Study on Antimicrobial and Cytotoxicity Activity of Methanol Extract of *Dracaena spicata*

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

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Declaration by the Research Candidate

I, Fatema Jannat, hereby declare that the dissertation entitled "**Study on Antimicrobial and Cytotoxic activity of Methanol Extract of *Dracaena spicata***" submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a complete record of original research work carried out by me during 2016, under the supervision and guidance of Ms 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 "**Study on Antimicrobial and Cytotoxic activity of Methanol Extract of *Dracaena spicata***" submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Bachelor of pharmacy was carried out by Fatema Jannat, ID# 2013-1-70-042 in 2016, 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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ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation, entitled "**Study on Antimicrobial and Cytotoxic activity of Methanol Extract of *Dracaena spicata***" is a bona fide research work done by Fatema Jannat (ID#2013-1-70-042), in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

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Dedication

This Research Paper is dedicated to my beloved parents, they are my biggest inspirations.

ABSTRACT

The purpose of the study was to evaluate the cytotoxic and antimicrobial activity of methanolic extract of *Dracaena spicata* of the family Asparagaceae.*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 ofvarious 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.Theantimicrobialactivitiesofmethalonicextractof*Dracaenaspicata*plantweretested againstthegram-positive(*Bacillus sereus, Bacillus megaterium,Staphylococcus aureaus*) andgram-negativebacterial(*Pseudomonas aureaus,Salmonella paratyphi, Salmonella typhi, Vibrio parahemolyticus, Vibrio mimicus, Sheigella dysenteriae, Escherichia coli)* strainsbyobservingthezoneofinhibition.Theantimicrobialtestwasperformedbydiscdiffusionmethod.Thecrudemethanolextractof*Dracaenaspicata* plant showedmoderate to strong (9mm-30mm) antimicrobial activitiesagainst themicroorganisms. Butmethanolextractof*Dracaenaspicata*showedstrong activityagainst*Pseudomonasaureaus* (30mm) and the lowest activity was found against *Bacillusmegaterium* (9mm). However, no activity was found against*Salmonella paratyphi, Salmonella typhi, Vibrio parahemolyticus, Staphylococcus aureaus, Escherichia coli, Sheigella dysenteriae.* The cytotoxic activity of the plant was done by using *Artemia saline* Leach. The LC50 was observed approximately as 46.4729 µg /mL with a R2 value of 0.772. Cytotoxicity of methanolic extract of *Dracaena spicata* was low to moderate. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation

Key Words

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

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CHAPTER ONE

INTRODUCTION

1.1 Introduction

According to the World Health Organization (WHO), 80% of the world's populations rely on traditional medicines. The practice of herbal medicine is common in rural areas where western medicines are too expensive or not available. Humans have frequently used plants to treat common infectious diseases and some of these traditional medicines are still part of the habitual treatment of various maladies. The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments. Hence, there is need to screen medicinal plants for promising biological activity. Drugs derived from unmodified natural products or drugs semi-synthetically obtained from natural sources corresponded to 78% of the new drugs approved by the FDA between 1983 and 1994.

Medicinal properties of plants were known even to pre-historic men and many of these plants have been used in traitional medicine for hundreds of years with reputation as efficacious remedies. Over the last century, ethnobotany has evolved into a specific discipline that looks at the people–plant relationship in a multidisciplinary way such as ecology,economic botany, pharmacology, public health and other disciplines as needed. A large number of plants are being used as medicinal agents all over the world. 1500 species in India, 5000 species in China and 1600 species in north-west Amazonia have been reported to possess medicinal uses. Limitations o diseases and the potential of plant based medicine as a more effective and cheaper alternative was probably responsible for the fast growing industry of herbal medicine. Many drugs that are currently in the market have come from folk medicine and traditional use of plants by indigenous communities (Ghani, 1998).

1.2 Medicinal plants

Any plant whose roots, leaves, seeds, bark, or plant part is used for therapeutic, tonic, purgative, or other health-promoting purposes. Plants used as natural medicines. Accordingly, the WHO consultative group on medicinal plants has formulated a definition of medicinal plants in the following way: " A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for

therapeutic purposes or which is a precursor for synthesizing of useful drugs" (Sofowora, A, 1982).

