“Anti-microbial and Thrombolytic activities of different solvent extracts 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 MASTERS OF PHARMACY

Submitted By

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Declaration by the Research Candidate

I, Afruja Sultana, hereby declare that the dissertation entitled **"Anti-microbial amd Thrombolytic activities of different solvent extracts 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 Masters of Pharmacy is a complete record of original research work carried out by me during 2016-2017, under the supervision and guidance of Nazia Hoque, Assistant Professor, 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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Certificate by the Supervisor

This is to certify that the thesis entitled **"Anti-microbial amd Thrombolytic activities of different solvent extracts of *Dracaena spicata*"** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Masters of Pharmacy was carried out by Afruja Sultana, ID# 2016-3-79-009 in 2017, under the supervision and guidance of me. 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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Dedication

This Research Paper is dedicated to my beloved Parents and my friends.

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 thrombolytic activity of *Dracaena spicata.*

The antimicrobial activities of different 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 different solvent extract of *Dracaena spicata* plant showed moderate to good antimicrobial activities against the microorganisms at concentrations of 300 μg/disc.

To check the efficacy of thrombolytic drugs or herbs one can compare the data with positive and negative control. In our study we took a known thrombolytic drug; streptokinase as a positive control and water as a negative control. The comparison of positive control with negative control clearly confirmed that clot dissolution does not occur when water was added to the clot. By comparing with this positive & negative control, a notable thrombolytic activity was observed after treating the clots with different solvent extracts of *Dracaena spicata*.

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

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List of Abbreviation

Meaning of abbreviated form	Abbreviated form
DS	*Dracaena spicata*
ME	Methanolic extract
PE	Petroleum ether
DCM	Dichloro methane
EA	Ethyl acetate
SK	Streptokinase
Gram	g or gm
Hour	hr
Microgram	µg
Micro liter	µl
Milligram	mg
Milliliter	ml
World Health Organization	WHO

Chapter 1: Introduction

1.1 Medicinal plant

Medicinal plants are plants which have a recognized medical use. It's containing essential bioactive ingredients are used to cure disease or disorder since time immortal. One of the aims of medicinal plant research is the isolation and identification of markers/ bioactive compounds. Isolation of the markers compounds and bioactive plant constituents has always been a challenging task for the researchers. Separation of these components from the medicinal plants includes the use of combination of chromatographic techniques such as column chromatography,preparative thin layer chromatography, preparative high performance liquid chromatography, droplet counter current chromatography, centrifugal thin layer chromatography, etc. Centrifugal thin layer chromatography which makes use of centrifugal force for separation of multi-component system offers extensive platform for the isolation of phytoconstituents from medicinal plants. This review focuses on basic principle, instrumentation and advantages of centrifugal thin layer chromatography (Ackerknecht, 1982).

1.1.1 Definition

The plants having therapeutic or medicinal effects are called medicinal plants. The term 'medicine' can be referred to a preparation or as compound containing one or more drugs or therapeutic agents which are used in the treatment, cure or mitigation of various diseases and external or internal injuries of man and other animals (Ghani, 2012).

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).

1.1.2 History of Plants in medicine

The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded plant remedies for various illnesses. The ancient Egyptian Ebers papyrus from 3500 year ago lists hundreds of remedies. The 'Pen Tsao' contains thousands of herbal cures attributed to Shen-nung, China's legendary emperor who lived 4500 years ago. In India, herbal medicine dates back several thousand years to the Rig-Veda, the collection

of Hindu sacred verses. The Badianus Manuscript is an illustrated document that reports the traditional medical knowledge of the Aztecs. Western medicine can be traced back to the Greek physician Hippocrates, who believed that disease had natural causes and used various herbal remedies in his treatments.

Early Roman writings also influenced the development of western medicine, especially the works of Dioscorides, who compiled information on more than 600 species of plants with medicinal value in De Materia Medica. Many of the herbal remedies used by the Greeks and Romans were effective treatments that have become incorporated into modern medicine (e.g., willow bark tea, the precursor to aspirin). Dioscorides' work remained the standard medical reference in most of Europe for the next 1500 years.

The beginning of the Renaissance saw a revival of herbalism in the identification of medicinally useful plants. This coupled with the invention of the printing press in 1450 ushered in the Age of Herbals. Many of the herbals were richly illustrated; all of them focused on the medicinal uses of plants, but also included much misinformation and superstition. The Doctrine of Signatures, for example, held that the medicinal use of plants could be ascertained by recognizing features of the plant that corresponded to human anatomy. For example, the red juice of bloodwort suggests that it should be used for blood disorders; the lobed appearance of liverworts suggests that it should be used to treat liver complaints; the "humanoid" form of mandrake root suggests that is should be used to promote male virility and ensure conception.

Many of the remedies employed by the herbalists provided effective treatments. Studies of foxglove for the treatment of dropsy (congestive heart failure) set the standard for pharmaceutical chemistry. In the 19th century, scientists began purifying the active extracts from medicinal plants (e.g. the isolation of morphine from the opium poppy). Advances in the field of pharmacology led to the formulation of the first purely synthetic drugs based on natural products in the middle of the 19th century. In 1839, for example, salicylic acid was identified as the active ingredient in a number of plants known for their pain-relieving qualities; salicylic acid was synthesized in 1853, eventually leading to the development of aspirin. It is estimated that 25% of prescriptions written in the U.S. contain plant-derived ingredients (close to 50% if fungal products are included); an even greater percentage are based on semi synthetic or wholly synthetic ingredients originally isolated from plants. While Western medicine strayed away from herbalism, 75% to 90%

of the rural population of the rest world still relies on herbal medicine as their only health care.

In many village marketplaces, medicinal herbs are sold alongside vegetables and other wares. The People's Republic of China is the leading country for incorporating traditional herbalmedicine into a modern health care system; the result is a blend of herbal medicine, acupuncture, and Western medicine. Plantations exist in China for the cultivation of medicinal plants, and thousands of species are thus available for the Chinese herbalist; prescriptions are filled with measured amounts of specific herbs rather than with pills or ointments. In India, traditional systems have remained quite separate from Western medicine. In addition to Ayurvedic medicine, which has a Hindu origin, Unani medicine, with its Muslim and Greek roots, is another widely practiced herbal tradition in India. The renewed interest in medicinal plants has focused on herbal cures among indigenous populations around the world, especially those in the tropical rain forests. It is hoped that these investigations will add new medicinal plants to the world's pharmacopoeia before they are lost forever. In addition to the destruction of the forests, the erosion of tribal cultures is also a threat to herbal practices (Ghani, 2012).

