# Antimicrobial and Cytotoxic Investigations of Petroleum ether Extract of *Dracaena spicata*

A Dissertation submitted to the Department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy.


**Submitted by**

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# Declaration by the Candidate

I**, Sumona Nazneen,** hereby declare that the dissertation entitled **"Antimicrobial and Cytotoxic Investigations of Petroleum ether 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, under the supervision and guidance of Ms. Nazia Hoque, Assistant Professor, Department of Pharmacy, East West University. The thesis paper 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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# Endorsement by the Chairperson

This is to certify that the thesis entitled "**Antimicrobial and Cytotoxic Investigations of Petroleum ether Extract of *Dracaena spicata***" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by Sumona Nazneen , ID: 2013-1-70-026.

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**Dr. Shamsun Nahar Khan**
**Associate Professor & Chairperson**
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# Dedication

This research paper is dedicated to my beloved Parents and my family members.

## Abstract

*Dracaena spicata* has been used as a medicinal plant for the general promotion of health and longevity by Asian tribal. It is used as a traditional medicine for the treatment of various diseases like cough, syphilis, conjunctivitis, constipation, 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 aim of the present study was to evaluate the antimicrobial activity and cytotoxic activity of petroleum ether extract of *Dracaena spicata.* The antimicrobial activities of petroleum ether solvent extract of *Dracaena spicata* plant were tested against the gram-positive (*Bacillus sereus, Bacillus megaterium, Staphylococcus aureaus*) and gram-negative bacterial (*Pseudomonas aureaus, Salmonella paratyphi, Salmonella typhi, Vibrio parahemolyticus, Vibrio mimicus, Sheigella dysenteriae, Escherichia coli)* strains by observing the zone of inhibition. The antimicrobial test was performed by disc diffusion method. The crude petroleum ether extract of *Dracaena spicata* plant showed strong (9mm-31mm) antimicrobial activities against the microorganisms .Petroleum ether extract of *Dracaena spicata* showed very good activity against *Bacillus sereus* (31 mm) and no activity was found against *Bacillus megaterium , Vibrio parahemolyticus* .The cytotoxic activity of the plant was done by using *Artemia saline* Leach. The $LC_{50}$ was observed approximately as 31.9845 μg/mL with a R2 value of 0.86836., which revealed mild to moderate cytotoxic 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 cytotoxic properties of *Dracaena spicata.*

**Key Words:**

*Dracaena spicata*, Antimicrobial, Cytotoxicity , *Artemia saline* Leach .

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# Chapter One

# INTRODUCTION

# 1.1 Introduction

Large human population in developing countries is dependent on plant resources for healthcare because allopathic medicine can cure a wide range of diseases, but its high prices and occasional side-effects are causing many people to return to herbal medicines which tend to have fewer side effects. In last few decades, traditional knowledge on primary healthcare has been widely acknowledged across the world. It is estimated that 60% of the world population and 80% of the population of developing countries rely on the traditional medicine, mostly plant drugs, for their primary health care needs . Therefore there is an urgent need to document the medicinal and aromatic plants associated traditional knowledge, because this knowledge orally passes on from one generation to the next; thus, have vulnerability to wiped out. The earliest recorded history of civilization from ancient culture of Africa, China, Egypt and Indus valley revealed evidences in support of the use of herbal medicine by dweller of those regions. Use of plants as a source of medicine has been inherited and is an important component of the health care system in Egypt. Keeping the traditional inherent knowledge, nowadays, Egyptians still depend on medicinal plants for primary health care needs. The documentation of medicinal plants prioritized by the local people, as well as their understanding of possible biodiversity loss and strategies of conservation are some of the under explored aspects in ethnobotanical studies .Medicinal plants are an integral component of ethnomedicine in Egypt and more than 342 species of medicinal plants are collected from wild. Studies involving on medicinal plants reveal decline of these resources .Information on the utilization of plants for primary healthcare in Egypt has been documented. (Rabe T. and Van Staden J., 1997)

## 1.1 Medicinal Plant

Plants have been used for medicinal purposes long before recorded history. Primitive men observed and appreciated the great diversity of plants available to them .Plants provide food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to be developed through observations of wild animals, and by trial and error.

In this essay we will explore the connection between plants, medicine, our food, and modern science.

The modern medical and pharmaceutical industry has dissected nature into its parts and along the way lost so much of the whole picture. What was once a trusted and natural approach to health thanks to plants, has today become nearly 100% synthetic. So why this great change the medicinal power of herbs in their area to its knowledge base. They methodically collected information on herbs and developed well defined herbal pharmacopoeias. Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants. Some people value these plants due to the ancient belief which says plants are created to supply man with food, medical treatment, and other effects. It is thought that about 80% of the 5.2 billion people of the world live in the less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis . There are nearly 2000 ethnic groups in the world, and almost every group has its own traditional medical knowledge and experiences. Iran is home to several indigenous tribes with a rich heritage of knowledge on the uses of medicinal plants. Iran has varied climates and geographical regions that have caused a wide distribution of individual medicinal plant species such that each tribe has its own plants and customs. Alamut is one of the most important geographic regions in Iran because of its ancient history of cultivating traditional medicinal plants. Alamut region and the several villages it encompasses are secluded from other cities in Iran, which is why the people living in this region have relied on indigenous medical knowledge and medicinal plants. In this study, we analyzed the medicinal plants with most therapeutic usage in the region. (Ahvazi and Zakeri, 2011).

## 1.2. Importance of Medicinal Plant

The plants are extremely useful. On the one hand they provide the oxygen needed to breathe. But they also provide nutrients that we eat. of plants for food has led to a

search from the beginning of the humanity of those species that are edible and those that are not . In this quest, the man has experienced in his own body and has found that a food can be converted into a deadly poison. Throughout history civilizations have moved in around the plants, making the living that have most influenced humanity. The conservation of seeds in clay helped liberate the collection of wild plants and the invention of agriculture with the consequent gradual disappearance of the nomadic cultures. The search for species allowed the discovery of the Americas and the emergence of colonialism. Similarly, the search for medicinal species, narcotics or aphrodisiac properties has led men to search the most remote. The importance of medicinal plants is most evident in the present in developing countries. In Pakistan an estimated 80% of people depend on these for treatment, 40% in China. In technologically advanced countries like the United States an estimated 60% of the medicinal plants is commonly used to combat certain diseases. In Japan there is more demand for medicinal plants.

Plants have been part of our lives since the beginning of time. We get numerous products from plants, most of them not only good and beneficial for our health, but also crucial to our exise and disconnect. After the post-war era, economies began to boom and an evermore sophisticated technology was spreading through every sector leading societies to change drastically. Our populations boomed and money and profit became the driving factors. Dabbling with plant medicines was pretty much looked upon as primitive and unscientific. Of course in order for something to be profitable today it needs a patent, and nature in its unmodified form cannot be patented. So we extracted what we wanted out of plants, synthesized it, and patented the final products as pharmaceuticals and various formulas were born. We even modified entire plants and their species, both physically and genetically, in order to make them more profitable. We were driven to meet the growing needs of the world populations, but also driven by greed. (Ahvazi and Zakeri ,2011).