1.3 Medicinal plants in Oncology

Plants used in oncological practice include Peucedanum morisonii, calendula, and bal d cypress. Madagascarperiwinkle (Catharanthus roseus), a cultivated herb, contains th e alkaloids vincaleukoblastine (vinblastine) and leurocristine(vincristine), which are u sed to treat lymphogranulomatosis, reticulosis, and leukosis. The crocus Colchicum sp eciosum, awild herb, contains the alkaloid colchamine in its corms, which is used in t he form of an ointment to treat skin cancer and intableform to treat cancer of the esop hagus (Faried, A.,et al, 2000).

1.4 Importance of Medicinal Plant

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes. The use of medicinal plants as a source for relief from 130 drugs, all single chemical entities extracted from illness can be traced back over five millennia to written higher plants, or modified further synthetically, are documents of the early civilization in China, India and the currently in use, though some of them are now being. Near east, but it is doubtless an art as old as made synthetically for economic reasons.

- Low cost: herbals are relatively inexpensive and the cost of pharmaceuticals to governments and individuals is rising
- Drug resistance: the need for alternative treatments for drug-resistant pathogens

- Limitations of medicine: the existence of ailments without an effective pharmaceutical treatment
- Medicinal value: laboratory and clinical corroboration of safety and efficacy for a growing number of medicinal plants
- Cultural exchange: expanding contact and growing respect for foreign cultures, including alternative systems of medicine
- Commercial value: growing appreciation of trade and other commercial economic opportunities represented by medicinal plants (Chowdhury, Et al,2008)

1.5 Historical Sources Relevant For Study of Medicinal Plants' Use:

The oldest written evidence of medicinal plants' usage for preparation of drugs has been found on a Sumerian clay slab from Nagpur, approximately 5000 years old. It comprised 12 recipes for drug preparation referring to over 250 various plants, some of them alkaloid such as poppy, henbane, and mandrake.

The Chinese book on roots and grasses "Pen T'Sao," written by Emperor Shen Nung circa 2500 BC, treats 365 drugs (dried parts of medicinal plants), many of which are used even nowadays such as the following: Rhei rhisoma, camphor, Theae folium, Podophyllum, the great yellow gentian, ginseng, jimson weed, cinnamon bark, and ephedra

The Indian holy books Vedas mention treatment with plants, which are abundant in that country. Numerous spice plants used even today originate from India: nutmeg, pepper, clove, etc(Queiroz, E., et al,2009).

The Ebers Papyrus, written circa 1550 BC, represents a collection of 800 proscriptions referring to 700 plant species and drugs used for therapy such as pomegranate, castor oil plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, common centaury.

According to data from the Bible and the holy Jewish book the Talmud, during various rituals accompanying a treatment, aromatic plants were utilized such as myrtle and incense.

In Homer's epics The Iliad and The Odysseys, created circa 800 BC, 63 plant species from the Minoan, Mycenaean, and Egyptian Assyrian pharmacotherapy were referred to. Some of them were given the names after mythological characters from these epics; for instance, Elecampane (Inula helenium L. Asteraceae) was named in honor of Elena.

Theophrast (371-287 BC) founded botanical science with his books "De Causis Plantarium"— Plant Etiology and "De Historia Plantarium"—Plant History. In the books, he generated a classification of more than 500 medicinal plants known at the time. In his work "De re medica" the renowned medical writer Celsus (25 BC–50 AD) quoted approximately 250 medicinal plants such as aloe, henbane, flax, poppy, pepper, cinnamon, the star gentian, cardamom, false hellebore, etc.

In ancient history, the most prominent writer on plant drugs was Dioscorides, "the father of pharmacognosy," who, as a military physician and pharmacognosist of Nero's Army, studied medicinal plants wherever he travelled with the Roman Army. Circa 77 AD he wrote the work "De Materia Medica." This classical work of ancient history, translated many times, offers plenty of data on the medicinal plants constituting the basic materia medica until the late middle Ages and the Renaissance. Of the total of 944 drugs described, 657 are of plant origin, with descriptions of the outward appearance, locality, mode of collection, making of the medicinal preparations, and their therapeutic effect (Singh, N.,etal, 2016).

Galen also introduced several new plant drugs in therapy that Dioscorides had not described, for instance, Uvae ursi folium, used as an uroantiseptic and a mild diuretic even in this day and age.

Paracelsus (1493-1541) was one of the proponents of chemically prepared drugs out of raw plants and mineral substances; nonetheless, he was a firm believer that the collection of those substances ought to be astrologically determined. He continuously emphasized his belief in observation, and simultaneously supported the "Signatura doctrinae"—the signature doctrine. (Jancic, 2002).