1.1.3 Medicinal Plants as Drugs

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal and human body are generally designated as medicinal plants.

According to the World Health Organization (WHO),

- 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 are precursors for chemo-pharmaceutical semi-synthesis. (Borsani, 2009).

When a plant is designated as medicinal, it is implied that the plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation.

- Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes‖. Many of the plants could be used as stimulants, poisons, hallucinogens or as medicine because of the presence of

unique or rich biological-active plant chemicals (i.e. Chemical compounds that have a biological effect on another organism.

1.1.4 Characteristics of Medicinal plants

Medicinal plants have many characteristics when used as a treatment, as follow:

- Synergic medicine- The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

- Support of official medicine- In the treatment of complex cases like cancer cancer diseases the components of the plants proved to be very effective.

- Preventive medicine- It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment (Balick, 1996).

1.1.5 Importances of studying medicinal plant

- A future medicine bank to discover. There are approximately half a million plants with flowers, most of which have not been investigated and which principles could be decisive in the treatment of present or future diseases.
- 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.

1.1.6 Use of Medicinal Plant in Bangladesh

In Bangladesh 5000 species of angiosperms are reported to occur (IUCN, 2003). The number of medicinal plants included in "Materia medica" of traditional medicine in this subcontinent at present stands as about 2,000. Since Bangladesh has an enormous resource of medicinal plants, majority of our population has to rely upon indigenous system of medication. The high cost of imported conventional drugs and inaccessibility to western health care facility, imply that traditional mode of health care is the only form of health care that is affordable and available to the rural people. On the other hand, even when western health facilities are available, traditional medicine is viewed as an efficient and an acceptable system from a cultural perspective and as a result, traditional medicines usually exist side by side with western forms of health care (Kritikar and Basu, 1980).

Bioactive compounds deposited in medicinal plants can serve as important raw materials for pharmaceutical manufacturing. Therefore, well-judged and scientific investigation of this wealth can significantly contribute to the public health. Again, it was observed that developed countries mostly imports raw materials of valuable medicinal plants from developing countries. Where they are screened, analyzed and used in drug preparations, and returned as high priced medicines to developing countries. Thus, being available commodity of commerce, a country can also earn a good amount of foreign currency by exporting this natural wealth to other countries (Borsani,. 2004).

1.2 Description of the *Dracaena spicata*

1.2.1 Plant Review

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.*

Table 1.1: Plant review of *Dracaena Spicata* (Ebbd.info, 2016)

Scientific name	*Dracaena Spicata Roxb.*
Family	Asparagaceae - Century-plant family
Group	Monocot
Growth habit	Shrub
Duration	Annual
Bangla/Vernacular Name:	Dracaena
Tribal Name	Kadorateng gaas(chakama,Tanchangya)
Planting month for zone 10 and 11	year round
Origin	native to Mayanmar, Bangladesh
Availability	generally available in many areas within its hardiness range
Synonym:	*Dracaena wallichii Kunth* *Draco spicata(Roxb.)Kuntze* *Pleomelespicata (Roxb.)N.E.Br.*
Parts utilized	Rhizomes, flowers, seeds, leaves, roots,fruits

1.2.2 Distribution

D. spicata is widely distributed in forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar, Andaman Islands and some parts of Myanmar (Ebbd.info, 2016).

1.2.3 Taxonomic position

Kingdom	: Plantae
Subkingdom	: Viridiplantae
Superdivision	: Embryophyta
Division	: Tracheophyta
Class	: Magnoliopsida
Order	: Asparagales
Family	: Asparagaceae
Genus	: *Dracaena*
Species	: *Dracaena spicata*

1.2.4 General Species Description

- **Height:** 8 to 15 feet
- **Spread:** 3 to 8 feet
- **Plant habit:** upright
- **Plant density:** open
- **Growth rate:** slow
- **Texture:** fine

Figure 1.1: *Dracaena spicata* plant. .

1.2.4.1 Leaves

- **Leaf arrangement:** alternate or crowded
- **Leaf type:** simple
- **Leaf margin:** entire
- **Leaf shape:** shortly petioled
- **Leaf venation:** parallel
- **Leaf type and persistence:** evergreen
- **Leaf blade length:** 18 to 36 inches
- **Leaf color:** green
- **Fall color:** no fall color change
- **Fall characteristic:** not showy

1.2.4.2 Inflorescence/Flowers

- **Flower color:** fascicles
- **Flower characteristic:** summer flowering

1.2.4.3 Fruits

- **Fruit shape:** round
- **Fruit length:** less than .5 inch
- **Fruit cover:** fleshy
- **Fruit color:**orange-red
- **Fruit characteristic:** inconspicuous and not showy

1.2.4.4 Trunk and Branches

- **Trunk/bark/branches:** showy; typically multi-trunked or clumping stems
- **Current year stem/twig color:** greenish
- **Current year stem/twig thickness: not** very thick

1.2.4.5 Culture

- **Light requirement:** plant grows in part shade/part sun
- **Soil tolerances:** clay; sand; acidic; slightly alkaline; loam
- **Drought tolerance:** high
- **Soil salt tolerances:** poor
- **Plant spacing:** 36 to 60 inches
- **Light:** Bright light. Avoid direct sunlight in summer.
- **Water:** Keep soil lightly moist spring through fall, slightly drier in winter. Do not let soil get waterlogged.
- **Humidity:** Average room humidity
- **Temperature:** Normal room temperatures. 60-75°F/16-24°C
- **Soil:** Good-quality, all-purpose potting mix.
- **Fertilizer:** Feed every 2 weeks in spring and summer with a 10-10-10 liquid fertilizer diluted by half.
- **Propagation:** Cut off the cane at any height and root them like stem cuttings.

1.3 Uses of *Dracaena spicata*

- Pills prepared with warm water twice daily for the treatment of meals by the Chakma
- A root extract of Dracaena spicata and Pandanus foetidus is taken togather and administered to healthy children during outbreaks of meals by Tanchangya

- Antimicrobial activity, Atiulcerant activity, Antit hrombolytic, Antipyretic activity
- Cooked as vegetable
- Also 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.