## 1.1.3. Medicinal Plant in Ancient Time

Plants have been used from ancient times to attempt cures for diseases and to relive physical suffering. Ancient peoples all had acquired some knowledge of medicinal plants. Oftentimes these primitive attempts at medicine were based on superstition

and speculation. Evil spirits in the body were thought to be the cause of medical problems. They could be driven out of the body through the use of poisonous or disagreeable plant substances that rendered the body a disagreeable habitat. Medicine men or women of a tribe were usually charged with knowledge of such plants. The progress of medicine has often been guided by the earlier observations and beliefs. Drug plants were always of especial interest. As early as 5,000 B.C. many drugs were in use in China. Sanskrit writings testify to methods of gathering and preparing drugs in these early times. The Babylonians, ancient Hebrews and Assyrians were all familiar with medicinal plants. From Egypt there are records dating to 1,600 B.C. naming many of the medicinal plants used by physicians of that period, among which myrr, opium, cannabis, aloes, cassia and hemlock are prominent. The Greeks were familiar with many of the drugs of today, evidenced by the works of Hippocrates, Theophrastus, Aristotle and Pythagoras. The supernatural element continued to remain prominent in their culture, however. Only a few individuals were thought able because of some special power to distinguish harmful from valuable plants. This "rhizotomoi" or root diggers were an important caste in ancient Greece. In Rome there was less interest in plants that had healing powers. But by 77 BC Dioscorides wrote in his treatise, "De Materia Medica," dealing with the nature and properties of all the medicinal substances known at that time. This work was highly esteemed for 15 centuries and to this day is valued in parts of Turkey and North Africa. Pliny and Galen also described the nature of some drug plants .

Following the Dark Ages there began a period of the encyclopedists and herbalists. The monasteries of Northern Europe produced large compendiums of information regarding plants, much of which was false. They stressed the medicinal value and folklore of plants. About the same time there appeared a "Doctrine of Signatures." This superstitious doctrine suggested that all plants possessed some sign, given by the Creator, which indicated the use for which they were intended. A plant with heart-shaped leaves was good for heart ailments; the liverleaf with its 3-lobed leaves was good for liver problems, etc. Many of the common names of plants owe their origin to this superstition. Names such as heartease, dogtooth violet, Solomon's seal and liverwort are examples. (Hill 1952).

## 1.1.4. Medicinal Plant in 21st Century

The development of artemisinin and related antimalarial compounds serves as a modern paradigm for the value of traditional medicines in drug discovery, and we assert the potential exists for additional discoveries of similar importance: of the estimated 250,000 – 500,000 extant plant species, only a fraction have been scientifically investigated for biological activity. Unexplored are untold numbers of species that are likely to be included in traditional medicines . Plants from widely separated regions of the world that are components of traditional medicines used to treat specific conditions such as malaria are phylogenetically clustered ; this principle has been recently described for *Pterocarpus,* which has significant cross-culture patterns that can inform drug development and supports the value of linking robust ethnobotanical and ethnomedical studies with 21st century 'omics technologies and systems analyses to speed identification of functionally relevant bioactivities .

Inclusion of traditional medicines in development of 21st century treatment paradigms can help assure their convenience, acceptability and accessibility. Furthermore, pharmacological synergism, a principle employed by many traditional medicines lessens the likelihood of development of genetic resistance by the pathogen or disease against drug monotherapies. Synergy research inspired by a "reverse pharmacological approach", could lead to a "new generation of phytopharmaceuticals". The use of powerful 'omics technologies facilitates disentangling such complexity: metabolomics analyses enable profiling of major and minor metabolites and bioactive components that contribute to synergism; and computational approaches for analysis of multiple-activity networks have become powerful tools for defining the principal components of mixtures with synergistic modes of action, for prediction of drug metabolism and toxicity, and for high-throughput prioritizing of agent combinations. Data mining approaches to identify active compounds in mixtures of natural products are being developed and will be essential for the development of effective multiple-agent drugs from traditional medicines.

While U.S. requirements for regulatory approval for health claims made for multi component medicines present significant challenges for the development of effective

multiple-agent drugs from traditional medicines, this is less so for Europe, and especially not for regions of the world highly impacted by Tb and malaria (and in which there are strong traditions of traditional medicine use). As described below, with the establishment of regional research facilities to confirm the safety and efficacy of traditional medicines through the use of 'omics tools and robust ethnobotanical and ethnomedical data, significant improvements in development of improved medicines that are accessible and affordable can be expected. (Hill 1952).

## 1.1.5. Present Form of Drug Medicinal Plant

Today, approximately 80% of antimicrobial, cardiovascular, immunosuppressive, and anticancer drugs are of plant origin; their sales exceeded US$ 65 billion in 2003. It is widely accepted that more than 80% of drug substances are either directly derived from natural products or developed from a natural compound .And, in fact, around 50% of pharmaceuticals are derived from compounds first identified or isolated from herbs/plants, including organisms, animals, and insects, as active ingredients.

As ancient humans adopted a plant-based (i.e., herbivorous) diet, the body function of humans may have been primed by a large number of secondary metabolites derived from plants.

Considering the extremely high cost and long time of new drug development, as well as the high drug attrition rate, an imminent task for pharmaceutical companies is to explore new ways for drug R&D. Therefore, more and more attention in the field of drug discovery has been focused on the herbal medicine. Herbal medicine as a source of new compounds for drugs is going to become a global trend in the pharmaceutical industry.

An impressive number of chemicals have been isolated either from medicinal plants or synthesized on the basis of natural lead compounds. For instance, schisandrin C present in Schisandrachinensis has led to the discovery and development of two potent drug derivatives, bifendate and bicyclol. Artemisinin isolated from Artemisia annua has generated at least ten new drugs on the market. Therefore, the use of

herbal/plant medicine has been the single most successful strategy for the development of novel therapeutic agents, and this trend will be continued in the future.

In an era of rapidly advancing science and technology, there is a tendency to ignore traditional values and knowledge, as well as traditional medicines at large. Although the "post genomic" era offers great opportunities for screening active compounds from medicinal plants, one should be aware of traditional knowledge in an attempt to discover drugs derived from herbal medicine. Many medicinal properties of plant species were revealed from experience accumulated from a long history of use in many traditional herbal therapies. Knowledge accumulated in traditional medicine, therefore, plays an important role in enhancing the success rate of drug discovery from herbal medicine. Generally, the success rate of the synthetic route for developing new medicinal agents may be 1/10,000; however, the success rate with search for new therapeutic moieties based on medical plants used in traditional medicinal system can be as high as 1/4 or more Last but not least, the principle of ecological ethics should be upheld by preserving biodiversity while exploiting natural resources for drug discovery. Man does not have the right to wipe out any species arbitrarily and mess with genes to create transgenic crops for their own benefits.

