healing, Skin burns & care, Ulcer.
Sada Bahar	*Vincea rosea*	Leukemia, Hypotensive , Antispasmodic , Antidote.
Vringraj	*Eclipta alba*	Anti-inflammatory, Digestive, Hair tonic
Neem	*Azardirchata indica*	Sedative, Analgesic, Epilepsy, Hypertensive
Anantamool/sariva	*Hemibi smus indicus*	Appetizer, Carminative, Aphrodisiac, Astringent
Kantakari	*Solanum xanthocarpum*	Diuretic, Anti-inflammatory, Appetizer, Stomachic.

Shankhamul	*Geodorum denciflorum*	Antidiabetic.

1.1.5: Tribal medicine

In different localities of Rangamati and Bandarban Districts of Bangladesh a survey was carried out between 2001 and 2002 to document medicinal plants. A total of 69 medicinal plants under 40 families were documented during this work, which the tribal use to treat about 50 diseases (Yusuf et al. 2006). Some examples are given below-

Table - 3: Some tribal medicinal plants & their uses (Yusuf et al. 2006)

Scientific Name	Tribal name	Locality	Disease
Annona mouricata L.(Annonaceae)	Marma Penchi	Hangshamapara Bandarban	Pain in head & leg
Kalanchoe pinnata (Crassulaceae)	Tanchongya-Rockkia	Naramuk Rajasthali	Cough and asthma of children
Leea indica (Leeacea)	Chakma Haskura	Toolaban Marissa	Sore, leprosy, eczema, itching, bone fracture.
Croton caudatus Geisel (Euphorbiaceae)	Chakma Sholokjara	Toolaban marissa	Arthritis , paralysis
Eupatorium odoratum (Asteraceae)	Tonchongya Demrapata gach	Naramuk Rajsthali	Bleeding

1.1.6: Resource of natural products for establishment of new drugs

Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species. Nature has been a source of therapeutic agents and a significant number of modern drugs have been developed from natural sources, many based on their use in traditional medicine. Over the last century, a remarkable number of top selling drugs have been derived from natural products (Vincristine from *Catharanthus roseus*, morphine form *Papaver somniferum*, quinine and quinidine from *Cinchona spp*.) Nowadays, approximately 40% of the modern drugs have been developed from natural source (Sarker et al. 2006). More precisely, 39% of the 520 new approved drugs between 1983 and 1994 were natural products or their derivatives, and 60-80% of antibacterial and anti –cancer drugs were from natural origin. In 2000, approximately 60% of all drugs in clinical trials for the multiplicity of cancer had natural origin. In 2001, eight (simvastatin, pravastatin, amoxicillin, clavulanic acid, azithromycin, ceftriaxone, cyclosporine and paclitaxe)

l) Of the 30 top - selling medicines were natural products or their derivatives (Newman et al. 2007).

In light of all these facts, natural product drug discovery process failed to generate little respect. As drug discovery has emerged into a highly competitive era in which the quality of chemical collections and the time taken from assay to drug development are crucial factors in the success of a company, combinatorial chemistry has become the darling of the pharmaceutical industry, bringing with it the promise of new level of chemical diversity (Strohl 2000). But this adoption of new strategy by the pharmaceutical companies gained little momentum. Biotechnology companies working in the fields of combinatorial biosynthesis, genetic engineering and met genomic approaches to identify novel natural product lead molecules have met with limited success. These disappointments have led the pharmaceutical industry to consider whether natural product chemical diversity can or will continue to generate valuable templates for drug development (Baker et al. 2007).

Natural products offer a potentially infinite source of chemical diversity unparalleled to any synthetic chemical collection or combinatorial chemistry approach. In addition to that, these

potent natural product compounds can have astounding chemical structures that can lead to unexpected, alternative medicinal chemistry programs based on important biological targets (Strohl 2000). In the past few years, new natural products with a wide variety of chemical classes have been reported in the scientific literatures. Moreover, a total of 19 natural product based drugs were approved for marketing worldwide in between the year 2005 to April 2010, among which 7 being classified as natural products, 10 semi-synthetic natural products and 2 natural product derived drugs (Mishra & Tiwari 2011)

Table - 4: Natural product derived drugs launched during 2005-2010; lead compounds, and therapeutic area(Mishra & Tiwari 2011)

Year	Trade name	Lead compound	Therapeutic use
2005	Dronabinol (Sativex™)	Dronabinol	Pain
2005	Fumagillin (Flisint™)	Fumagillin	Antiparasitic
2005	Tigecycline (Tygacil™)	Tetracycline	Antibacterial
2005	Zotarolimus (Endeavor™)	Sirolimus	Cardiovascular
2006	Anidulafungin (Eraxis™)	Echinocandin	Anti-fungal
2006	Exenatide (Byetta™)	Exenatide-4	Diabetes
2007	Lisdexamfetamine (Vyvanse™)	Amphetamine	ADHD
2007	Temsirolimus (Torisel™)	Sirolimus	Oncology
2008	Methylnaltrexone (Relistor™)	Naltrexone	Pain
2009	Telavancin (Vibativ™)	Vancomycin	Antibacterial
2009	Romidepsin (Istodax™)	Romidepsin	Oncology
2010	Monobactam aztreonam (Cayston™)	Monobactam aztreonam	Antibacterial

1.1.7: Approaches of drug development

The major portion of the present day knowledge of the medicinal properties of plants is the sum total of some observations and experiences. According to some generous estimates, almost 80 percent of the present day medicines are directly or indirectly obtained from plants (Ghani 1998).