1.4 Some Speices

Species Name	Description	Pictures
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.	 Fig 1.2: *Dracaena fragrans*
Gold Dust Dracaena (*Dracaena godseffiana*)	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.	 Fig 1.3: *Dracaena godseffiana*

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. 2) antiviral activity of methanol extract of resin of *Dracaena cinnabari* against Herpes simplex virus and Human influenza virus. 3)Al-Fatimi et al. (2005) reported cytotoxic activity of resin of *Dracaena cinnabari* from Yemen against human ECV-304 cells. 4) Juranek et al. (1993) have reported antioxidant activity of three homoisoflavans isolated from resin of *Dracaena cinnabari*. Machala et al. (2001) studied homoisoflavonoids and chalcones, isolated from the *Dracaena cinnabari* , for their potential to inhibit cytochrome P4501A (CYP1A) enzymes and Fe (II)/NADPH dependent in vitro peroxidation of microsomal lipids	 Fig1.4: *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. Steroidal saponins, (25R)-spirost-5-en-3-ol-3-O-{O—l-rhamnopyranosyl-(1→2)-d- glucopyranoside }and (23S,24S) - spirosta-5,25(27)-diene-1,3,23,24-tetrol 1- 0-{O-2,3,4-tri-O-acetyl—1--rhamnopyranosyl-(1→2)-l-arabinopyranosyl}24-O--d-fucopyranoside, isolated from the aerial parts of Dracaena draco are reported to show potent cytostatic activity against HL-60 cells with IC50 value being 1.3 and 2.6 g/ml	 Fig1.5: *Dracaena draco*
Dracaena cochinchinensis	Resin from *Dracaena cochinchinensis* has been produced by infection with Fusarium and Cladosporiumspp. (Wang et al., 1999)	 Fig1.6:*Dracaena cochinchinensis*

1.5 Other Speices

There are around 110 species of *Dracaena*, including:

Dracaena afromontana – Afromontane dragon tree

Dracaena americana – Central America dragon tree

Dracaena aletriformis (Haw. Bos)

Dracaena arborea – tree dracaena

Dracaena aubryana Brongn. ex E.Morren (syn.*D. thalioides*)

Dracaena bicolor Hook.

Dracaena braunii Engl. – ribbon dracaena, marketed as "lucky bamboo"

Dracaena camerooniana Baker

Dracaena cincta

Dracaena cinnabari Balf.f. – Socotra dragon tree

*Dracaena concinna*Kunth

Dracaena draco (L.) L. – Canary Islands dragon tree *Dracaena elliptica*

Dracaena fragrans (L.) Ker Gawl. (syn. *D. deremensis*) – striped dracaena, compact dracaena, corn plant, cornstalk dracaena

*Dracaena goldieana*W.Bull

Dracaena hookeriana

Dracaena kaweesakii Wilkin &Suksathan

Dracaena mannii

Dracaena marginata Lam. – red-edged dracaena or Madagascar dragon tree: see *Dracaena reflexa* var. *angustifolia*

Dracaena marmorata

Dracaena ombet – Gabal Elba dragon tree

Dracaena phrynioides

Dracaena reflexa Lam. – Pleomele dracaena or "Song of India"

*Dracaena surculosa*Lindl. – Spotted or gold dust *dracaena*, formerly *D. godseffiana* (E-monocot.org, 2016).

1.6 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 (Mishra, 2011).

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

1.7 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).

1.7.1 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.

Characteristics of antibiotic:

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

- They should have the ability to destroy or inhibit many different species of pathogenic microorganism.
- They should prevent the ready development of resistant forms of the parasites.
- 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).
- They should not eliminate the normal microbial flora of the host.

1.7.2 Classification of Antibiotics

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.

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

Table 1.2: Classification of antibiotics (Barry et al. 1976)

Based on chemical structure	**I. Sulfonamide & relative drugs** **II. Diaminopyrimidines** **III. Quinolones** **IV. β- lactam antibiotics** **V. Amino glycosides** **VI. Polypeptide antibiotics** **VII. Nitrofuran derivatives etc**
Based on mode of action	Inhibition of cell wall synthesis. Drug – penicillin, bacitracin. 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)

1.8 Thrombolysis

Thrombolysis, also known as thrombolytic therapy, is a treatment to dissolve dangerous clots in blood vessels, improve blood flow, and prevent damage to tissues and organs. Thrombolysis may involve the injection of clot-busting drugs through an intravenous (IV) line or through a long catheter that delivers drugs directly to the site of the blockage. It also may involve the use of a long catheter with a mechanical device attached to the tip that either removes the clot or physically breaks it up.

Thrombolysis is often used as an emergency treatment to dissolve blood clots that form in arteries feeding the heart and brain -- the main cause of heart attacks and ischemic strokes and in the arteries of the lungs (acute pulmonary embolism).

Thrombolysis is also used to treat blood clots in:

- Veins that cause deep vein thrombosis (DVT) or clots in the legs, pelvic area, and upper extremities; if left untreated, pieces of the clot can break off and travel to an artery in the lungs, resulting in an acute pulmonary embolism.
- Bypass grafts
- Dialysis catheters

1.8.1 Thrombolytic Drug

Thrombolysis therapy uses thrombolytic drugs that dissolve blood clots. Most of these drugs target fibrin (one of the main constituent of blood clots) and are therefore called fibrinolytics. These drugs are either derived from *Streptococcus* species, or, more recently, using recombinant biotechnology whereby tPA is manufactured using cell culture, resulting in a recombinant tissue plasminogen activator or rtPA.

Desirable features of ideal thrombolytic drug

- Fibrin specificity
- Longer half life (bolus administration)
- Good patency
- Low or no reocclusion rate and systemic bleeding

- Resistant to plasminogen activator inhibitor-1(PAI-1)
- Nonantigenic and cost effective

1.8.2 Classification of Thrombolytic Drugs

Thrombolytic agents can be categorized in several ways. Classification schemes can be devised on the basis of the source of the agent, the propensity for enhanced enzymatic activity on fibrin or cell surface or the mechanism of action (enzymatic versus nonenzymatic) or different generation wise.

Table 1.3: Classification of Thrombolytic Drugs (Guta. 2008)

Generation of Thrombolytic Drug	Fibrin Specific	Nonfibrin Specific
First	— —	Streptokinase Urokinase
Second	Recombinant tissue plasminogen activator (t-PA)	Prourokinase (scu-PA)
Third	Alteplase Tenecteplase (TNK-tPA) Reteplase Monteplase Lanoteplase Pamiteplase Staphylokinase Desmoteplase (Bat-PA) Chimeric thrombolytics	APSAC — — — — — — —

1.9 Aims of the Present Study

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 and evaluate the antimicrobial activity and thrombolytic activity of different solvent extract of *Dracaena spicata*.