People are just one of the residents on Earth. As articulated by the ancient Chinese philosopher Lao Zi: "Mother Nature is benevolent to all living things even a stray dog on earth." Modern anthropocentrism with value of philosophical significance for sustainable development should be implemented in the processes of herbal medicine R&D.( Sofowora et al,1982 )

## 1.1.6. Traditional System of Medicine

Ayurveda is an ancient health care system which evolved in India dates back to about 5000 years ago. As per the ancient literatures on Ayurveda, it was practiced during Vedic period of India About 700 plants were described in Charaka Samhita and Sushruta Samhita during the 1$^{st}$ millennium BC. This medical system is widely

practiced in other parts of the world as a form of complementary medicine. Ayurvedic System of India aims to preserve, promote and sustain good health and preventing diseases through healthy lifestyle practices. The literal meaning of ayurveda is the "Science of life". It is estimated that about 7,500 plants are used in local health traditions in most rural and tribal villages in India. Herbal treatments are the most popular form of Traditional Medical System. The plant based traditional medicine systems continue to play a crucial role in the health care system. The demand of herbal based medicine ,health products, pharmaceuticals, food supplements, nutraceuticals, cosmetics are increasing worldwide. In the 21$^{st}$ century, natural products represent more than 50% of all drugs in clinical use. Up to 50% the approved herbal drugs during the last 3 decades are from either directly or indirectly from natural products including plants, microorganisms, fungi and animals. As per the records of the National Medicinal Plant Board (NMPB), the Indian herbal industry may like to increase in order of Rs. 80 to 90 billion by 2020. However, India is moving forward in popularising of the Traditional Medical System of AYUSH (Ayurveda, Yoga, Unani, Siddha and Homeopathy) in health care sector through global networks.(Kirtikar and Basu 1918).

## 1.1.7. Future of Medicinal chemistry

The history of drug research over a period of a century , since Paul Ehrlich introduced the concept of chemotherapeutic agents, is an amazing journey of accomplishments including the serendipitous success of antibiotic. Drug discovery has changed over the years but the goal remains the same: to find safer medicines for the deadliest diseases. Traditionally, the discovery of new drugs has arisen from observations that various plant extracts possess interesting biological effects. However, early users of such plant extracts did not understand or realize which components in the material were responsible for achieving these therapeutic benefits. The main difference between modern and age-old medicine is identifying the composition of matter, or the active form, within the medicine itself. This modern drug discovery and development is mostly a complex, expensive, time consuming and market-driven process with very few novel drug candidates actually making it through the Food and Drug Administration (FDA) for approval. Discovery and development of new drugs does

not rely so much on miracles or serendipity anymore, but instead utilizes highly planned processes involving cutting edge technologies. The previous era of modern drug discovery was dominated by chemistry, whereas now a more rational approach is employed where knowledge about enzymes and receptors has required a unique dialogue between chemists and biologists.( Sofowora et al,1982 )

Finding viable drug targets (the so-called biological approach) has become increasingly used in recent decades with the advancements in the field of genomics and proteomics. A medicinal chemist uses this information to seek out relevant targets capable of being affected by the addition of compounds. These specifically synthesized drug molecules are proposed, synthesized and tested for direct action on these protein targets in order to effectively treat a wide variety of illnesses. In previous times, "classical medicinal chemists" would modify the existing bio-active molecules from natural products. These natural products were the source of most of the active ingredients in most medicine .

In summary, new biological targets, methodologies and advanced computing have improved modern drug discovery and have given medicinal chemists a more profound skill set and toolkit to grasp the nuances of disease pathophysiology. Driven now by target identification and specificity of action, these new molecules and their development are revolutionizing healthcare. Not only are these new techniques and approaches innovative, but they are cost-effective as well. Medicinal chemists are essential players in this process and are relying heavily on new scientific literature to drive this process forward more efficiently. Open access journals such as organic chemistry: current research from OMICS group is playing a very important role by providing essential up-to-date research information to scientific community worldwide. The hope here is that these journals will add to the every growing knowledge created by the medicinal chemist to have a great impact in drug discovery process. (Shivaputra, A., 2012)

## 1.1.8. Medicinal Plant In Bangladesh

Nature has provided innumerable number of culturally important medicinal plants that

have been indispensable for the treatment of various diseases and maintaining health. Plants produce a wide variety and high diversity of secondary metabolites, which are not required for the immediate survival of the plant but which are synthesized in response to stress as a means to protect themselves from organisms, diseases or the environment . Medicinal uses of plants have been documented in approximately 10,000 to 15,000 of world′s plants and roughly 150-200 have been incorporated in western medicine . And, it is currently estimated that approximately 420,000 plant species exist in nature . A good number of secondary metabolites from plants possess interesting biological activities with various applications, such as pharmaceutical ingredients, insecticides, dyes, flavors, and fragrances . Despite decades of research, active compounds of plant remain poorly characterized.( Sofowora et al,1982 )

Usage of natural substances as therapeutic agents in modern medicine has sharply declined from the predominant position held in the early decades of last century, but search for bioactive molecules from plants continues to play an important role in fashioning new medicinal agents. With the advent of modern techniques, instrumentation and automation in isolation and structural characterization, we have on hand an enormous repository of natural compounds. In parallel to this, biology has also made tremendous progress in expanding its frontiers of knowledge. The interplay of these two disciplines constitutes the modern thrust in research in the realm of compounds elaborated by nature. ( Moghadam, 2012)

## 1.1.9. Classification of Medicinal Plant

There is a large number of medicinal and aromatic plants in the nature which are used for medicinal and aromatic purposes. Moreover, medicinal plants are sometimes used for aromatic purposes similarly aromatic plants may also be used for medicinal purpose! Hence, classification of medicinal and aromatic plants is difficult. Since there is a large number of plants in these two groups an attempt has been made in this chapter to facilitate for further study. Medicinal plants are generally classified on the basis of their growth habit. It may be either a tree, shrub, herb, annuals, biennial, tubers, rhizomes .

1) Tree

Table 1: Tree

| | Common Name | Botanical Name | Parts Used |
|---|---|---|---|
| 1 | Babul | *Acacia nilotice Delite* | Pods, leaves, bark, gum |
| 2 | Bael | *Aeglemarmelos L. Corr.* | Roots, leaves, fruit |
| 3 | Neerh | *Azaflirachtaindica* | Bark leaves, flowers, seed, oil |
| 4 | Palas | *Buteamonossperma (Lam.)* | Bark, leaves, flowers, seed, gum |
| 5 | Gugul | *CommiphoramukulEngh J* | Resinous gum |
| 6 | Olive | *Oleaeuropeae* | Leaves, Oil |
| 7 | Arjun | *Terminaliaarjuan Roxb.* | Bark |
| 8 | Behela | *TerminaliabelliricaGaertu* | Bark, fruit |
| 9 | Hirda | *TerminaliabelliricaGaertu* | Fruits |
| 10 | Nagakesar | *Mesuaferrea L.* | Blowers, oil . |
| 11 | Markingnut | *Semecarpus `& anacardium L.* | Fruits |

2. Medicinal Shrubs:

Table 2: . Medicinal Shrubs

| Sr.No. | Common Name | Botanical Name | Parts Used |
|---|---|---|---|
| 1 | Davana | *Artemisia nilagirica* | Leaves, flowering top |
| 2 | Safedmusli | *AparagusadscendensRoxbi* | Tuberous roots |
| 3 | Belladonna | *Atropa belladonna* | Leaves and roots |
| 4 | Lavender | *Lavandulaofficinalis* | Flowers |
| 5 | Sarpagandha | *Rauvalfiaserpentina L.* | Roots |
| 6 | Chitrak | *Plumbagezeylanica L.* | Leaves, roots |