Steps of drug development from plant sources given below:

Selection of plant species:

- Preliminary screening of traditionally used plants
- Review literature and scientific result
- Authentication of data for their validity and comprehensiveness

Evaluation of toxicity:

- Gather data concerning toxicity and if demonstrate no toxicity then proceed to next step
- If toxicity data is not exit, select an appropriate test for toxicity analysis
- Develop and prepare bioassay protocol for safety and toxicity

Preparation of plant sample and element analysis:

- Collection of plant sample
- Extraction
    1. compare the selective and yield
    2. Use various extraction technique
- Analysis for elemental contents

Biological Testing:

- Selection of appropriate biological test
- Development protocol for biological test
- Analyze biological activity in- vivo
- Determine type and level of biological activity

Isolating active compounds:

- Isolating and characterization of compounds responsible for
- observed biological activity
- Evaluation of active compounds singularly and in combination with others to explore existence of activity and/or synergy of biological effect

In-vivo analysis:

- Use animal model for bioactivity analysis of active compounds
- Analyze again safety and toxicity but in in-vivo
- Conduct human studies

Commercialization:

- Develop appropriate dose delivery system
- Analyze cost-effectiveness
- Sustainable industrial production

1.3: Antimicrobial screening

Antimicrobial screening is performed to determine the susceptibility of the pathogenic microorganisms to test compound which, in turn is used to selection of the compound as a therapeutic agent. In general, antimicrobial screening in-vitro is undertaken in following two steps**:**

i) **Primary assay** It is essentially a qualitative or semi qualitative test that indicates the sensitivity or resistance of microorganisms to the compound. However this technique cannot be used to distinguish between bacteriostatic and bactericidal agents (Reiner et al. 1982). The primary assay can be performed in vitro by disk diffusion assay method**,** which includes

- ❖ Plate Diffusion test
- ❖ Streak test

The plate diffusion test utilizes different concentrations of a test compound absorbed on sterile filter paper disks on the same plate whereas the streak test permits the determination of the antibacterial effect of a test compound on several microorganisms simultaneously and is suitable for the estimation of the spectrum of the activity. However, the plate diffusion test is commonly used (Reiner et al. 1982).

ii) Secondary assay It quantifies the relative potency such as minimum inhibitory concentration (MIC). The lowest concentration of an antimicrobial agent required to inhibit the growth of the microorganisms in vitro is referred to as minimum inhibitory concentration (MIC). It is done by serial dilution technique (Reiner et al. 1982).

1.3.1: Antimicrobial drug

Antimicrobial drug/Antibiotics are the greatest contribution at the present century at therapeutic. Antibiotics are special kind of chemotherapeutic agent usually obtained from living organism.

The term chemotherapeutic agent means "All chemical substance that destroy all kind of cell

wall such as bacterial cell wall, viral cell wall even human cell wall". Antibiotics one kind of chemotherapeutic agent, but it does not destroy the human cell wall, it destroy the bacterial & viral cell wall. So all antibiotics are chemotherapeutic agent but all chemotherapeutic agents are not antibiotic. The word antibiotic come to refer to a metabolic of one microorganism that is very small amount is detrimental or inhibitory to their microorganism. The term antibiosis was first defined by Guillemin in 1889.The first systematic search for & study of antibiotics made by Gratia & both about 1924. In 1929 Alexander Fleming discovers one kind of antibiotics named by penicillin from the penicillium tree (Cui et al. 2011).

Characteristics of antibiotic: To be useful as chemotherapeutic agent antibiotics must have the following qualities:

(1) They should have the ability to destroy or inhibit many different species of pathogenic microorganism.

(2) They should prevent the ready development of resistant forms of the parasites.

(3) The should not produced undesirable side effects in the host, such as sensibility or allergic reaction, never damage or irritation of the kidneys & gastrointestinal tract(G.I.T).

(4) They should not eliminate the normal microbial flora of the host (Cui et al. 2011).

Antibiotic drugs are classified in several way, for example, some are bactericidal & some are bacteriostatic . Bactericidal means stop the bacterial growth & it also kill the bacteria and bacteriostatic mean stop the bacterial growth but cannot kill the bacteria.

1.3.2: Classification of antibiotics:

Antibiotic can be classified according to the chemical structure and their mode of action.

Table - 5: Classification of antibiotics (Barry et al. 1976)

Based on chemical structure	**Sulfonamide & relative drugs** **Diaminopyrimidines** **Quinolones** **β- lactam antibiotics** **Amino glycosides** **Polypeptide antibiotics** **Nitrofuran derivatives etc**
Based on mode of action	Inhibition of cell wall synthesis. Drug – penicillin, bacitracin. Damage to the cytoplasmic membrane. Drug – polymyxins, hamycin Inhibition of nucleic acid & protein synthesis. Drug – tetracycline, clindamycin Inhibition of specific enzyme system. Drug – pyridine , pyrimidine Interfere with DNA synthesis. Drug – Acyclovir Interfere with intermediary metabolism. Drug – Sulfonamides, PAS (Para amino salicylic acid)

1.4: Antioxidant activity

The main goal of antioxidant activity test is to find the oxidation- reducing power of the plant extract.

Oxidation in living organisms is essential for the acquirement of energy in catabolism. However, oxygen-centered free radicals and other reactive oxygen species, which are continuously, produced in vivo result in cell death and tissue damage. Oxidative damage caused by free radicals may be related to aging, and diseases such as atherosclerosis, diabetes, cancer and cirrhosis (Halliwell & Gutteridge 1999).

Free radicals are natural by-products of human metabolism. These are charged molecules which attack cells, breaking cellular membranes and reacting with the nucleic acids, proteins, and enzymes present in the cells. These attacks by free radicals, collectively known as oxidative stress, are capable of causing cells to lose their structure, function and eventually result in cell dysfunction. They are continuously produced by our body's use of oxygen, such as in respiration and some cell-mediated immune functions. Free radicals are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air pollution, pesticides, etc (Li & Trush 1994). Normally, there is a balance between the quantity of free radicals generated in the body and the antioxidant defense systems which scavenge these free radicals preventing them from causing deleterious effects in the body (Nose 2000). The antioxidant defense systems in the body can only protect the body when the quantity of free radicals is within the normal physiological level. But when this balance is shifted towards more free radicals, increasing their burden in the body either due to environmental conditions or infections, it leads to oxidative stress (Finkel & Holbrook 2000).