1.10 Study Area

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

Chapter 2: Literature Review

2.1 Evaluation of Antimicrobial Activities of Some Bangladeshi Medicinal Plants

The crude methanol extracts of aerial parts of Abrus precatorius L., leaf of Magnolia pterocarpa Roxb., Dracaena spicata Roxb. and Ravenala madagascariensis Sonn. as well as their hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for disc diffusion assay. Among the test samples of A. precatorius, the highest zone of inhibition (15.0mm) was exhibited by the carbon tetrachloride soluble fraction against Pseudomonas aeruginosa. The M. pterocarpa extractives exhibited significant zone of inhibition ranging from 7.0 to 23.0mm against the test organisms. The highest zone of inhibition (23.0mm) was demonstrated by the carbon tetrachloride solublefraction against Pseudomonas aeruginosa. This fraction also exhibited 20.0mm zone of inhibition against the gram positive bacteria Staphylococcus aureus and gram negative bacteria Vibrio parahemolyticus. Among the test samples of D. spicata, the highest (18.0mm) zone of inhibition was demonstrated by the aqueous soluble fraction against Pseudomonas aeruginosa. The test samples of R. madagascariensis exhibited weak antimicrobial activity with zone of inhibition ranging from 2.0 to 9.0mm (Sharmin et al., 2014)

2.2 Evaluation of Thrombolytic and membrane Stabilizing Activities of Four Medicinal Plants of Bangladesh

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. Among the extractives of *A. precatorius*, the crude methanol extract exhibited the highest thrombolytic activity (34.92±0.54 %) while the carbon tetrachloride soluble fraction of *M. pterocarpa* exhibited 22.59±0.88 % clot lysis. *D. spicata* extractives showed mild thrombolytic activity. The methanolic crude extract of *R. madagascariensis* leaf and the aqueous soluble fraction of *R. madagascariensis* bark extract showed 45.32±0.82 % and 32.67±0.74% clot lysis, respectively. In hypotonic solution and heat induced conditions, the crude methanol extract of *A. precatorius* and the hexane soluble fraction of crude methanol extract of *M. pterocarpa* inhibited 63.46±0.84

% & 36.54±0.21 % and 66.12±0.66 % & 40.54±0.02 % haemolysis of RBCs, respectively as compared to 71.90 % and 42.12 % inhibition by acetyl salicylic acid (0.10 mg/ml), respectively. The crude methanol extract of *D. spicata* demonstrated 64.44±0.68 % and 36.52±0.19 % inhibition of hypotonic solution and heat induced hemolysis, respectively. The chloroform soluble fraction of *R. madagascariensis* leaf extract demonstrated 28.72±0.61 % & 39.97±0.39 % and the hexane soluble fraction of *R. madagascariensis* bark extract revealed 53.78±0.17 % & 41.83±0.61 % inhibition of hypotonic solution and heat induced hemolysis of RBCs, respectively (Chowdhury *et al*., 2013).

2.3 Ethnobotanical Survey of the Rakhain Tribe Inhabiting the Chittagong Hill Tracts Region of Bangladesh

The Rakhains belong to the Bhotbarmi community of the Mongoloids. In Bangladesh, they form a small tribal community inhabiting the Chittagong Hill Tracts region. Their traditional healers are noted for their knowledge of medicinal plants. A survey was conducted on this topic among the Rakhain traditional healers to obtain information on medicinal plants used to treat various ailments. The plants used by the Rakhain traditional healers include Dracaena spicata, Blumea sinuata, Eclipta prostrata, Ananas comosus, Terminalia arjuna, Eupatorium odoratum, Cuscuta reflexa, Dillenia indica, Dryopteris filix-mas, Emblica of icinalis, Pedilanthus tithymaloides, Bambusa multiplex, Bambusa oldhamii, Leucas aspera, Caesalpinia nuga, Crotalaria incana, Cassia sophera, Abutilon indicum, Hibiscus rosa sinensis, Urena lobata, Artocarpus heterophyllus, Musa sapientum, Psidium guajava, Syzygium cumini, Syzygium jambos, Zizyphus oenoplia, Aegle marmelos, Clerodendrum indicum, Clerodendrum viscosum, and Alpine nigra. The plants are used to treat ailments like constipation, diarrhea, stomach pain, acidity, flatulence, piles, blood with stool, loss of appetite, helminthiasis, vomiting tendency, toothache, colds, cough, mucus, fever, asthma, bronchitis, throat pain, tonsillitis, dizziness, wounds, inflammation in any part of the body, abscess, scabies, psoriasis, ringworm, burning sensation in hand or feet, lesions within the nose, nose bleeds, poisonous animal or insect bites, body pain, rheumatic pain, muscle pain, urinary tract disorders (burning sensation in urinary tract, frequent or infrequent urination), elephantitis, jaundice, malaria, kalazar, low sperm count, kidney disorders, hypertension, heart palpitations, weakness, and paralysis. A notable feature of the Rakhain traditional healers is that they us e the same p la n t or plant parts to cure multiple ailments. In this

aspect they are more knowledgeable on the different medicinal properties of plant parts like leaf, stem, flower, and fruit. They have found that the leaf of Dracaena spicata has been used for fever, dizziness. Leaf paste is applied to forehead. (Hanif et al., 2009).

2.4 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)

2.5 Biological activity of saponins from two *Dracaena* species

Many species of the west African "soap tree" *Dracaena* are used in traditional medicine for the treatment of a variety of diseases. In continuation of our search for anti-infective agents from plants implicated in traditional medicine, we evaluated the biological activities of saponins from extracts of *Dracaena mannii* and *Dracaena arborea* by using a battery of test systems such as radiorespirometry, Cytosensor bioautography, and agar dilution methods and molluscicidal tests. Bioassay-directed fractionation of the methanol extracts of seed pulp using a combination of chromatographic techniques, gel filtration, droplet counter current chromatography (DCCC), and low-pressure liquid chromatography (Lobar), led to the isolation and characterization of spiroconazole A, a pennogenin triglycoside [3 beta-O-[(alpha-L-rhamnopyranosyl(1-->2), alpha-L-rhamnopyranosyl(1-->3)-beta-D-glucopyranosyl]-17 alpha-hydroxyl-spirost-5-ene] As the active constituent, spiroconazole A exhibited pronounced antileishmanial,

antimalarial, and molluscicidal activities. It also reports on the fungistatic, fungicidal and bacteriostatic activity of spiroconazole A against 17 species of fungi and 4 of bacteria (Okunji *et al*., 2016).