3. Medicinal Herbs:

Table 3: Medicinal Herbs

| Sr.No. | Common Name | Botanical Name | Parts Used |
|---|---|---|---|
| 1. | Brahmi | *Bacopamonnieri L.* | Whole plant |
| 2. | Am haldi | *Curcuma amada Roxb.* | Rhizomes |
| 3. | Haldi | *Curcuma domestica Valet* | Rhizomes |
| 4. | Datura | *Daturametel L.* | Leaves, flowers |

4. Medicinal Annuals:

Table 4: Medicinal Annuals

| Sr.No. | Common Name | Botanical Name | Parts Used |
|---|---|---|---|
| 1. | Jangalimuli | *Blumealacera* | Whole plant |

| | | | |
|---|---|---|---|
| 2. | Cockscomb | *Celosia cristala L.* | Inflorescence |
| 3. | Red poppy | *Papaverrhoeas* | Flowers |
| 4. | Bhuiamla | *Phyllantiusniruri* | Whole plant |

5. Biennial:

Table 5: Biennial

| Sr.No. | Common Name | Botanical Name | Parts Used |
|---|---|---|---|
| 1 | Bankultthi | *Cassia abus L.* | Leaves, seeds |
| 2 | Caper spurge | *Euphorbia lathyrus* | Seed latex |
| 3 | Catchfly | *Melandriumfirmum* | Whole plant |

6. Tubers and Rhizomes:

Table 6: Tubers and Rhizomes

| Sr.No. | Common Name | Botanical Name | Parts Used |
|---|---|---|---|
| 1 | Satavar | *Asparagus adscendens Roxb* | Tubers |
| 2 | Safedmusli | *Chlorophytum borivilianum* | Tubers |
| 3 | Puskarmul | *Inularacemosa Hook* | Roots |
| 4 | Sakarkhand | *Manihote sculentacrantz* | Tubers |

## 1.2 plant information

The name Dracaena is derived from the Greek word ‘drakainia’ meaning a female dragon (Stern, 1992). The most striking source is the *Dracaena cinnabari* Balf. f. which is endemic to the island of Socotra (Yemen) west of Somalia. Pal-inurus, a

survey ship of Leut. J.R. Wellsted of the East India Company gave first description of the Dragon's blood tree, *Dracaena cinnabari*, calling it *Pterocarpus draco* while under-taking a survey of Socotra for the Indian Government in 1835.However, the species was first named and described by the Scot-tish botanist Sir lsaac Bailey Balfour when he visited the island in 1880. Three grades of Dracaena resin were identified by Balfour (1883), the most valuable being tear-like in appearance, followed by one made of small chips and frag-ments, and the cheapest being a molten mixture of fragments and refuse. Voyagers to the Canary Islands in the 15th century obtained Dragon's blood as dried garnet colored drops from another species *Dracaena draco* (L.) L., a native to the Canary Islands and Morocco. The *canarian* dragon tree *Dracaena draco* was first described in 1402. The resin is exuded from the wounded trunk or branches of the tree. *Dracaena cochinchinensis* (Lour.) S.C. Chen is another species used in China as source of Dragon's blood. 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. Dracaenas are generally rugged, carefree houseplants with a robust and tropical appearance. They are widely used for both home and office plantings. Many tolerate low light conditions. (Gupta, 2008)

Figure -1 : *Dracaena Spicata*

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

**Family** Asparagaceae - Century-plant family

**Group:** Monocot

**Growth habit:** Shrub

**Duration**: Annual

**Bangla/Vernacular Name:** Dracaena

**Tribal Name:** Kadoratenggaas (chakama, Tanchangya)

**Synonym:**

*Dracaena wallichii Kunth*

*Draco spicata (Roxb.)*

*Kuntze Pleomele spicata (Roxb.) N.E.Br.*

## 1.2.1. 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, recurved bractes 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. Calyxnone, corolonepetalled, 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 . (Okunji et al,2016).

## 1.2.2. Distribution

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

## 1.2.3. Parts utilized

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

## 1.2.4. Bengali name

Ognikund, commonly known as dragon tree, is a tree of Asparagaceae family.

## 1.2.5. Taxonomy

Kingdom : Plantae

Phylum : Magnoliophyta

Class : Liliopsida

Order :Asparagales

Family: Asparagaceae

Genus : Dracaena

Species : *Dracaena spicata*

## 1.2.6. Height/Spread

Dracaenas can grow 2 to 10 feet tall, depending on the cultivar. It is easy to maintain these plants at shorter heights if desired. Upright types will usually be no more than 2 feet wide. *Dracaena* (*Dracaena spp*.) is grown for its dramatic foliage and carefree nature. This large group of plants includes many species that can grow up to 6 feet tall with long, strap-like leaves, often with red and yellow variegation. *Dracaena* is an undemanding plant that tolerates low light and low humidity and it will forgive the occasional missed watering. As the plant grows, the lower leaves drop off and the trunk scars over, creating an interesting pattern of markings. *D. fragrans*, which is the familiar corn plant and *D. marginata*, commonly known as the rainbow plant, are two of the more familiar *Dracaena species.*

## 1.2.6. Some Common Species

| Species Name | Description | Pictures |
|---|---|---|
| *Dracaena cinnabari* | 1) Antimicrobial activity of chloroform and methanol extract of *Dracaena cinnabari* resin from island Soqotra against Staphylococcus aureus, Bacillus subtilis, Micrococcus flavus and Escherichia coli.<br>2) antiviral activity of methanol extract of resin of *Dracaena cinnabari* against Herpes simplex virus and Human influenza virus.<br>3) Juranek et al. (1993) have reported antioxidant activity of three homoisoflavans isolated from resin of Dracaena cinnabari. |  |
| *Dracaena draco* | *Dracaena draco* has been found to be a rich source of cytotoxic steroidal saponins Darias et al. (1989) reported, for the first time, the use of sap of Dracaena draco as an anticarcinogen. |  |

| | | |
|---|---|---|
| *Dracaena cochinchinensis* | Resin from *Dracaena cochinchinensis* has been produced by infection with Fusarium and Cladosporiumspp. ( Wang et al., 1999 ) |  |
| *Dracaena fragrans* 'Massangeana' | 'Massangeana' is the most commonly grown cultivar. Its glossy green, arching leaves have a wide central stripe of yellow. The plants grow 4 to 5 feet tall with a 2-foot spread on stout tan stems. |  |
| Gold Dust Dracaena (*Dracaen godseffian a*) | This small dracaena is shrub like in appearance. It grows 2½ feet tall with 3-to 4- inch long leaves spiraled around thin-wiry stems. The leaves are liberally speckled creamy yellow that fades to white as the leaves mature. |  |

## Other species:

1) *Dracaena Lindenii*

2) *Dracaena Rothiana*

3) Green Dracaena *(Dracaena deremensis)*

4) 'Janet Craig' (*Dracaena deremensis )*

5) *Dracaena Compacta*

6) *Dracaena Bausei*

7) *Dracaena Warneckii*

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.(Xing, et al, 2011).