When the production of reactive oxygen species (ROS) exceeds the antioxidant capacity of the system, oxidative stress occurs in cellular system, including the superoxide anion radical, the hydroxyl radical, hydrogen peroxide and the peroxyl are greatly reactive molecules, which consequently generate metabolic products that attack lipids in cel1 membrane or DNA (Halliwell & Gutteridge 1999). Oxidative stress, involves a series of free radical chain reaction

processes, is associated with several types of biological damage, DNA damage, diabetes, respiratory tract disorders, carcinogenesis and cellular degeneration related to aging (Anderson et al. 2000). Continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them and cause irreversible oxidative damage (Tseng et al. 1997). Improved antioxidant status helps to minimize the oxidative damage and thus can delay or decrease the risk for developing many chronic age related, free radical induced diseases (Karuna et al. 2009). The interest in natural antioxidants, especially of plant origin, has greatly increased in recent years as the possibility of toxicity of synthetic antioxidants has been criticized. Plants (fruits, vegetables, medicinal herbs, etc.) may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity (Zheng & Wang 2001). Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a greater or lesser extent (Owen et al. 2000).

1.4.1: Types of Antioxidants

There are two types of antioxidants**:**

- **Natural Antioxidants** : Ascorbic acid, Gallic acid, Vitamin C & E, Coenzyme Q 10, Lipoic acid, Gluthatione
- **Synthetic Antioxidants**: Butylated hydroxyanisole (BHA), Butylated
- hydroxytoluene (BHT), Tert butylhydroquinone Propyl gallate

1.5: Plant review

Figure - 2: ***Dracaena spicata***

Scientific name: ***Dracaena Spicata Roxb.***

1.5.1. Family Asparagaceae - Century-plant family

1.5.2. Group: Monocot

1.5.3. Growth habit: Shrub

1.5.4. Duration: Annual

1.5.5Bangla/Vernacular Name: Dracaena

1.5.6 Tribal Name: Kadorateng gaas(chakama,Tanchangya

1.5.7. Description of the plant:

Caulescent, Leaves lanceolate, drooping, Spikes terminal, bracts many flowered, Corolcylindric, at last becoming twisted, Stigma three-lobed.

A native of Chittagong, and from thence introduced into this Garden by Dr. Buchanan, where it blossoms in april .Root fibrous, stem erect, toward the top succulent, perennial, marked with the cicatrices of the fallen leaves, as in the other *Dracaena*. Leaves crowded about the extremity of the plant,sheathing, lanceolate, drooping, entire, pointed; smooth on both sides; from six to twelve inches long, and two or three broad. Spikes terminal, bent a little to one side; numerous pointed, recurvedbractes surround the base, and a few shorter, oppressed ones from thence to the flower-bearing position. Flowers numerous, sessile, collected in small fascicles, each fascicle having a small, cordate, pointed bracte immediately under it.Calyx none.corol onepetalled, cylindric divided half way down into three exterior, and three interior slender, linear, equal, straight segments; color pale greenish yellow, as they advance in age the tube becomes twisted. Filaments inserted on the base of the segments of the corol, and of their length. Stigma three-lobed. Berry with from one to three, distinct, round, and smooth lobes; while immature, a deep olive green, when ripe, deep reddish orange; each lobe containing a single large, round, smooth, white, horny seed(Botanicus.org, 1832)..

1.5.8. Distribution:

Forests of Chittagong, Chittagong Hill Tracts and Cox's Bazar Andaman Islands and Myanmar.

1.5.9: Parts utilized

Rhizomes, flowers, seeds, leaves, roots,fruits.

[

1.5.9. Synonym:

Dracaena wallichii Kunth

Draco spicata(Roxb.)Kuntze

Pleomelespicata (Roxb.)N.E.Br..

1.5.11. Taxonomy :

Kingdom : Plantae

Phylum : Magnoliophyta

Class : Liliopsida

Order : Asparagales

Family Asparagaceae

Genus : Dracaena

Species : *Dracaena spicata*

1.6: Aim of this experiment

Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi processed plant produce drugs and medicines. Thus huge foreign exchanges can be saved if the manufacturers, to satisfy their needs, utilize the indigenous medicinal plants or their semi processed products.

Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against the harmful diseases. The increasing failure of chemotherapeutics, severe adverse effects with increase doses and repeated use of drugs ,problems with multiple dosage regimens and antibiotic resistance exhibited by pathogenic microbial infectious agents and emergence of new diseases has led to the screening of medicinal plants throughout the world for their potential activity.

The main objective of this study was to discovery of new medicinal compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases.

Dracaena spicata is a medicinal plant used traditionally in Bangladesh. Upon significant literature survey it was found only a little research work has been performed on this plant to evaluate its medicinal value and active constituents those are responsible for its pharmacological activities. Therefore, taking into consideration the traditional uses of the plant and facilities

available for conducting the study, this research work was performed on this plant.

The principal aim of the present study was to investigate the scientific basis of the traditional uses of the plant. The methanolic extract of *Dracaena spicata* to evaluate their in- vitro pharmacological activities (like antioxidant, antimicrobial).

1.6.1: Study Area:

The research was carried out in the Research Lab, Microbiology Lab and Pharmacognosy Lab of Department of Pharmacy, East West University, Dhaka

1.6.2: Data Collection:

All the relevant data has been collected from two types of sources:

1. Primary sources: direct personal contact and observations of the experiments carried out in The laboratory.
2. Secondary sources: various publications like journals, papers, documents and websites

Chapter - 2

LITERATURE REVIEW

2.1: Literature review

Using information :

- Leaf juice is used to cure long term fever, coughs and mucus in nose by traditional healers of the Marma tribe of Naikhongchhari, Bandarban District.
- The sap from the root is taken for conjunctivitis and constipation (Chakma).
- A leaf extract is taken and a paste of the leaves is applied to the whole body for the treatment of hysteria (Tanchangya).
- Pills prepared from the leaves are taken with warm water twice daily for the treatment of measles by the Chakma;
- A root extract of *Dracaena spicata* and *Pandanus foetidus* is taken together and administrated to healthy children during outbreaks of measles by the Tanchangya
- Rhizome juice is prescribed against leucorrhoea.
- Powdered rhizome with honey is given for piles, dysentery and dyspepsia.
- Root juice is drunk to keep stomach cool and to get relief from burning sensation during urination.
- Root paste of the red flowered plant is given for treating menorrhagia