2.6 Anticoagulant and Antioxidant Activities of *Dracaena arborea* Leaves

The crude methanol extract of *Dracaena arborea* leaves induced significant ($p<0.01$) increase in the clotting times of 21 ± 0.54 sec and 25 ± 1.1 sec at 5% and 10% concentrations of the extract respectively compared to the baseline clotting time of 7 ± 0.63 sec for the blood sample. The extract also exhibited potent in vivo and in vitro anticoagulant activities. Increased doses (100 and 200 mg/kg) of the extract, heparin (0.75 and 1.5 mg/kg) and aspirin (1.0 and 2.0 mg/kg) were found to have significantly ($p < 0.01$) prolonged the mean bleeding times with respect to the baseline in rabbits. However, in thrombin-induced clotting assay, the extract demonstrated a reduced potency compared to heparin. DPPH (1, 1-Diphenyl-2-picrylhydrazyl) and FRAP (Ferric reducing/antioxidant power) spetrophotometric assays revealed that the crude leaf extract possesses appreciable high antioxidant potentials. *Dracaena arborea* leaves could be a source of novel anticoagulant and antioxidant compounds for the management of various hematological disorders (Chinaka *et al.*, 2013).

2.7 Anthracnose of lucky bamboo *Dracaena sanderiana* caused by the fungus Colletotrichum dracaenophilum in Egypt

Dracaena sanderiana, of the family Liliaceae, is among the ornamental plants most frequently imported into Egypt. Typical anthracnose symptoms were observed on the stems of imported *D. sanderiana* samples. The pathogen was isolated, demonstrated to be pathogenic based on Koch's rule and identified as *Colletotrichum dracaenophilum*. The optimum temperature for its growth ranges from 25 to 30 °C, maintained for 8 days. Kemazed 50% wettable powder (WP) was the most effective fungicide against the pathogen, as no fungal growth was observed over 100 ppm. The biocontrol agents *Trichoderma harzianum* and *Trichoderma viride* followed by *Bacillus subtilis* and *Bacillus pumilus* caused the highest reduction in fungal growth. This report describes the first time that this pathogen was observed on *D. sanderiana* in Egypt (Ibrahim , 2016).

2.8 Phytochemical Screening, Proximate Analysis and Antioxidant Activity of *Dracaena reflexa Lam.* Leaves

In the present study, the antioxidant activity of successive leaf extracts of *Dracaena reflexa* was investigated using the scavenging activity on 1,1-diphenyl-2-picrylhydrazyl and reducing power by ferric reducing antioxidant power assay. Methanol extract was found potent in both the assays. IC50 values of 1,1-diphenyl-2-picrylhydrazyl assay for methanol extract was 0.97 mg/ml and ferric reducing antioxidant power value for the same is 1.19. Phytochemical screening, proximate analysis and total phenolic content were also determined. Qualitative screening for phytochemical showed the presence of alkaloids, flavonoids, terpenoids, glycosides and saponins. Highest phenolic content was shown by methanol extract (49.69 mg gallic acid equivalent/g dry weight). Proximate analysis showed moisture content (3.31%), ash content (8.02%), crude fibre (1.31%), crude fat (0.97%), total protein (3.70%), total carbohydrate (86.01) and nutritive value (367.56 kcal/100 g), which would make it a potential nutraceutical. This study suggested that *Dracaena reflexa*, a potential natural free radical scavenger, which could find use as an antioxidative (Shukla *et al.,* 2016)

2.9 Cell Cycle Arrest and Apoptosis Induction via Modulation of Mitochondrial Integrity by Bcl-2 Family Members and Caspase Dependence in Dracaena cinnabari-Treated H400 Human Oral Squamous Cell Carcinoma

Dracaena cinnabari Balf.f. is a red resin endemic to Socotra Island, Yemen. Although there have been several reports on its therapeutic properties, information on its cytotoxicity and anticancer effects is very limited. This study utilized a bioassay-guided fractionation approach to determine the cytotoxic and apoptosis-inducing effects of *D. cinnabari* on human oral squamous cell carcinoma (OSCC). The cytotoxic effects of *D. cinnabari* crude extract were observed in a panel of OSCC cell lines and were most pronounced in H400. Only fractions DCc and DCd were active on H400 cells; subfractions DCc15 and DCd16 exhibited the greatest cytotoxicity against H400 cells and D. cinnabari inhibited cells proliferation in a time-dependent manner. This was achieved primarily via apoptosis where externalization of phospholipid phosphatidylserine was

observed using DAPI/Annexin V fluorescence double staining mechanism studied through mitochondrial membrane potential assay cytochrome c enzyme-linked immunosorbent and caspases activities revealed depolarization of mitochondrial membrane potential (MMP) and significant activation of caspases 9 and 3/7, concomitant with S phase arrest. Apoptotic proteins array suggested that MMP was regulated by Bcl-2 proteins family as results demonstrated an upregulation of Bax, Bad, and Bid as well as downregulation of Bcl-2. Hence, *D. cinnabari* has the potential to be developed as an anticancer agent (Alabsi *et al.,* 2016).

2.10 Antimicrobial activity test

Antimicrobial screening: Antimicrobial activity of the extractives was determined against gram positive and gram negative bacteria and fungi by the disc diffusion method Measured amount of the test samples were dissolved in definite volume of solvent (chloroform or methanol) and applied to sterile discs and carefully dried to evaporate the residual solvent. In this investigation, ciprofloxacin (30µg/disc) disc was used as the reference.

Result: The test samples of *D. Spicata* exhibited zone of inhibition ranging from 7.0 to 18.0mm against the test organisms. The highest (18.0mm) zone of inhibition was demonstrated by the aqueous soluble fraction against *Pseudomonas aeruginosa*. Against gram positive bacteria *Staphylococcus aureus*, the carbon tetrachloride and aqueous soluble extractives revealed 15.0mm zone of inhibition

2.11 Thrombolytic activity

The thrombolytic activity was evaluated by the method developed by by using streptokinase as positive control.

Result: The crude methanol extracts of aerial parts of leaf of *D. spicata* as well as its hexane, 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 promote lysis of blood clot from natural resources, the extractives *D. spicata* were assessed for thrombolytic activity. Addition of 100 µl streptokinase, a positive control (30,000 I.U.) to the clots of human blood and subsequent

incubation for 90 minutes at 37°C showed 66.77 % lysis of clot. On the other hand, distilled water, treated as negative control, revealed a negligible lysis of clot (3.79 %). *D. spicata* extractives showed mild thrombolytic activity and the highest thrombolytic activity was demonstrated by the carbon tetrachloride soluble fraction (21.05±0.23 %).