# Chapter Two

# LITRETURE REVIEW

# 2.1: Literature Review

## 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 (300 µg /disc) displayed antibacterial activity against tested microorganisms *Escherichia .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 petroleum ether extract of *Dracaena spicata* showed that plant might be useful sources for the development of new potent antimicrobial agents (Ghosh, et al , 2008)

## 2.2. Antithrombal 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 petroleum ether extracts of aerial parts of *Abrusprecatorius L*., leaf of *Magnolia pterocarpa Roxb.* And *Dracaena spicata Roxb.* and leaf and bark of *Ravenalamada gascariensis Sonn*. as well as their hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were

subjected to screenings for thrombolytic and membrane stabilizing activities.. *Dracaena spicata* extractives showed mild thrombolytic activity. . (Omale and Okafor, 2008)

## 2.3. Membrane Stabilizing Activity

The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution and heat induced hemolysis 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 *Dracaena 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 petroleum ether extract of *Dracaena spicata* extractives showed mild thrombolytic activity .( Omale and Okafor, 2008 ).

## 2.4. 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 ,Escherichia coli, Staphylococcus aureus.* Petroleum ether extract of *Dracaena spicata* is more significant in producing antifungal activities. . (Ghosh et al, 2008) .

## 2.5. Anti-ulcer activity

Aqueous extract of leaves of *Dracaena spicata* was investigated for anti ulceractivity .Aqueous extract of *Dracaena spicata* at doses of 50 and 250 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. Root juice is drunk to keep stomach cool and to get relief from burning sensation during urination. (Encyclopedia Britannica, 2013).

## 2.6. Anti-tussive activity

Petroleum ether extract of fruits of *Dracaena 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 *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 (Nangare et al, 2009).

## 2.7. Analgesic and anti pyretic activity

Fever, dizziness. Leaf paste is applied to forehead. (Encyclopedia Britannica, 2013).

## 2.8. Anti –paralytic drug

*Dracaena spicata* (local name: Agunikundu) Leaf used in paralysis. Leaf juice is massaged to affected area twice daily for 1 week. (Shakti et al., 2009).

## 2.9. Cures: Cough, Syphilis

**Preparation & use for cough treatment**

Mix dry leaves of *Dracaena 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. (Sultana , 2012)

**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. (Chowdhury *et al*, 2013)

## 2.10. Antioxidant activity

Methanolic extract, aqueous extract and powder of the leaves of *Dracaena spicata* were tested for antioxidant activity. Powder form and petroleum ether extract showed good antioxidant property whereas aqueous extract did not showed significant activity (Shyam et al., 2010). The methanol extract of *Dracaena spicata* contains glycoside and flavonoid. The antioxidant activity of *Dracaena 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, 2012)

## 2.11. 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 *Dracaena 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).A tea is made from the leaves and used to treat

fevers, cough and cold molecular cooking for the preparation of distillates and extracts. (Chowdhury *et al*., 2013).

# Chapter Three

# METHODOLOGY

# 3.1 Preparation of Plant Extract for Experiments

## 3.1.1 Materials

### 3.1.1.1 Reagent

1. Petroleum ether

2. Dichloromethane

### 3.1.1.2 Equipments

1. Beaker

2. Funnel

3. Glass rod

4. Grinding machine

5. Filter paper

6. Cotton

7. Separating funnel

8. Measuring cylinder

9. Cotton cloth

## 3.1.2 Collection & Preparation of Plant Material

*Dracaena spicata* plant was collected from Chittagong Hill tracts. 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.

The leaves of the plant were sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried

leaves was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Department of Pharmacy, East West University.

### 3.1.3 Washing and Drying of *Dracaena spicata*

At first the leaves were thoroughly washed with tap water to remove dust, soil, bird's droppings etc. within them. The leaves were dried under sunlight for one week. But, due to rainy season sun drying was avoided. Instead, the leaves were dried in hot air oven at 500C for 2 hours.

### 3.1.4 Grinding and Storage of Dried Samples

The dried parts 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. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder. The total weight of the dried powdered leaf was 800g and was measured using electronic balance.

### 3.1.5 Extraction of the Dried Powdered Sample

The fine powder of *Dracaena spicata* whole plant was dissolved in 3000 ml petroleum ether 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.

### 3.1.6 Filtration of the Extract

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 volumetric flask and covered with aluminum foil paper was prepared for rotary evaporation.

### 3.1.7 Solvent Evaporation

The filtrate was kept in rotary evaporator for complete evaporation of the solvent. The solution was also kept in the hot plate and stirred frequently for solvent evaporation. After running this procedure, a gummy extraction was obtained which was preserve refrigerator.

## 3.2 Principle of a Rotary Evaporator

A rotary evaporator is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation. Rotary evaporators are also used in molecular cooking for the preparation of distillates and extracts.

Figure 7: Rotary evaporator device

A simple rotary evaporator system was invented by Lyman C. Craig. It was first commercialized by the Swiss company Büchi in 1957. Other common evaporator brands are Heidolph, LabTech, Stuart, Hydrion Scientific, senco, ika, andeyela. In research the most common form is the 1L bench-top unit, whereas large scale (e.g., 20L-50L) versions are used in pilot plants in commercial chemical operations.

### 3.3.1. 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 988). 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 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)**.**

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 test compound on several microorganisms simultaneously and is suitable for the estimation a 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 as concentration of an antimicrobial agent required to inhibit the growth of the microorganisms in vitro is referred to minimum inhibitory concentration (MIC). It is done by serial dilution technique (Reiner et al. 1982).

## 3.3.2. Test materials used for the study

- The petroleum ether crude extracts of *Dracaena spicata* for the investigation of antimicrobial activity.
- Solvent (petroleum ether) were used for dissolving the compounds.

 Kanamycin (30 µg/disc) as 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 standard disc.

### 3.3.3. Reagents

 Rectified spirit

 Agar purified powder

 Petroleum ether

### 3.3.4. Materials

 Micropipette

 Electric balance 4 digits)

 Nose mask and hand gloves

 Spirit burner and match box

 Laminar air flow unit

 Incubator

 Refrigerator

 Autoclave

## 3.3.5. 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.

**List of the test pathogenic bacteria/*test organism***

**Table 7: Test organism name**

| Numbers | Microorganism |
|---|---|
| 1 | *Bacillus sereus* |
| 2 | *Bacillus megaterium* |
| 3 | *Bacillus subtilis* |
| 4 | *Salmonella paratyphi* |
| 5 | *Salmonella typhi* |
| 6 | *Vibrio parahemolyticus* |
| 7 | *Vibrio mimicus* |
| 8 | *Staphylococcus aureaus* |
| 9 | *Escherichia coli* |
| 10 | *Sheigella dysenteriae* |
| 11 | *Pseudomonas aureaus* |

## 3.3.6: 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.

**Composition of nutrient agar medium** (Barry 1976)

| Ingredients | amount |
|---|---|
| Bacto peptone | 0.5 gm |
| Sodium chloride | 0.5 gm |
| Bacto yeast extract | 1 gm |
| Bacto agar | 2 gm |
| Distilled water qs. | 100 gm |