2.1.1: Antimicrobial and Antioxidant activities of *Dracaena spicata*

This study was investigated the antibacterial activities of methanolic extracts of leaves of medicinal plant, *Dracaena spicata Roxb* (Family: Asparagaceae) available in Bangladesh in the part of Chittagong,Chittagong hill tracks and Cox's. Extracts obtained from leaves and roots were examined for their antimicrobial activities against some gram positive bacteria such as *Bacillus sereus, Bacillus megaterium* , also gram negative strains of *Escherichia coli, Salmonella typhi,* and fungus *Aspergillus niger*. Agar disc diffusion method was applied to observe the antibacterial efficacy of the extracts. Results indicated that plant extracts (600 µg /disc) displayed antibacterial activity against tested microorganisms *E.coli* and *Aspergillus niger*. These results were also compared with the zones of inhibition produced by commercially available standard antibiotic, Kanamycin at concentration of 30 µg/disc. Observed antimicrobial properties of the methanolic extract of *Dracaena spicata* showed that plant might be useful sources for the development of new potent antimicrobial agents (Roy et al.) .

1. Antimicrobial activity:

The bioactive compounds of leaves of *Dracaena spicata* were investigated for antimicrobial activity against some pathogenic bacteria. The aqueous extracts did not show much significant activity, while the organic extracts (Dichloromethane and methanol) showed the highest activity against the test bacteria. The activity was more pronounced on gram-negative organisms and against fungus..Fungus *Aspergillus niger* being more susceptible and Salmonella paratyphi being more resistant .Disc diffusion technique was used for in vitro antimicrobial screening against gram positive and gram negative human pathogenic bacteria. Here kanamycin disc (30µg/disc) was used as standard. The methanol extract of *Dracaena spicata* showed good antimicrobial activity with the average zone of inhibition 9-12mm.

2. Anti thrombal activity:

In this study, investigated that thrombous formation inside the blood vessels obstructs blood flow through the circulatory system leading hypertension, stroke to the heart, anoxia and so on. The complete deprivation of oxygen and infarction is a mode of cell death. Crude biologicals and their components possessing anti-thrombotic activity have been reported before. This study was aimed to investigate thrombolytic activity of methanol extracts of four traditionally used medicinal plants. For this an in-vitro thrombolytic study was carried out along with Kanamycin, and methanol was taken as reference standard and negative control, respectively.

The crude methanol extracts of aerial parts of *Abrus precatorius L*., leaf of *Magnolia pterocarpa Roxb.* and *Dracaena spicata Roxb.* and leaf and bark of *Ravenala madagascariensis Sonn*. as well as their hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for thrombolytic and membrane stabilizing activities. *D. spicata* extractives showed mild thrombolytic activity.

3. Membrane Stabilizing Activity:

The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution and heat induced haemolysis of human erythrocytes following the method developed by Omale et al (2008) The crude methanol extracts of aerial parts of *A. precatorius*, leaf of *M. pterocarpa* and *D. spicata* .Carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for thrombolytic and membrane stabilizing potentials. In order to identify the drugs with the ability to promoteolysis of blood clot from natural resources, the crude methanol extract of *D. spicata* extractives showed mild thrombolytic activity .

4. Antifungal activity:

In the year of 2010 a study performed to evaluate antifungal activity of *Dracaena spicata* leaves extract. The study performed *against Salmonella typhi,Basillus subtilis ,E.coli,Staphylococcus aureus.* Methanol extract of *Dracaena spicata* is more significant in producing antifungal activities.

5. Anti-ulcer activity:

- Aqueous extract of leaves of *Dracaena spicata* was investigated for anti ulcer activity
- Aqueous extract of *Dracaena spicata* at doses of 50 and2 50 mg/kg produced significant inhibition of the gastric lesions induced by pylorus ligation induced ulcer and ethanol induced gastric ulcer. The extract showed significant reduction in ulcer index, free acidity. (Girish et al., 2011)
- Root juice is drunk to keep stomach cool and to get relief from burning sensation during urination. (Girish et al., 2011)

6. Anti-tussive activity:

Methanol extract of fruits of *D.spicata* and *Dracaena steudneri* , with two different concentrations (2.5% and 5% w/v) was tested for anti-tussive activity by counting number of cough.. The extract showed significant inhibition of cough, like the standard drug (codeine phosphate) in dose-dependent manner. Thus the extract might be acting via the central nervous system, but the exact mechanism of action cannot be withdrawn from the study. From this investigation, it can be concluded that on preliminary screening the extract of *D.spicata* produced a significant antitussive effect and thus the claim of using the plant as an anti-cough agent in ancient folklore e medicine was established (Shakti et al., 2009).

7. Analgesic and anti –pyretic activity:

- Fever, dizziness. Leaf paste is applied to forehead.
- A tea is made from the leaves and used to treat fevers, cough and cold

8. Anti –paralytic drug:

Dracaena spicata (local name:Agunikundu) Leaf used in paralysis. Leaf juice is massaged to affected area twice daily for 1 week.

9. Cures:cough,syphilis:

Preparation & use for cough treatment: Mix dry leaves of *D. spicata* and *C. papaya* (pawpaw) then burn to ashes. Add small amount of lake salt to the ash. Add some little water to the ash plus salt mixture then drink the mixture.

Preparation and use for syphilis treatment: Crush sizeable amount of the bark of the tree and boil in water for about an hour. Let it cool then strain and drink the liquid.

10. Antifungul activity: This plant showed good jone of inhibition against fungus ***Aspergillus niger.***

11. Antioxidant activity:

Methanolic extract, aqueous extract and powder of the leaves of *D. spicata* were tested for antioxidant activity. Powder form and methanolic extract showed good antioxidant property whereas aqueous extract did not showed significant activity (Shyam et al., 2010).