2.12 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

Result: The membrane stabilizing activity of D. spicata extractives was also determined. The hexane soluble fraction of crude methanol extract of D. Spicata demonstrated 64.44±0.68 % & 36.52 % inhibition of hypotonic solution and heat induced hemolysis, respectively. *D. spicata* exhibited significant membrane stabilizing activity 2.4.Antipyretic activity: Root extract of the plant possesses antipyretic activity mild.

2.13 Antiulcerant activity

The tribal people make juice from the leaf of the plant and it is used for ulcer and stomachaches (EncyclopediaBritannica, 2013).

Chapter 3 : Methods & Materials

3.1 Preparation of Plant Extract for Experiments

3.1.1 Materials

3.1.1.1 Reagent

- Petroleum ether
- Dichloromethane
- Ethyl acetate
- MeOH

3.1.1.2 Equipments

- Beaker
- Funnel
- Glass rod
- Grinding machine
- Filter paper
- Cotton
- Separating funnel
- Measuring cylinder
- Cotton cloth

3.2 Collection and 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 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.

3.2.1 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.

3.2.2 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.

3.2.3 Extraction of the Dried Powdered Sample

The 200gm fine powder of *Dracaena spicata* whole plant was dissolved in 500 ml of pet. Ether DCM, EA, methanol separately 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.

3.2.4 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

3.2.5 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 preserved in refrigerator.

3.3 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.

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 and EYELA. 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.

Figure 3.1: Rotary evaporator devaice

3.4 Antimicrobial screening

Antimicrobial activity of the extractives was determined against gram positive and gram negative bacteria and fungi by the disc diffusion method. Measured amount of the test samples were dissolved in definite volume of solvent (chloroform or methanol) and

applied to sterile discs and carefully dried to evaporate the residual solvent. In this investigation, kanamycine (30µg/disc) disc was used as the standard

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).

3.5 Test materials used for the study

The methanol 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.

3.5.1 Reagents:

- Rectified spirit
- Agar purified powder

- Methanol
- Dichloromethane

3.5.2 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 unitϖ Incubator
- Refrigerator

3.6 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 – 3.1: List of the test pathogenic bacteria Name of the test organism

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*

3.7 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.

3.8 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

Figure – 3.2: Laminar hood

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.

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 – 3.3: Incubator

3.10 Preparation of the test plate

The test organisms were transferred from the subculture to petri dish 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 petri dish with the help of this cotton bud.

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 (400µg/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.

3.11.1 List of Test Materials

Plant	Sample Code	Test Sample
Dracaena spicata (whole plant)	DSPE	Petroleum ether soluble fraction
	DSDCM	Dichloromethane soluble fraction
	DSEA	Ethyl acetate soluble fraction
	DSME	MeOH soluble fraction

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.

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

3.14 Materials and Method

3.14.1 Preparation of Sample

The thrombolytic activity of all extractives was evaluated by a method using streptokinase (SK) standard substance. 10 mg of different solvent extracts of *Dracaena spicata* were taken in different vials to which 1 ml distilled water was added.

3.14.2 Streptokinase

Streptokinase (15, 00,000 I.U.,) used as a standard which was collected from Beacon pharmaceutical Ltd, Bangladesh. 5 ml sterile distilled water was added to streptokinase vial and mixed properly. From this suspension100µl (30,000 I.U) was used for *in vitro* thrombolysis.

3.14.3 Blood Sample

Blood samples were collected from healthy human volunteers (n=5) by maintaining aseptic condition without a history of oral contraceptive or anticoagulant therapy. 1ml of blood was transferred to the previously weighed vials tubes to form clots.

3.14.4 Thrombolytic Activity

Aliquots (5 ml) venous blood were drawn from healthy volunteers which were distributed in five different pre weighed sterile vials (1 ml/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed without disturbing the clot and each vial having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone).

To each vial containing pre-weighed clot, 100 µl aqueous solutions of different partitions along with the crude extract were added separately.As a positive control, 100 µl of streptokinase (SK) and as a negative non thrombolytic control, 100 µl of distilled water were

separately added to the controls vials. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, the released fluid was discarded and vials were again weighed to observe the difference in weight after clot disruption. Finally percentage of clot lysis was determined as followings:

$$\% \text{ of clot lysis} = (\text{wt of released clot} / \text{clot wt}) \times 100$$

3.14.5 List of Test Materials

Plant	Sample Code	Test Sample
Dracaena spicata (whole plant)	DSPE	Petroleum ether soluble fraction
	DSDCM	Dichloromethane soluble fraction
	DSEA	Ethyl acetate soluble fraction
	DSME	MeOH soluble fraction

3.14.6 Statistical Analysis

The significance between % lysis of clot by Streptokinase and each of the extracts of the plant by means of weight difference was carried out three times in blood samples of fifty different healthy volunteers and results are expressed as mean ± standard deviation.

Chapter 4 : Results & Discussion

4.1 Results of Anti-microbial Screening

Table 4.1: Antimicrobial test (result in mm)

Microorganism	PE (400 µg/ disk)	DCM (400 µg/ disk)	EA (400 µg/ disk)	MeOH (400 µg/ disk)	Kanamycin (30 µg/ml)
Bacillus sereus	31	16	20	26	**35**
Bacillus megaterium	0	26	15	9	**37**
Bacillus subtilis	9	20	13	10	**35**
Salmonella paratyphi	11	30	22	20	**35**
Salmonella typhi	30	20	14	14	**34**
Vibrio parahemolyticus	0	16	17	13	**40**
Vibrio mimicus	27	30	25	29	**36**
Staphylococcus aureaus	11	10	9	8	**38**
Escherichia coli	9	0	6	9	**35**
Sheigella dysenteriae	8	16	11	10	**34**
Pseudomonas aureaus	**25**	**28**	**20**	**30**	**36**

4.1.1 Discussion of Anti-microbial Screening

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.

Dracaena spicata extractives exhibited moderate to strong antimicrobial activity. The test samples of *D. spicata* exhibited zone of inhibition ranging from 9.0 to 30.0mm against the test organisms.

In the present study, the petroleum ether extracts of *Dracaena spicata* showed the activity against *Bacillus sereus* having the highest zone of inhibition 31mm, *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 analysis.

The DCM soluble fraction of D. spicata exhibited zone of inhibition ranging from moderate to good against the test organisms. The highest (30.0mm) zone of inhibition was demonstrated against Vibrio mimicus and Salmonella paratyphi. But there was no zone of inhibition found against E. coli. A good zone of inhibition were exhibited against Bacillus megaterium, Bacillus subtilis, Salmonella typhi and Pseudomonas aureaus .On the other hand against Pseudomonas aureaus, Vibrio parahemolyticus, Vibrio parahemolyticus and Sheigella dysenteriae , a moderate zone of inhibition was seen.