Agar medium having this composition was directly brought from the market

## 3.3.7. 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.

## 3.3.8. 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.

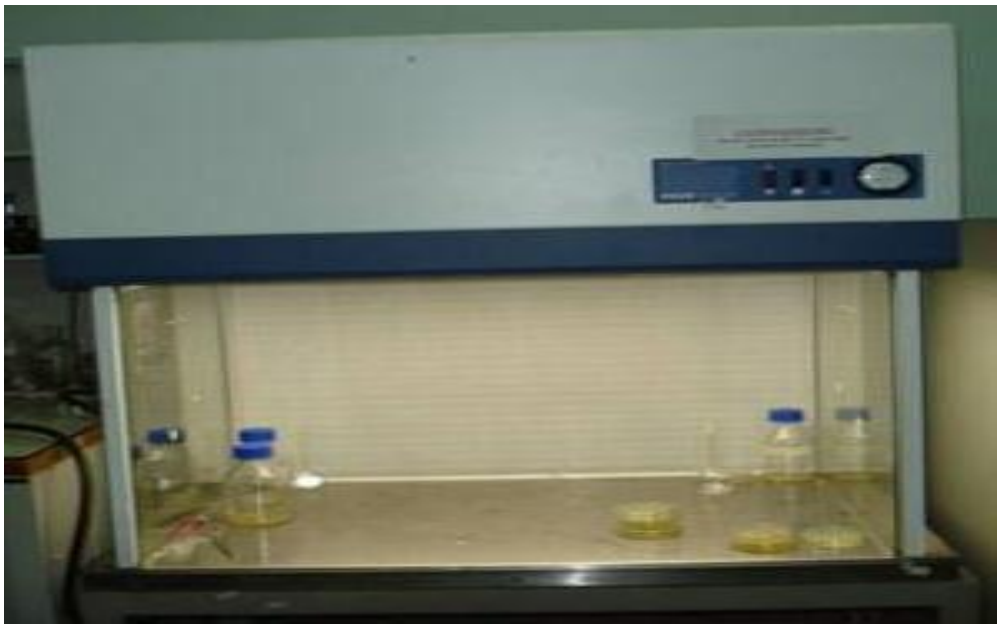


Figure – 8: Laminar hood

### 3.3.9. 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 – 9: Incubator

### 3.3.10. 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.

### 3.3.11. 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 (300 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.

### 3.3.12. 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.

### 3.3.13. 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.

### 3.3.14: 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 .

## 3.4. Cytotoxic Test

The purpose of this study is to evaluate the cytotoxicity of test chemicals using the BALB/c 3T3 Neutral Red Uptake (NRU) cytotoxicity test. The data will be used to evaluate the intra- and inter laboratory reproducibility of the assay and effectiveness of the cytotoxicity assay to predict the starting doses for rodent acute oral systemic toxicity assays. This test method protocol outlines the procedures for performing the cytotoxicity test and supports the in vitro validation study organized by NICEATM and the European

Centre for the Validation of Alternative Methods (ECVAM) and sponsored by NIEHS, U.S. Environmental Protection Agency, and ECVAM. This test method protocol applies to all personnel involved with performing the cytotoxicity assay. (Polovich, M., 2004)

### 3.4.1. Test Procedure

The NRU cytotoxicity assay procedure is a cell survival/viability chemosensitivity assay based on the ability of viable cells to incorporate and bind neutral red (NR), a supravital dye. NR is a weak cationic dye that readily penetrates cell membranes by non-ionic diffusion and accumulates intracellularly in lysosomes. Alterations of the cell surface or the sensitive lysosomal membrane lead to lysosomal fragility and other changes that gradually become irreversible. Such changes brought about by the action of xenobiotics result in a decreased uptake and binding of NR. It is thus possible to distinguish between viable, damaged, or dead cells, which is the basis of this assay. Healthy mammalian cells, when maintained in culture, continuously divide and multiply over time. A toxic chemical, regardless of site or mechanism of action, will interfere with this process and result in a reduction of the growth rate as reflected by cell number. Cytotoxicity is expressed as a concentration dependent reduction of the uptake of the NR after chemical exposure thus providing a sensitive, integrated signal of both cell integrity and growth inhibition.

## Technical Equipment

a) Shaker for microtiter plates

b) Cell counter or hemocytometer

c) Pipetting aid

d) Pipettes, pipettors

e) pH paper (wide and narrow range)

f) Multi channel reagent reservoir

g) Water bath sonicator

h) Dimethyl sulfoxide (DMSO)

**Preparation of Seawater**

38 g sea salt (pure NaCl) was weighed, dissolved in 1 litre of distilled water adjusted to pH 8.5 using 1N NaOH and was filtered off to get clear solution.

**Hatching of Brine Shrimps**

*Artemia salina* Leach (Brine Shrimp eggs) collected from pet shops was used as the test organism. Artificial seawater was taken in the small tank and Shrimp eggs were added to one side of the tank and then that side was covered. The tank was kept under constant aeration for 48 hrs to hatch the Shrimp and to be matured as nauplii. The hatched Shrimps were attracted to the lamp through the perforated dam and with the help of a Pasteur pipette 10 living shrimps were added to each of the test tubes containing 5 ml of Brine solution.

**Preparation of Test Solutions**

3.2mg of each sample is measured sample was dissolved in 2ml of DMSO. A series of solutions of lower concentrations were prepared by serial dilution with DMSO. From each of these test solutions 2ml were added to pre-marked glass vials/test tubes containing 6 ml of seawater and 10 shrimp nauplii. So, the final concentration of samples in the vials/test tubes were 320 µg/ml, 160 µg/ml, 80µg/ml,40 µg/ml, 20 µg/ml, 10 µg/ml, 5 µg/ml, 2.5 µg/ml and 1.25 µg/ml for 9 dilution.

**Counting of Nauplii and Analysis of Data**

After 24 hours, the test tubes were inspected using a magnifying glass and the number of survivors was counted. The percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed by using Microsoft Excel. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration ($LC_{50}$) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

## 3.5. 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 drug , 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 petroleum ether extract of *Dracaena spicata* to evaluate their in- vitro pharmacological activities (like antioxidant, antimicrobial).

### 3.5.1. Study Area

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

### 3.5.2. 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 Four

# RESULT AND DISCUSSION

## 4.1 Results and Discussions of Antimicrobial Screening

*Dracaena spicata* extractives exhibited mild to moderate antimicrobial activity. The test samples of *Dracaena spicata* exhibited zone of inhibition ranging from 9.0 to 31.0mm against the test organisms (*Bacillus sereus, Salmonella paratyphi, Salmonella typhi, Vibrio mimicus, Staphylococcus aureaus, Pseudomonas aureaus).* The highest (31.0mm) zone of inhibition was demonstrated by the aqueous soluble fraction against Bacillus sereus lowest result was found against *Bacillus megaterium , Vibrio parahemolyticus* .