The methanol extract of *D.spicata* contains glycoside and flavonoid. The antioxidant activity of *D.spicata* is due to the reducing power ability (Moideen et al., 2011). Preliminary chemical group identification revealed the presence of alkaloids, glycosides, steroids, terpenoids, tannins and reducing sugars important secondary metabolites (Sultana et al. 2012).

12. Ethnomedical Studies of Chakma Communities of chittagong Hill Tracts, Bangladesh

The use of local medicinal knowledge as herbal remedy is a part of traditional heritage in any rural areas of Bangladesh, especially among forest inhabitations. It has unequivocal emphasis on welfare of the high land communities of Chittagong Hill Tracts (CHT), Bangladesh. Present investigation revealed that Chakmas have strong belief in traditional system of medicine and still use herbal medicines prescribed by local healers. A total of 146 plant species are regularly used, one of these plants is *D. spicata*. These plants are used to treat diverse maladies like fever diarrhea ,jaundice ,rheumatism ,bronchitis, leprosy ,snake bite ,cancer ,tuberculosis ,blood pressure ,measles etc. i.e. from simple common cold to cancer like diseases. Among plant parts, leaves and roots were found to be used in maximum herbal preparations. Most of these formulations were prescribed as pastes ,extracts ,and juices while 16 species were reported to have more than one therapeutic use. 130 species were reported to have activity against single specific ailment(Khisha et al., 2012).

Chapter - 3

METHODS AND MATERIALS

3.1: Preparation of plant extract for experiments:

3.1.1: Collection:

Dracaena spicata is not so available throughout the country. The plant collected from Chittagong hill tract area. The plant was taxonomically identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka, where a voucher specimen (Accession No. 40633) has been deposited for future reference.

3.1.2: Process of powdering:

At first the plants were cleaned to remove dust, soil etc within them. After this the whole amount of plant was dried. The dried plants were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. The amount of powder was 550g. During powdering of sample, the blander was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the blander.

3.1.3: Extraction:

The fine powder of plants was dissolved in 2 liter methanol and it was thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass jar for 7 days and shaken several times during the process for more interaction between the powdered particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar.

3.1.4: Filtration:

After the extraction process the plant extracts was filtered with sterilized cotton filter and filter paper. The filtrate was collected in a beaker. The filtration process was repeated three times by using cotton and filter paper. Then the filtrate was taken into a conical flask and covered with aluminum foil paper was prepared for rotary evaporation.

3.1.5: Evaporation and extract preparation:

For evaporating the solvent and collect for reuse I have used rotary evaporator machine with a vacuum pump which helped to reduce the pressure of the inside of glass tube coil, as well as the whole system. Reduction of pressure causes quick evaporation. On the other part condenser recommenced the solvent so that I could reused it. For this solvent almost 70% solvent get back into liquid form. The extraction was collected from the evaporating flask and the solvent is collected from the receiving flask. Extract transferred into a 50 ml beaker and covered with aluminum foil.

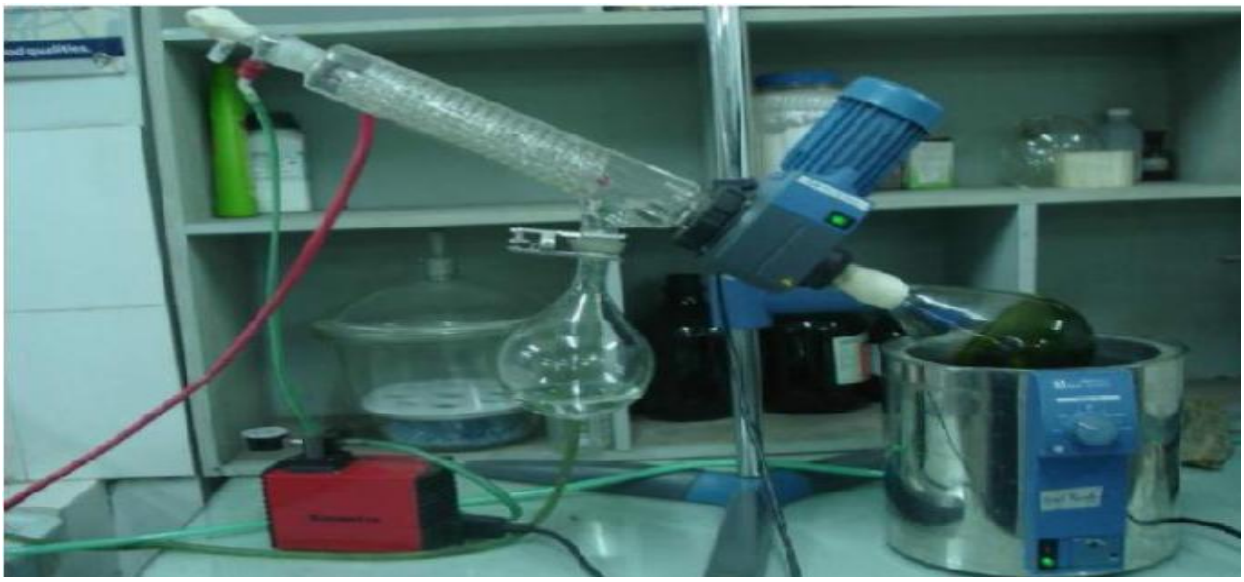

Figure - 3: Rotary evaporator.