In 18th century, in his work Species Plantarium (1753), Linnaeus (1707-1788) provided a brief description and classification of the species described until then. The

species were described and named without taking into consideration whether some of them had previously been described somewhere.

Early 19th century was a turning point in the knowledge and use of medicinal plants. The discovery, substantiation, and isolation of alkaloids from poppy (1806), ipecacuanha (1817), strychnos (1817), quinine (1820), pomegranate (1878), and other plants, then the isolation of glycosides, marked the beginning of scientific pharmacy.

In late 19th and early 20th centuries, there was a great danger of elimination of medicinal plants from therapy (Blumenthal, 1998).

1.6 Modern Medicinal Chemistry:

In present days, almost all pharmacopoeias in the world—Ph Eur 6,USP XXXI,BP 2007—proscribe plant drugs of real medicinal value. There are countries (the United Kingdom, Russia, Germany) that have separate herbal pharmacopoeias. Yet, in practice, a much higher number of unofficial drugs are always used. Their application is grounded on the experiences of popular medicine (traditional or popular medicine) or on the new scientific research and experimental results (conventional medicine). Many medicinal plants are applied through self-medication or at the recommendation of a physician or pharmacist. They are used independently or in combination with synthetic drugs (complementary medicine). For the sake of adequate and successfully applied therapy, knowledge of the precise diagnosis of the illness as well as of medicinal plants, i.e. the pharmacological effect of their components is essential. Plant drugs and phytopreparations, most commonly with defined active components, verified action and, sometimes, therapeutic efficiency, are applied as therapeutic means. In the major European producer and consumer of herbal preparations—Germany, rational phytotherapy is employed, based on applications of preparations whose efficiency depends on the applied dose and identified active components, and their efficiency has been corroborated by experimental and clinical tests. Those preparations have been manufactured from standardized plant drug extracts, and they adhere to all requirements for pharmaceutical quality of drugs (Barboza, G.E.,etal, 2009).

1.7 Traditional medicines:

Traditional medicine (also known as indigenous or folk medicine) comprises medical aspects of traditional knowledge that developed over generations within various societies before the era of modern medicine. The World Health Organization (WHO) defines traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness." (Patwardhan, B.,et al, 2000).

1.8 Classification of Medicinal Plants:

Medicinal plants are generally classified on the basis of their growth habit. It may be either a tree, shrub, herb, annuals, biennial, tubers, rhizomes and climbers.

1. Table:

	Common Name	**Botanical Name**	**Parts Used**
1	Babul	*Acacia nilotice Delite*	Pods, leaves, bark, gum
2	Bael	*Aegle marmelos L. Corr.*	Roots, leaves, fruit
3	Neerh	*Azaflirachta indica*	Bark leaves, flowers, seed, oil
4	Palas	*Butea monossperma (Lam.)*	Bark, leaves, flowers, seed, gum
5	Gugul	*Commiphora mukulEngh J*	Resinous gum
6	Olive	*Olea europeae*	Leaves, Oil
7	Arjun	*Terminalia arjuan Roxb.*	Bark

8	Behela	*Terminalia bellirica Gaertu*	Bark, fruit
9	Hirda	*Terminalia bellirica Gaertu*	Fruits
10	Nagakesar	*Mesua ferrea L.*	Blowers, oil .
11	Markingnut	*Semecarpus & anacardium L.*	Fruits

2. Medicinal Shrubs:

Sr.No.	Common Name	Botanical Name	Parts Used
1	Davana	*Artemisia nilagirica*	Leaves, flowering top
2	Safed musli	*Aparagus adscendens Roxbi*	Tuberous roots
3	Belladonna	*Atropa belladonna*	Leaves and roots
4	Lavender	*Lavandula officinalis*	Flowers
5	Sarpagandha	*Rauvalfia serpentina L.*	Roots
6	Chitrak	*Plumbage zeylanica L.*	Leaves, roots

3. Medicinal Herbs:

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

5.	Kalazira	*Nigella sativa L.*	Seed
6.	Afim	*Papaver somniferum L.*	Latex, seed
8.	Babchi	*Psoralea corylifolia*	Seed, Fruit

4. Medicinal Annuals:

Sr.No.	Common Name	Botamical Name	Parts Used
1.	Jangali muli	*Blumea lacera*	Whole plant
2.	Cockscomb	*Celosia cristala L.*	Inflorescence
3.	Red poppy	*Papaver rhoeas*	Flowers
4.	Bhui amla	*Phyllantius niruri*	Whole plant