The highest zone of inhibition was demonstrated by the EA soluble fraction against *Vibrio mimicus* (25.0mm)and lowest result was found against *Staphylococcus aureaus* (6 mm).

The highest zone of inhibition was demonstrated by the aqueous soluble fraction against *Pseudomonas aureaus*(30.0mm)and lowest result was found against *Bacillus megaterium* (9 mm).

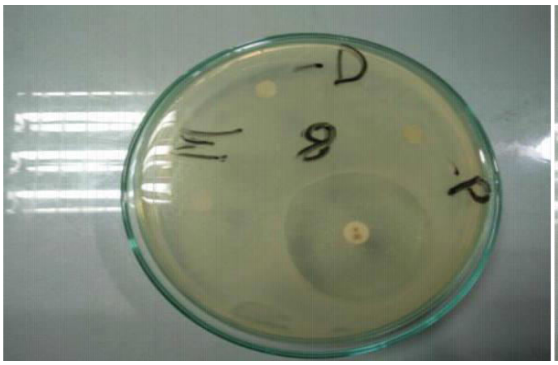

Fig 4.1: (*Staphylococcusaureaus*)

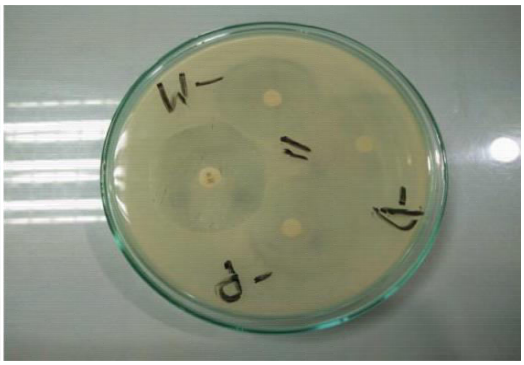

Fig 4.2: (*Pseudomonas aureaus*)

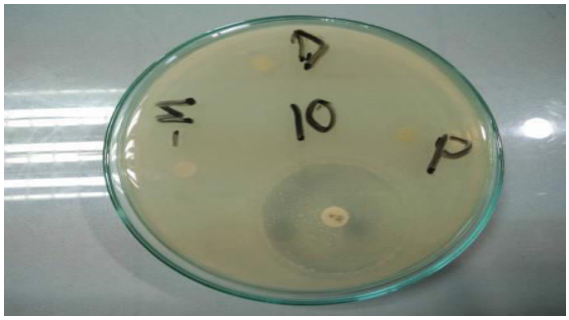

Fig 4.3: (*Sheigella dysenteriae*)

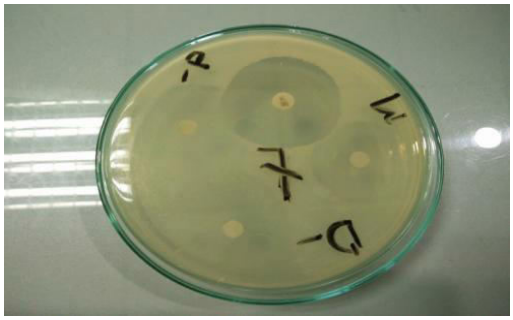

Fig 4.4: (*Vibrio mimicus*)

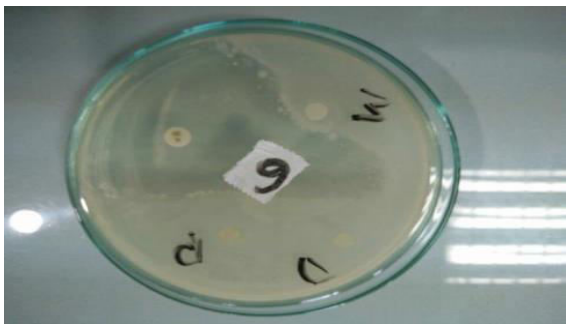

Fig 4.5: Test plate 6(*Vibrio parahemolyticus*)

Fig 4.6: *Salmonella typhi*

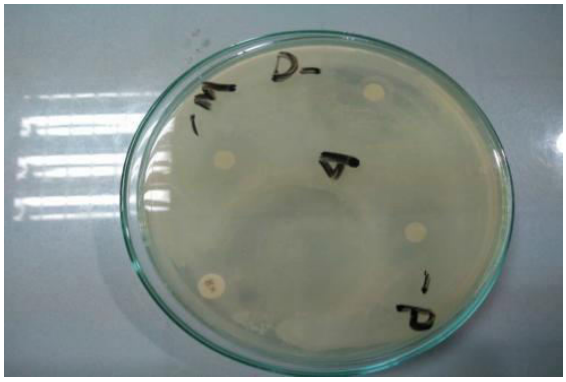

Fig 4.7: test plate 4(*Salmonella paratyphi*)

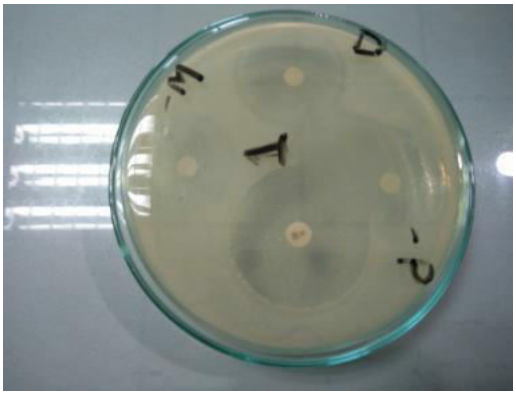

Fig 4.8: test plate 1 (*Bacillussereus*)

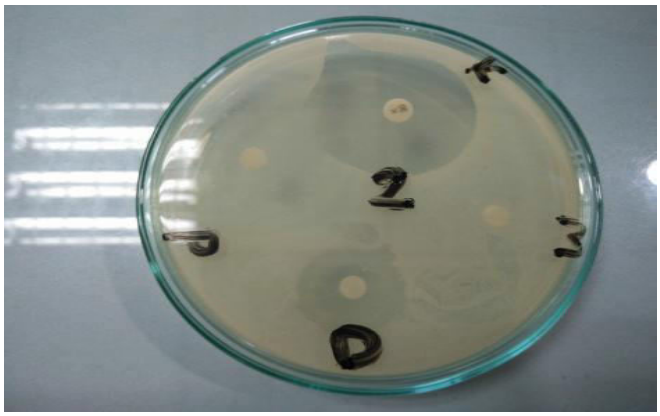

Fig 4.9: test plate 2 (*Bacillusmegaterium*)