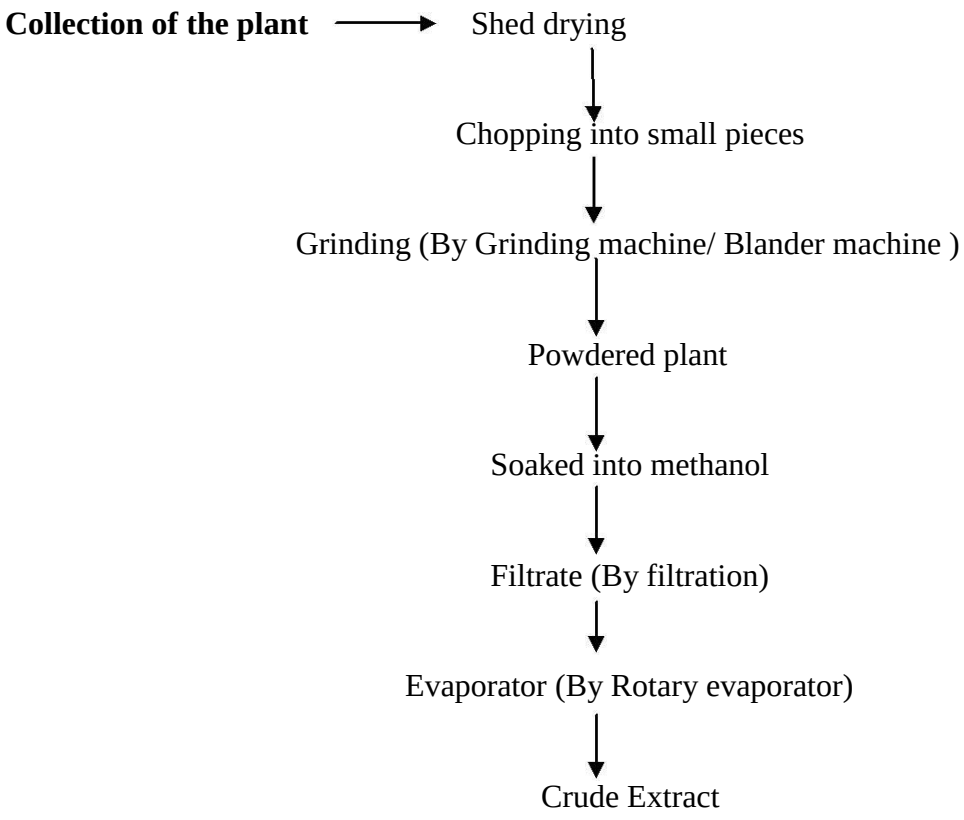

Figure - 4: Schematic presentation of the crude preparation from the plant

3.1.6: Antimicrobial Screening

The antimicrobial assay was performed by disc diffusion technique. Disc diffusion technique is highly effective for rapidly growing microorganisms. In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient (Bauer et al. 1988). Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms.

Standard antibiotic discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media (Barry 1976)**.** The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter (Barry 1976)**.** In the present study the crude extracts, fractions as well as some pure compounds were tested for antimicrobial activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is required (Ahmed & Azam 2011)**.**

3.4.1: Test materials used for the study:

- The methanolic crude extracts of *Dracaena spicata* for the investigation of antimicrobial activity.
- Solvent (methanol) were used for dissolving the compounds.
- Kanamycin (30 μg/disc) as standard disc.

3.4.2: Reagents:

- Rectified spirit
- Agar purified powder
- Methanol
- Dichloromethane

3.4.3: Apparatus:

- Filter paper discs (sterilized)
- Petri dishes
- Inoculating loop
- Sterile cotton
- Test tubes
- Sterile forceps
- Micropipette
- Electric balance(4 digits)
- Nose mask and hand gloves
- Spirit burner and match box
- Laminar air flow unit
- Incubator
- Refrigerator
- Autoclave

3.4.4: Test Organisms:

The bacterial strains used for the experiment were collected as pure cultures from the East West University microbiology laboratory. Both gram positive and gram-negative organisms were taken for the test and they are listed in the following table.

Table - 6: List of the test pathogenic bacteria

Name of the test organism
Gram Positive
Bacillus sereus
Bacillus megaterium
Gram Negative
E.Coli
Salmonella typhi
Fungus
Aspergillus niger

3.4.5: Culture Medium and their composition:

The nutrient agar medium was used normally to demonstrate the antimicrobial activity and to make subculture of the test organisms. Nutrient agar medium contains following things:

Table - 7: Composition of nutrient agar medium (Barry 1976)

Ingredients	Amount
Bacto peptone	**0.5 gm**
Sodium chloride	**0.5 gm**
Bacto yeast extract	**1.0 gm**
Bacto agar	**2.0 gm**
Distilled water q.s.	**100 ml**

Agar medium having this composition was directly brought from the market.

3.4.6: Preparation of the Medium:

To prepare required volume of this medium, calculated amount of agar medium was taken in a bottle with a cap and distilled water was added to it to make the required volume. The contents were then autoclaved to make a clear solution.

3.4.7: Sterilization Procedure:

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in laminar hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the laminar hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121ºC and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

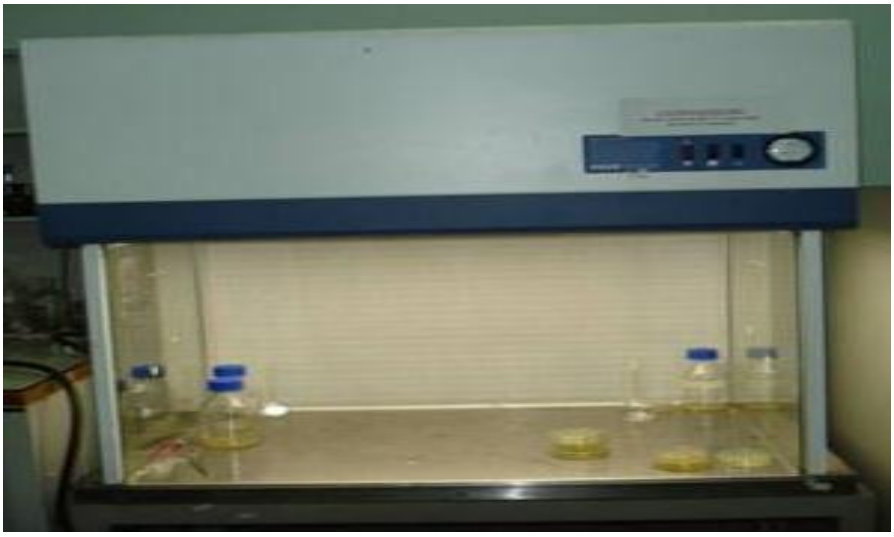

Figure – 5: Laminar hood

3.4.8: Preparation of subculture:

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

Figure – 6: Incubator

3.4.9: Preparation of the test plate:

The test organisms were transferred from the subculture to petridish containing about 10 ml of melted and sterilized agar medium.