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	*Melandrium firmum*	Whole plant

6. Tubers and Rhizomes:

Sr.No.	Common Name	Botanical Name	Parts Used
1	Satavar	*Asparagus adscendens*	Tubers

		Roxb	
2	Safed musli	*Chlorophytum borivilianum*	Tubers
3	Puskarmul	*Inula racemosa Hook*	Roots

7. Biennial:

Sr.No.	**Common Name**	**Botanical Name**	**Parts Used**
1	Chocloate vine	*Akebia quinata Deene*	Stem, fruit
2	Malkunki	*Celustrus paniculatus Wild*	Bark, leaves, seed
3	Hajodi	*Cissus quadrangularis L.*	Whole plant
4	Khira	*Cucumis sativus L.*	Fruit, seed
5	Gudmar	*Gymnema sylvestre Retzx*	Whole plant, leves
6	Kali mirch	*Piper nigrum L.*	Fruit

Plant review

Figure 1: *Dracaena spicata*

1.9 Plant information

The name Dracaena is derived from the Greek word ‘drakainia’ meaning a female dragon. The most striking source is the Dracaena cinnabari, 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 dracowhile under-taking a survey of Socotra for the Indian Government in 1835.However, the species was first named and described by the Scottish botanist Sir lsaac Bailey Balfour when he visited the island in 1880. Three grades of Dracaena resin, the most valuable being tear-like in appearance, followed by one made of small chips and fragments, and the cheapest being a molten mixture of fragmentsand refuse.Voyagers to the Canary Islands in the 15th century obtainedDragon’s blood as dried garnet colored drops from another species Dracaena draco, 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 usedin China as source of Dragon’s blood (Gupta, 2008).

1.9.1 Synonyms:

D. wallichii Kunth., Draco spicata Roxb. Kuntze.

1.9.2 Bengali name:

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

1.9.3 Distribution:

The plant is distributed in Assam, Bangladesh, Andaman Islands and Myanmar. The leaf extract is used by the chakma communities in the treatment of measles. Leaf juice is used to cure long term fever, coughs and mucus in nose by traditional healers of the Marma tribe of Naikhongchhari, Bandarban District.

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.

1.9.4 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.

1.9.5 Taxonomy:

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Liliopsida

Order: Asparagales

Family Asparagaceae

Genus: Dracaena

Species: Dracaena spicata

1.9.6 Description of the plant:

Caulescent, Leaves lanceolate, drooping, Spikes terminal, bracts many flowered, Corolcylindric, at last becoming twisted, Stigma three-lobed. A native of Chittagong, and from thence introduced into this Garden by Dr. Buchanan, where it blossoms in april .Root fibrous, stem erect, toward the top succulent, perennial, marked with the cicatrices of the fallen leaves, as in the other Dracaena. Leaves crowded about the extremity of the plant,sheathing, lanceolate, drooping, entire, pointed; smooth on both sides; from six to twelve inches long, and two or three broad. Spikes terminal, bent a little to one side; numerous pointed, recurvedbractes surround the base, and a few shorter, oppressed ones from thence to the flower-bearing position. Flowers numerous, sessile, collected in small fascicles, each fascicle having a small, cordate, pointed bracte immediately under it.Calyx none.corol onepetalled, cylindric divided half way down into three exterior, and three interior slender, linear, equal, straight segments; color pale greenish yellow, as they advance in age the tube becomes twisted. Filaments inserted on the base of the segments of the corol, and of their

length. Stigma three-lobed. Berry with from one to three, distinct, round, and smooth lobes; while immature, a deep olive green, when ripe, deep reddish orange; each lobe containing a single large, round, smooth, white, horny seed (Borsani, C.,et al, 2004).

1.10 Species & Cultivars:

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. 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	 Fig: 1.1: *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	 Fig: 2 *Dracaena draco*
Dracaena cochinchinensis	Resin from *Dracaena cochinchinensis* has been produced by infection with Fusarium and Cladosporiumspp. (Wang et al., 1999)	 Fig:3 *Dracaena cochinchinensis*

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 4: *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.	 Fig5: *Dracaena godseffiana*

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*

1.11: Aim of this experiment

Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi processed plant produce drugs and medicines. Thus huge foreign exchanges can be saved if the manufacturers, to satisfy their needs, utilize the indigenous medicinal plants or their semi processed products. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against the harmful diseases. The increasing failure of chemotherapeutics, severe adverse effects with increase doses and repeated use of drugs, problems with multiple dosage regimens and antibiotic resistance exhibited by pathogenic microbial infectious agents and emergence of new diseases has led to the screening of medicinal plants throughout the world for their potential activity. The main objective of this study was to discovery of new medicinal compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases.