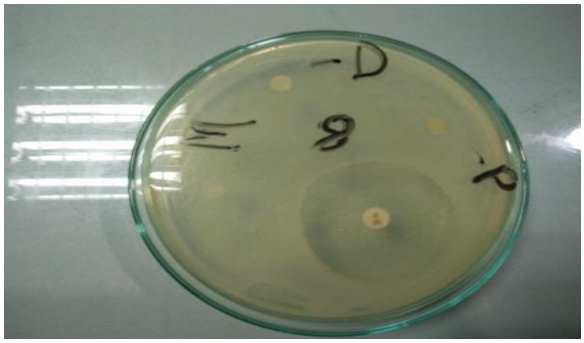


Figure 9: Test plate 8 (*Staphylococcus aureaus*)

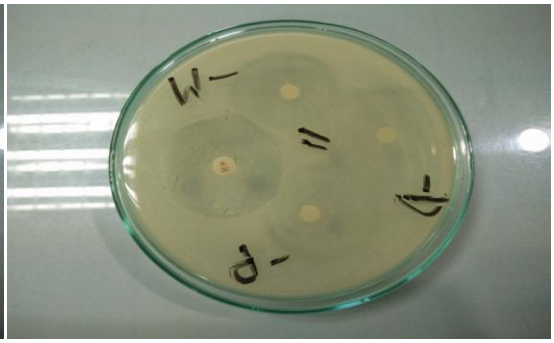


Figure10: Test plate 11 (*Pseudomonas aureaus*)

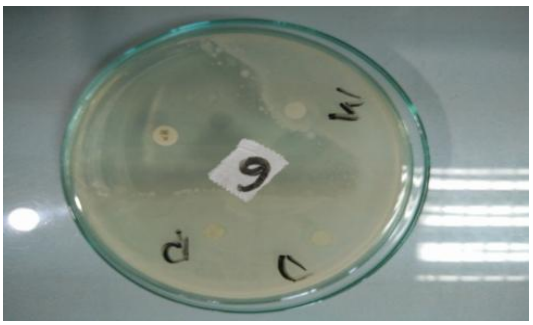


Figure 13: test plate 6( *Vibrio parahemolyticus )*

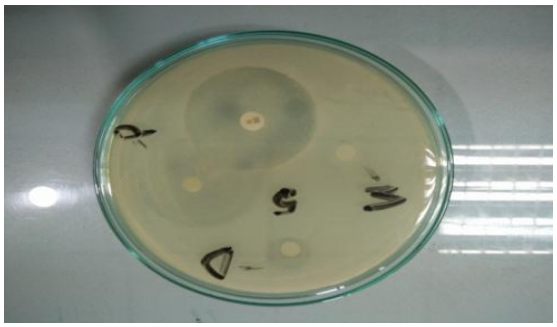


Figure 14: test plate 5 (*Salmonella typhi*)

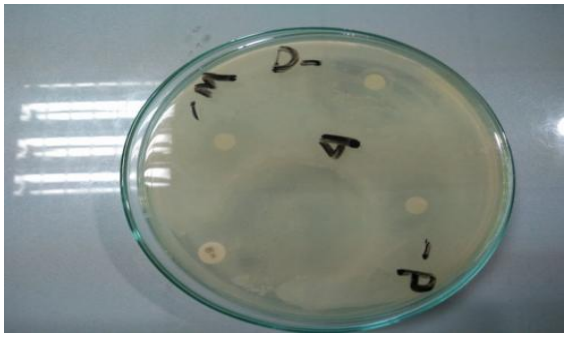


Figure15: test plate 4(*Salmonella paratyphi*)

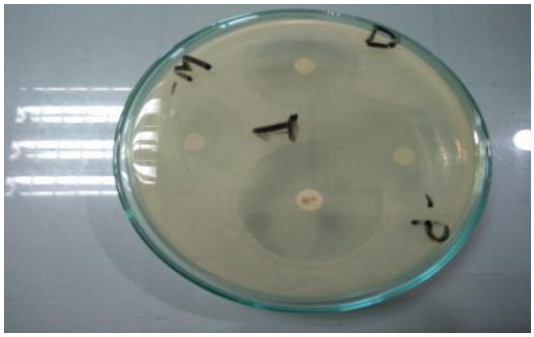


Figure16: test plate 1 (*Bacillus sereus*)

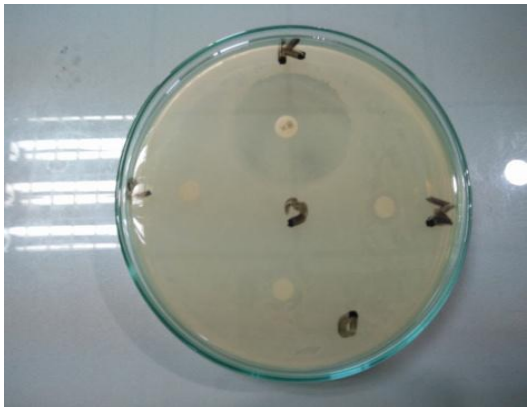


Figure 17: test plate 2 (*Bacillus megaterium*)

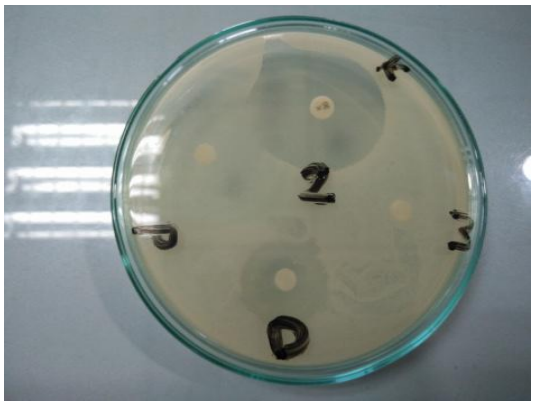


Figure 18: test plate 3 (*Bacillus subtilis*)

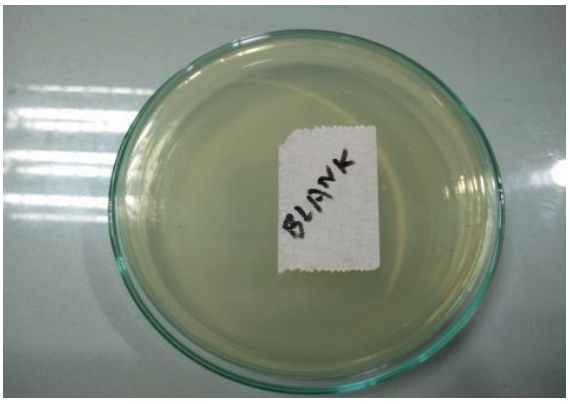



Figure 19 : Blank

**Table 16:The antimicrobial activity (in vitro) of Petroleum etherl extract of *Dracaena spicata* and standard Kanamycin discs (result in mm)**