The bacterial suspension was taken by a loop a mixed with normal saline with the help of vortex machine. Then a sterilized cotton bud was taken and dipped into the bacterial suspension. Then the bacterial sample is applied to the petridish with the help of this cotton bud.

3.4.10: Preparation of discs:

- **Standard discs**

These were used to compare the antibacterial activity of the test material. In the present study, I used Kanamycin 30 µg/disc were used as a standard disc for comparison purpose.

❖ **Sample discs**

Sterilized filter paper discs (6 mm in diameter) were taken by the forceps in the plates. Sample solutions of desired concentrations (600 µg/disc) were applied in the disc with the help of the micropipette in an aseptic condition. These discs were left for a few minutes in aseptic condition for complete removal of the solvent.

3.4.11: Diffusion and incubation:

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria. The plates were then kept in a refrigerator at 4ºC for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37ºC for 24 hours.

3.4.12: Determination of antimicrobial activity by measuring the zone of inhibition:

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

3.4.13: Precaution:

The discs were placed in such a way that they were not closer than 15 mm to the edge of the plate and for enough apart to prevent overlapping the zones of inhibition.

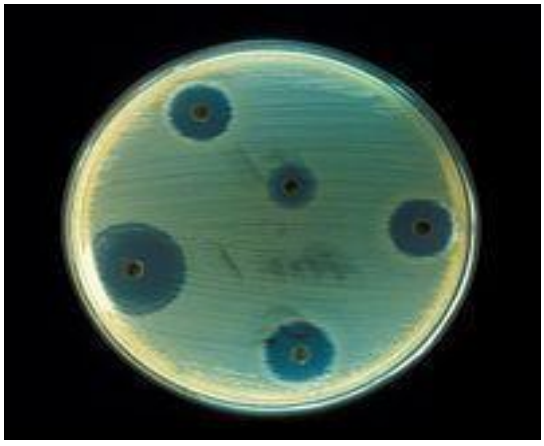

Figure - 7: Zone of inhibition

3.5: Antioxidant test:

3.5.1: Reducing power determination:

Reducing power of the plant fractions was determined following the method described by Oyaizu (1986).

Reagents and chemicals:

List of the reagents used in reducing power test and their sources.

- Potassium ferricyanide May and Backer, Dagenham, UK
- Trichloro Acetic acid Fine Chemicals, India
- Ferric Chloride ($FeCl_3$) Fine Chemicals, India
- Ascorbic acid as standard SD Fine chem. Ltd., Biosar, India

Principle:

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe3+/ferricyanide complex to the ferrous form. Therefore, Fe2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Oyaizu, 1986).

Experimental procedure:

1. 2.0 ml of each fraction and standard (ascorbic acid) in different concentrations were taken in test tubes.
2. 2.5 ml of potassium ferricyanide [K3Fe(CN)6] 1% solution was added into the test tubes.
3. Then the test tubes were incubated for 10 minutes at 50 0C to complete reaction.
4. 2.5 ml of trichloro acetic acid (10%) was added into the test tubes.
5. The total mixture was centrifuged at 3000 rpm for 10 minutes.
6. 2.5 ml supernatant solution was withdrawn from the mixture and mixed with 2.5 ml of distilled water.
7. 0.5 ml of ferric chloride (0.1%) solution was added.
8. Then the absorbance of the solution was measured at 700 nm using a spectrophotometer (Shimadzu UV PC-1600) against blank.

9. A typical blank solution contained the same solution mixture without plant extract or standard and it was incubated under the same conditions as the rest of the sample solution.
10. The absorbance of the blank solution was measured at 700 nm against the solvent used in solution preparation was also taken.

Chapter – 4

RESULT

4.1: Result of Antimicrobial Screening

4.2.1: The Results of antimicrobial screening:

The extract of methanol was not so active against all the test organism . The methanol extract (600 µg/disc) of *Dracaena spicata* showed antibacterial activity against fungus and gram negative bacteria.

Table:8:The antimicrobial activity (in vitro) of methanol extract of *Dracaena spicata* and standard Kanamycin discs.

Name of the test organism	**Diameter of the zone of inhibition(in mm)**	
	Methanol extract (600µg/disc)	Kanamycin disc (30µg/disc)
Gram positive bacteria		
Bacillus sereus	8	-
Bacillus megaterium	0	-
Gram negative bacteria		
Salmonella paratyphi	0	-
Eschericia coli	8	8
***Aspergillus niger*(FUNGUS)**	16	10

4.3: Result of Antioxidant Activity

4.3.1: Determination of reducing power:

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.. The presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe3+/ferricyanide complex to the ferrous form.Therefore, Fe2+ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Oyaizu, 1986).

Reducing power of *Dracaena spicata* was calculated using the standard curve of ascorbic acid .Then the absorbance at 700 nm was determined. These data were used to estimate the reducing power of the contents using a standard curve obtained from various concentration of ascorbic acid. The reducing power of *Dracaena spicata* content was expressed as mg of ascorbic acid equivalent.