Dracaena spicata is a medicinal plant used traditionally in Bangladesh. Upon significant literature survey it was found only a little research work has been performed on this plant to evaluate its medicinal value and active constituents those are responsible for its pharmacological activities. Therefore, taking into consideration the traditional uses of the plant and facilities available for conducting the study, this research work was performed on this plant. The principal aim of the present study was to investigate the scientific basis of the traditional uses of the plant and evaluate the antimicrobial activity and cytotoxic activity of methanol extract of *Dracaena spicata*.

1.11.1: Study Area:

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

1.11.2: 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

1.12 Approaches of drug development

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

Steps of drug development from plant sources given below:

1.12.1 Selection of plant species:

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

1.12.2 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

1.12.3 Preparation of plant sample and element analysis:

1) Collection of plant sample Extraction Compare the selective and yield

2. Use various extraction techniques 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

1.12.4 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

1.13 Antibiotics:

Antibiotics are chemical compounds used to kill or inhibit the growth of bacteria. Strictly speaking, antibiotics are a subgroup of organic anti-infective agents that are derived from bacteria or moulds that are toxic to other bacteria. However, the term antibiotic is now used loosely to include anti-infectives produced from synthetic and semisynthetic compounds.

The term antibiotic may be used interchangeably with the term antibacterial. However, it is incorrect to use the term antibiotic when referring to antiviral, antiprotozoal and antifungal agents.

1.14 Natural Antibiotics:

Acacia, Aloe, Cryptolepsis, Echinacea, Eucalyptus, Garlic, Ginger, Goldenseal, Grapefruit Seed Extract, Honey, Juniper, Licorice, Sage, Usnea, Wormwood

1.15 Classification of antibiotics:

Antibiotics can be classified in several ways. The most common method classifies them according to their chemical structure as antibiotics sharing the same or similar chemical structure will generally show similar patterns of antibacterial activity, effectiveness, toxicity and allergic potential (Sharmin, T., et al, 2004)**.**

8. Table: classification of Antibiotics

Class(chemical structure)	**Mechanism of action**	**Examples**
B-lactam antibiotics **Penicillins** **Cephalosporin**	Inhibit bacterial cell wall synthesis	Penicillins, Penicillin G, Amoxicillin, Flucloxacillin, Cephalosporins, Cefoxitin, Cefotaxime, Ceftriaxone, Carbapenem, Imipenem
Macrolides	Inhibit bacterial protein synthesis	Erythromycin, Azithromycin, Clarithromycin
Tetracyclines	Inhibit bacterial protein synthesis	Tetracycline, Minocycline, Doxycycline Lymecycline
Fluoroquinolones	Inhibit bacterial DNA synthesis	Norfloxacin, Ciprofloxacin, Enoxacin, Ofloxacin
Sulphonamides	Blocks bacterial cell metabolism by inhibiting enzymes	Co-trimoxazole, Trimethoprim
Aminoglycosides	Inhibit bacterial protein synthesis	Gentamicin, Amikacin

Imidazoles	Inhibit bacterial DNA synthesis	Metronidazole
Peptides	Inhibit bacterial cell wall synthesis	Bacitracin
Lincosamides	Inhibit bacterial protein synthesis	Clindamycin, Lincomycin
Other	Inhibit bacterial protein synthesis	Fusidic acid, Mupirocin

(Sharmin, T., et al, 2004)

1.16 Uses of antibiotic:

Antibiotics only work against infections caused by bacteria. Bacterial infections are much less common than viral infections. Most coughs and colds are of viral origin so antibiotics should not be prescribed for these. Antibiotics should only be used when absolutely necessary, because:

- There is increasing resistance of bacteria to treatment
- Resistant bacteria are selected out by the use of antibiotics
- Antibiotics may have serious adverse effects in some people

1.17 Cytotoxic drugs:

Any drug that has a toxic effect on cells. These can be used in the treatment of cancer because their effect is greatest on cells which are reproducing most rapidly. The cytot oxic drugs include alkylating agents,such as cyclophosphamide, melphalan and chlora mbucil, that interfere with cell growth differentiation and function; cytotoxicantibios, such as dactinomycin, daunorubicin and doxorubicin, that bind to DNA blocking its tr anscription and the Topoisomerase1 inhibitors, such as etoposide,anthracyclines and a nthrapyrazoles, that interfere with nuclear enzymes required for DNA replication and the separation ofdaughter chromosomes (Schimmel, et al, 2004).