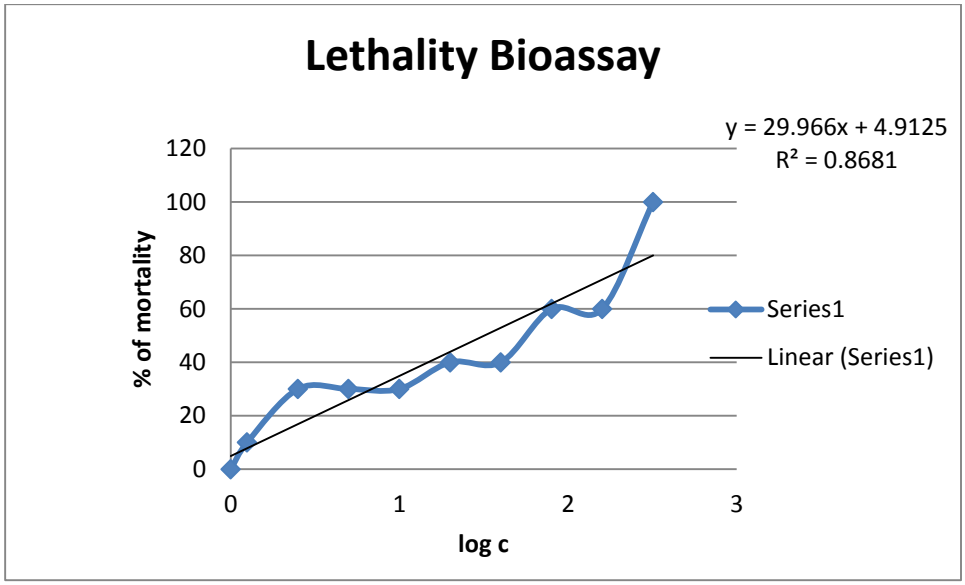
| Microorganism | Petroleum Ether (300 µg/ disk) | Kanamycin (30 µg/ml ) |
|---|---|---|
| *Bacillus sereus* | 31 | 35 |
| *Bacillus megaterium* | 0 | 37 |
| *Bacillus subtilis* | 9 | 35 |
| *Salmonella paratyphi* | 11 | 35 |
| *Salmonella typhi* | 30 | 34 |
| *Vibrio parahemolyticus* | 0 | 40 |
| *Vibrio mimicus* | 27 | 36 |
| *Staphylococcus aureaus* | 11 | 38 |
| *Escherichia coli* | 9 | 35 |
| *Sheigella dysenteriae* | 8 | 34 |
| *Pseudomonas aureaus* | 25 | 36 |

## 4.2. Result of Cytotoxicity Assay of the *Dracaena spicata*

The results of brine shrimp lethality bioassay are shown in the table. Test samples showed different mortality rate at different concentration. The mortality rate of brine shrimp napulii was found to be increased with the increase with the concentration of the sample. The median lethal concentration ($LC_{50}$) was calculated. The $LC_{50}$ values of petroleum ether extract of *Dracaena spicata* are 31.9845 µg/ml. So,it is evident that the petroleum ether extract of *Dracaena spicata* was cytotoxic as well as biologically active.

**Table 17 :Effect of Dracaena spicata (petroleum ether extract ) on shrimp nauplii**

| Concentration( µg/ml ) | Log C | No of naupli taken | No of dead | % mortality | Value of x (log $LC_{50}$) | $LC_{50}$ |
|---|---|---|---|---|---|---|
| 320 | 2.50515 | 10 | 10 | 100 | | |
| 160 | 2.20412 | 10 | 9 | 90 | | |
| 80 | 1.90309 | 10 | 3 | 30 | | |
| 40 | 1.60206 | 10 | 3 | 30 | | |
| 20 | 1.30103 | 10 | 3 | 30 | 1.50494 | 31.9845 |
| 10 | 1 | 10 | 2 | 20 | | |
| 5 | 0.69897 | 10 | 1 | 10 | | |
| 2.5 | 0.39794 | 10 | 1 | 10 | | |
| 1.25 | 0.09691 | 10 | 1 | 10 | | |
| 0 | 0 | 10 | 0 | 0 | | |




Fiqure 20: % mortality brine shrimp of *Dracaena spicata*

## 4.3. Cytotoxicity assay of the petroleum ether extract of *Dracaena spicata*

The brine shrimp lethality bioassay was performed to evaluate the cytotoxicity activity of the petroleum ether extract of the *Dracaena spicata* by their brine shrimp lethality. From this test, the concentration required for killing 50% percent of the brine shrimp test larva or $LC_{50}$ of the petroleum ether extract of the *Dracaena spicata* was calculated approximately as 31.9845 µg/mL with a R2 value of 0.868. So, Cytotoxicity of petroleum ether extract of *Dracaena spicata* was not good. Cytotoxicity of petroleum ether extract of *Dracaena spicata* was mild to moderate, since according to Wattanapiromsakul, Wangsintaweekul, Sangprapan, Itharat, & Keawpradub (2005) $LC_{50}$ value. So, further studies are needed to evaluate the cytotoxicity of isolated pure compounds .

## 4.3 Conclusion

*Dracaena spicata* has been used as a medicinal plant for the general promotion of health and longevity by Asian tribal. 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. Although active phytochemicals may have been identified, in general, many pathways for the biosynthesis of specific medicinal compounds and the factors (biotic and abiotic) regulating their production remain unclear. At present, a major concern with the use of phytomedicines regards the maintenance of consistent medicinal quality in botanical medicines. Therefore, plant materials can be potential sources of chemically interesting and biologically important drug entrant. And for this purpose the plant can be further screened against various diseases in order to find out its unexplored efficacy with a gaze to the future with a great deal of expectation petroleum ether extract of *Dracaena spicata*

of the family Asparagaceae tribally used in various disease conditions. In my experiment it shows very positive result for Antimicrobial activity. The plant also shows moderate antimicrobial activity. The antimicrobial activity of the plant extracts were tested against eleven potentially bacterial pathogenic by using disc diffusion method at different concentrations of the extracts of *Dracaena spicata* to understand the most effective activity. In case of anticancer drug preparation this plant extracts may treated as a good candidate as it has notable cytotoxic effect .The plant possesses cytotoxic activity. It was observed that brine shrimp was died at different concentrations such as-1.25 µg/ml , 2.5, µg/ml 5, µg/ml ,10, µg/ml 20 µg/ml, 40 µg/ml, 80 µg/ml, 160 µg/ml and, 320 µg/ml. There are some established research reports regarding the antimicrobial and pharmacological properties of this plant. This is only a preliminary study but the plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.

## 5.1 Discussion

*Dracaena spicata* has been used as a medicinal plant for the general promotion of health and longevity by Asian tribal. It is used as a traditional medicine for the treatment of various diseases like cough, syphilis, conjunctivitis, constipation, boils, eczema, scabies, septic abscess, itching and skin allergy, burns, chicken pox, warts and leucoderma, fungal and bacterial infections, including healing cuts and wounds .Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

In the present study, the petroleum ether extracts of *Dracaena spicata* showed the activity against *Bacillus sereus, Salmonella paratyphi, Salmonella typhi, Vibrio mimicus, Staphylococcus aureaus, Pseudomonas aureaus E. coli, Bacillus subtilis* .

For instance, petroleum ether extracts of *Dracaena spicata* exhibited inhibitory activity against all the strains of *Bacillus sereus, Pseudomonas aureaus ,Vibrio mimicus* . Petroleum ether extract was subsequently fractioned and monitored by bioassay leading to the isolation of active fraction by further cytotoxicity analysis. Apart from antimicrobial activities, the plant extracts are also exploited for therapeutic purpose to cure several carcinogenic disorder. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs. Therefore, plant materials can be potential sources of chemically interesting and biologically important drug entrant. And for this purpose the plant can be further screened against various diseases in order to find out its unexplored efficacy with a gaze to the future with a great deal of expectation Petroleum ether extract of *Dracaena spicata* of the family Asparagaceae tribally used in various disease conditions.

Cytotoxicity of petroleum ether extract of *Dracaena spicata* was mild to moderate. So, further studies are needed to evaluate the cytotoxicity of isolated pure compounds. In conclusion, further investigations are needed to identify the active constituents and the exact mechanism(s) of action responsible for the reported antimicrobial and cytotoxic properties of *Dracaena spicata.*

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