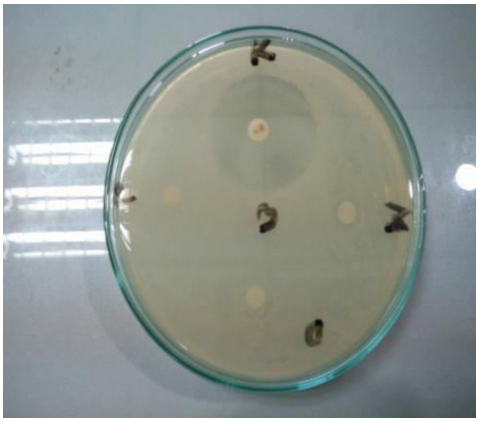

Fig 4.10: test plate 3 (*Bacillus subtilis*)

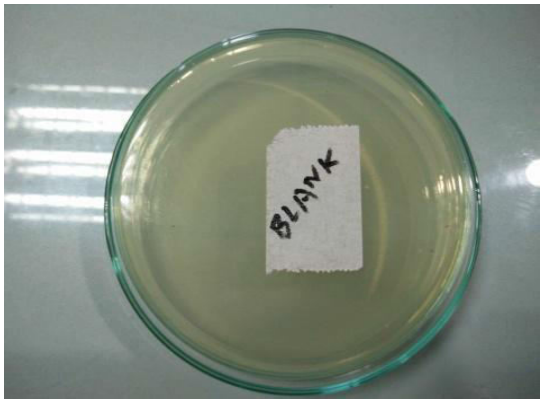

Figure 4.11: blank

Here, M= methanol extract, P= Petroleum ether, D= DCM

4.2 Results of Thrombolytic Activity

Table 4.12: Thrombolytic activity (in terms of % of clot lysis) of *Dracaena spicata*

Fractions	Weight of empty vial, W_1 g	Weight of clot containing vial before clot disruption, W_2 g	Weight of clot containing vial after clot disruption, W_3 g	% of clot lysis $\frac{W_2-W_3}{W_2-W_1}\times 100$
DSPE	6.3320	7.1169	6.9005	27.57
DSDCM	6.3772	7.0468	6.8890	23.57
DSEA	6.2073	6.8449	6.6984	22.98
DSME	6.1981	6.8028	6.6811	20.12
Blank	6.1674	6.7281	6.6988	5.22
SK	6.2728	6.7390	6.4397	64.19

4.2.2 Discussion of Thrombolytic Activity

In investigating cardio protective drugs from natural sources the extracts obtained from *Dracaena spicata* were assessed for thrombolytic activity and the results are presented in Table 4.12. After addition of 100 µl SK, a positive control (30,000 I.U),to the clots, the system was incubated for 90 minutes at 37 °which then exhibited 64.19% lysis of clot. On the other hand, distilled water was applied as negative control which exhibited a negligible percentage of lysis of clot (5.22%). The mean difference in clot lysis percentage between positive and negative control was found statistically very notable. In this study, Pet ether soluble fraction (DSPE) of *Dracaena spicata* demonstrated highest thrombolytic activity (27.57%), although it is not much significant. This was followed by Dichloromethane soluble fraction (DSDCM) (23.57%) and Ethyl acetate soluble fraction (DSEA) (22.98%), again not much significant. The other partitions of methanol

extract of *Dracaena spicata* showed little thrombolytic activity. The comparison of thrombolytic activities of different extractives is shown in Figure 4.13.

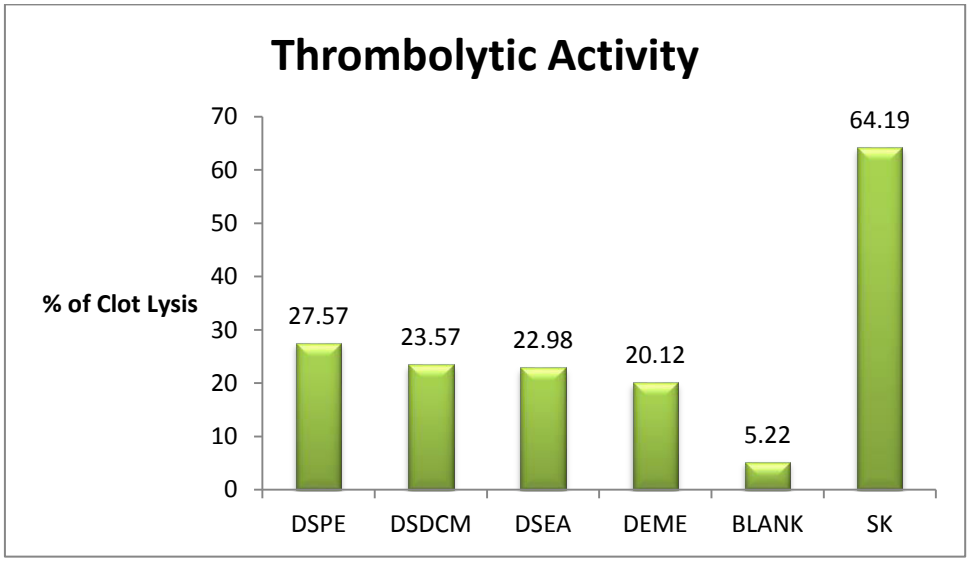

Figure 4.13: Thrombolytic activity of *Dracaena spicata*

Chapter 5 : Conclusion

5.1 Conclusion

The present study was carried out to investigate anti-microbial and thrombolytic activity of *Dracaena spicata* available in Bangladesh.

From the result of my study, it can be concluded that, using in vitro experiments established that different solvnt extracts of *Dracaena spicata* inhibits the bacterial growth. The antimicrobial activity of the plant extracts were tested against 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.

To check the efficacy of thrombolytic drugs or herbs one can compare the data with positive and negative control. In our study we took a known thrombolytic drug; streptokinase as a positive control and water as a negative control. The comparison of positive control with negative control clearly confirmed that clot dissolution does not occur when water was added to the clot. By comparing with this positive & negative control, a significant thrombolytic activity was observed after treating the clots with *Dracaena spicata* extracts.

In conclusion from our recorded data, it can be demonstrated that our findings may have notable implications in cardiovascular health. In addition, this finding may indicate the possibility of developing novel thrombolytic compounds from *Dracaena spicata* extracts and our study could be an instant and effective methodology to study clot lytic effect of newly developed drugs as well as known drugs. Further studies are ongoing to isolate and characterize the compounds responsible for thrombolytic activity

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.

Chapter 6: Reference

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