CHAPTER TWO

LITERATURE REVIEW

2.1: Literature review

2.2 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 Evaluation of Locomotor activity of Dichloromethane Extract of *Dracaena spicata* by Open Field Method 35 agents from plants implicated in traditional medicine, we evaluated the biological activities of saponins from extracts of *Dracaena spicata* 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-Lrhamnopyranosyl(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).

2.3 Anti thrombal activity

In this study, investigated that thrombus formation inside the blood vessels obstructs blood flow through the circulatory system leading hypertension, stroke to the heart, anoxia and so on. The complete deprivation of oxygen and infarction is a mode of cell death. Crude biologicals and their components possessing anti-thrombotic activity have been reported before. This study was aimed to investigate thrombolytic activity of methanol extracts of four traditionally used medicinal plants. For this an in-vitro thrombolytic study was carried out along with Kanamycin, and methanol was taken as reference standard and negative control, respectively. The crude methanol extracts of aerial parts of *Abrus precatorius L*., leaf of *Magnolia pterocarpa Roxb.* and *Dracaena spicata Roxb.* and leaf and bark of *Ravenala madagascariensis Sonn*. as well as their hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for thrombolytic and membrane stabilizing activities. *D. spicata* extractives showed mild thrombolytic activity (Likhitwitayawuid, K., et al, 2002).

2.4 Membrane Stabilizing Activity

The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution and heat induced haemolysis of human erythrocytes following the method developed by Omale et al (2008) The crude methanol extracts of aerial parts of *A. precatorius*, leaf of *M. pterocarpa* and *D. spicata* .Carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for thrombolytic and membrane stabilizing potentials. In order to identify the drugs with the ability to promoteolysis of blood clot from natural resources, the crude methanol extract of *D. spicata* extractives showed mild thrombolytic activity (Sharmin, T., et al, 2004)**.**

2.5 Anti-ulcer activity

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

2.6 Anti-tussive activity

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

2.7 Antioxidant activity

Methanolic extract, aqueous extract and powder of the leaves of *D. spicata* were tested for antioxidant activity. Powder form and methanolic extract showed good antioxidant property whereas aqueous extract did not showed significant activity (Shyam et al., 2010). The methanol extract of *D.spicata* contains glycoside and flavonoid. The antioxidant activity of *D.spicata* is due to the reducing power ability (Moideen et al., 2011). Preliminary chemical group identification revealed the presence of alkaloids, glycosides, steroids, terpenoids, tannins and reducing sugars important secondary metabolites (Sultana et al. 2012).

2.8 Phytochemical evaluation:

The results show varying quantities of the phytochemicals in the leaves, stems and roots of the two Dracaena species (*Dracaena spicata* and *Dracaena mannii*) with some parts lacking some of the phytochemicals. The highest quantity of the phytochemicals was contained in the leaves of both species when compared to other parts respectively. The result also revealed no significant statistical difference in the phytochemistry of the two Dracaena species. The implication is that the two species are closely related and this justified their placement in the same genus Dracaena while the slight differences between them support their separation into different species. The result also indicated that the two species could be used in ethnomedicine for the treatment of diseases. In addition, these parts could be the possible sources of these phytochemicals (McCormick, et al, 2001).

2.9 Antibacterial activity

The crude methanol extracts of aerial parts of *Dracaena spicata* Roxb 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 *D. spicata*, the highest (18.0mm) zone of inhibition was demonstrated by the aqueous soluble fraction against *Pseudomonas aeruginosa.* (Sharmin, T., et al, 2004)

CHAPTER THEE

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Collection of plant materials and extraction:

The aerial parts of *Dracaena spicata* were collected in March 2012 from Dhaka. The collected plant materials were cleaned, sun dried and pulverized. The powdered materials (800g each) of the collected plants were separately soaked in 3.0 liters of methanol at room temperature for 7 days. The extracts were then filtered through fresh cotton bed and finally with Whatman filter paper number 1 and concentrated with a rotary evaporator at reduced temperature and pressure. The residues were then stored in a refrigerator until further use.