Table: Reducing power determination table of ascorbic acid and *Dracaena spicata*

Sample	Concentration	Absorbance of ascorbic acid	Absorbance of *Dracaena spicata*
Methanolic extract of *Dracaena spicata*	0µg/ml	0	0
	50µg/ml	0.237	0.099
	100µg/ml	0.290	0.285
	150µg/ml	0.386	0.293
	200µg/ml	0.489	0.308

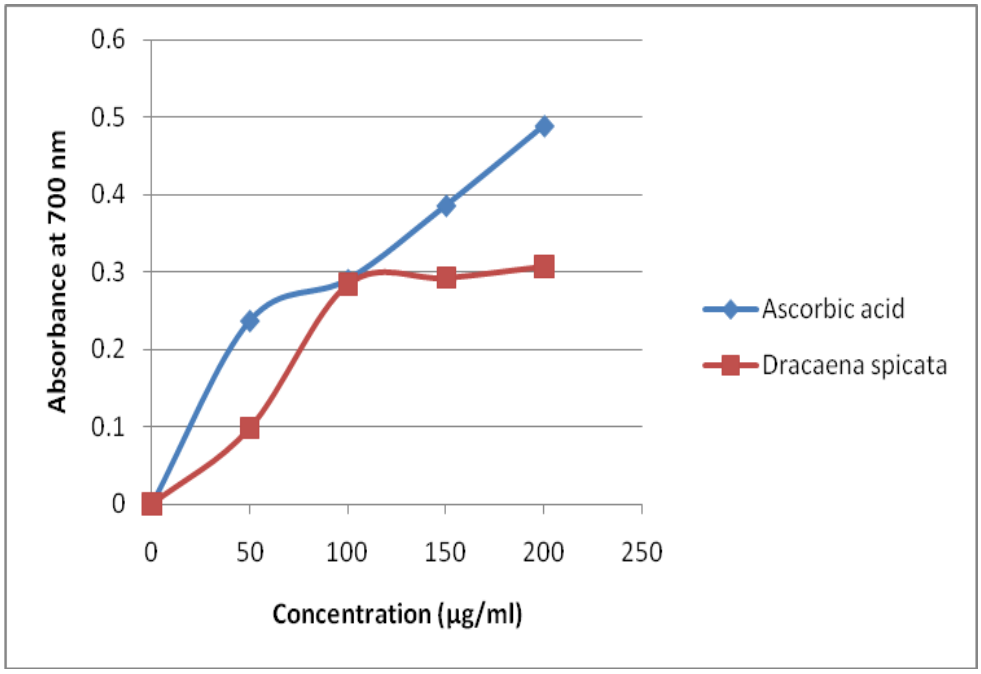

Fig:Reducing power assay of ascorbic acid(standard) and *Dracaena spicata.*

Chapter - 5

DISCUSSION

5.1: Discussion

The plant *Dracaena spicata* has been used for the general promotion of health and longevity by asian tribal(specially Chakma, Marma and Tanchunga). It is used as a traditional medicine for the treatment of various diseases cough, syphilis , conjunctivitis, constipation, pills prepared from the leaves are taken with warm water twice daily for the treatment of measles by the Chakma etc.The aim of the present study was to evaluate the antimicrobial activity and antioxidant activity of methanolic extract of *Dracaena spicata.*

Due to its huge therapeutic use by the tribal I get interested to do experiment on this plant (Rahman et al. 2007).The therapeutic value of medicinal plants lies in the various chemical constituents in it.

Antimicrobial assay of the methanol extract of *Dracaena spicata*:

The Antimicrobial Activity of the methanol extract of *Dracaena spicata* was tested against 2 Gram positive bacteria ,2 gram negative bacteria and a fungus. The highest antimicrobial activity was shown against fungus ***Aspergillus niger***, the diameter of zone of inhibition was 10 mm compared to the 25 mm of diameter of zone of inhibition of the standard Kanamycin 30 μg/disc. In case of gram negative micro-organism, zone of inhibition of *Dracaena spicata* was 8 for ***E.coli*** where the standard Kanamycin zone of inhibition was 30 mm. So, the methanol extract of the *Dracaena spicata* showed moderate antimicrobial activity of the against the selected microorganisms and thus further studies must be conducted to isolate the pure compounds and to evaluate their antimicrobial activity by using more advanced methods.

Reducing power determination of the methanolic extract of *Dracaena spicata:*

The reducing power of methanolic extract of *Dracaena spicata* was determined by comparing it with the standard ascorbic acid using the potassium ferricyanide reduction method.. The present result suggest that the tested plant extracts have potent antioxidant activity.Since a variety of constituents is present in the extracts studied. Reducing power of extract of *Dracaena spicata* was very potent and the power of the extract was increased with quantity of sample. The plant extract could reduce the most Fe3+ ions, which had a lesser reductive activity than the standard of Ascorbic. It becomes difficult to describe the all properties selectively to any one

group of constituents without further studies, which are beyond the scope of this paper. Thus, further extensive investigations are necessary to find out the active principles present in these plants.

Chapter – 6

CONCLUSION

6.1: Conclusion:

From the result of my study, it can be concluded that, using in vitro experiments established that methanol extract of *Dracaena spicata* inhibits the bacterial growth. In case of anticancer drug preparation this plant extracts may treated as a good candidate as it has notable cytotoxic effect. In case of antioxidant preparation this plant extracts may be treated as potent antioxidant as it has potent antioxidant effect.

In oxygen involving metabolism, Reactive Oxygen Species (ROS) are natural byproducts. Such typical ROSs is superoxides, hydroxyl, peroxyl, and alkoxy free radicals. Under relaxed condition, production and scavenging of ROS is in equilibrium. However, different environmental stresses like pollution, drought, temperature, excessive light intensities and nutritional limitations are able to increase ROS production. Such stressful conditions are called oxidative stress. Oxidative stress can contribute to diseases such as cancer and cardiovascular disease. So, it a dying need to find out the antioxidant potential of the compounds found from the natural sources.

For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities, and plant physiological studies would indeed have a major role to play in this burgeoning field. With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. . In my experiment it shows very positive result for anti-oxidant activity, antimicrobial activity,. The plant also shows poor antimicrobial activity. The antimicrobial activity of the plant extracts were tested against four potentially bacterial pathogenic by using disc diffusion method at different concentrations of the extracts of *Dracaena spicata* to understand the most effective activity. There are some established research reports regarding the phytochemical and pharmacological properties of this plant. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.

Chapter - 7

REFERECE

7.1: Reference

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