3.1.1. Plant Material:

Dracaena spicata is not so available throughout the country. The plant collected from Chittagong Hill tract area. The plant was taxonomically identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka. A voucher specimen of Dracaena spicata: was provided on July, 2016 by the research instructor Ms Nazia Hoque, Asistant professor, Department of Pharmacy, East-West University, Dhaka, Bangladesh

3.2. Preparation of plant extract for experiments:

3.2.1. Process of powdering:

At first the plants were cleaned to remove dust, soil etc within them. The collected plant materials were cleaned, sun dried and pulverized. After this the whole amount of plant was dried. The dried plants were ground to coarse powder with the help of the blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. The amount of powder was 800g. During powdering of sample, the blander was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the blander

3.2.2 Extraction:

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

3.2.3. Filtration:

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

3.2.4. Evaporation and extract preparation:

For evaporating the solvent and collect for reuse I have used rotary evaporator machine with a vacuum pump,in 60 degree Celsius which helped to reduce the pressure of the inside of glass tube coil, as well as the whole system. Reduction of pressure causes quick evaporation. On the other part condenser recommenced the solvent so that I could reused it. For this solvent almost 70% solvent get back into liquid form. The extraction was collected from the evaporating flask and the solvent is collected from the receiving flask. Extract transferred into a 50 ml beaker and covered with aluminum foil.

3.2.5 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).

3.3 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.

3.3.1 Reagents:

- Rectified spirit
- Agar purified powder
- Methanol
- Dichloromethane

3.4 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

3.5 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.

Table – 9: 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*

3.6 Preparation of the Medium

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

3.7 Sterilization Procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in laminar hood and all types of precautions were highly maintained. UV light was switched on one hour before

Figure – 8: 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.

3.8 Preparation of subculture:

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

.

Figure – 20: Incubator

3.9 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.

3.10 Preparation of discs:

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

Sample discs: Sterilized filter paper discs (6 mm in diameter) were taken by the forceps in the plates. Sample solutions of desired concentrations (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.

3.11 Diffusion and incubation:

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

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

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

Cytotoxicity Test

Experimental procedure

3.13 Principle

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, and pharmacological activities of natural products etc. Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus (in-vivo) lethality, a simple zoological organism, (Brine shrimp napulii- *Artemia salina*) can be used as a convenient monitoring for screening and fractionation in the discovery of new bioactive natural products. Natural product extracts, fractions or pure compounds can be tested for their bioactivity by this method. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of natural products. Brine shrimp is the English name of the genus Artemia of aquatic crustaceans. Artemia is the only genus in the family Artemiidae (Olowa, L.F, et al, 2013).

3.13.1 Preparation of seawater

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

3.13.2 Hatching of Brine Shrimps

*Artemiasa lina*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 addedto one side of the tank and then that side was covered. The tank was kept underconstant aeration for 48 hrs to hatch the Shrimp and to be matured as nauplii. Thehatched Shrimps were attracted to the lamp through the perforated dam and with thehelp of a Pasteur pipette 10 living shrimps were added to each of the test tubescontaining 5 ml of Brine solution.

3.13.3 Preparation of test solutions

3.2mg of each sample is measured sample was dissolved in 2ml of DMSO with 2ml sea water. A series of solutions of lower concentrations were prepared by serial dilution with DMSO. From each of these test solutions 3ml were added to pre-marked glassvials/test tubes containing 2ml of seawater and 10 Shrimp nauplii. So, the final concentration of samples in the vials/test tubes were 320µg/ml, 160 µg/ml, 80µg/ml,40 µg/ml, 20 µg/ml, 10 µg/ml, 5 µg/ml, 2.5 µg/ml and 1.25µg/mlfor 9 dilution.

3.13.4 Counting of nauplii and analysis of data

After 8 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.

Percentage of Death (%): (Total naupii - Alive naupli) x 100%/Total naupli

CHATER FOUR

RESULTS AND DISCUSSION

4.1 Results and Discussions of antimicrobial screening:

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. 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).

Table 10: Antimicrobial test (result in mm)

Serial	Microorganism	MeOH (300µg/disc)	Kanamycine (30µg/disc)
1	*Bacillus sereus*	26	35
2	*Bacillus megaterium*	9	37
3	*Bacillus subtilis*	10	35
4	*Salmonella paratyphi*	0	35
5	*Salmonella typhi*	0	34
6	*Vibrio parahemolyticus*	0	40
7	*Vibrio mimicus*	29	36
8	*Staphylococcus aureaus*	0	38
9	*Escherichia coli*	0	35
10	*Sheigella dysenteriae*	0	34
11	*Pseudomonas aureaus*	30	36

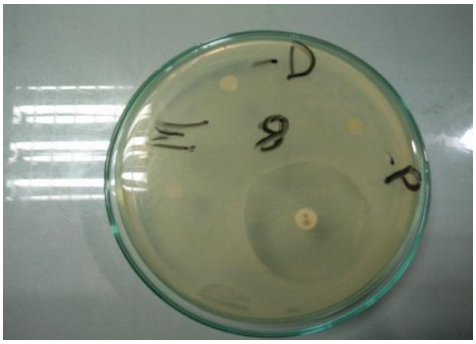

Fig: 9 (*Staphylococcusaureas*)

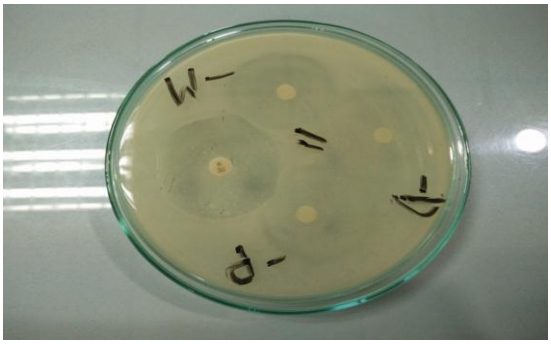

fig10: (*Pseudomonas aureaus*)

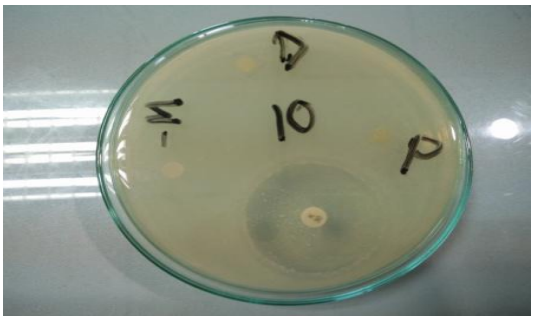

Fig11: (*Sheigella dysenteriae*)

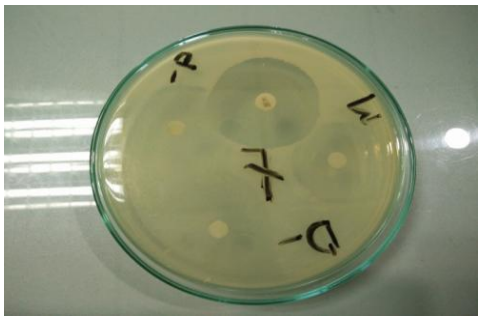

fig12: (*Vibrio mimicus*)

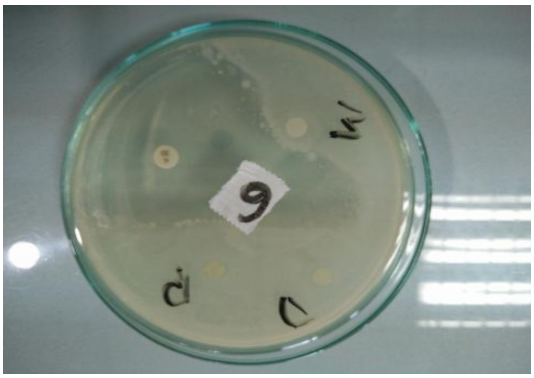

Fig 13: Test plate 6(*Vibrio parahemolyticus*)

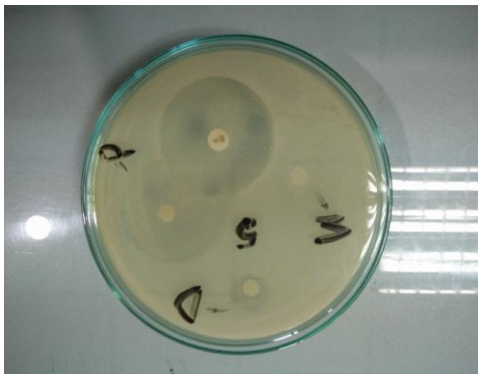

Fig 14: *Salmonella typhi*

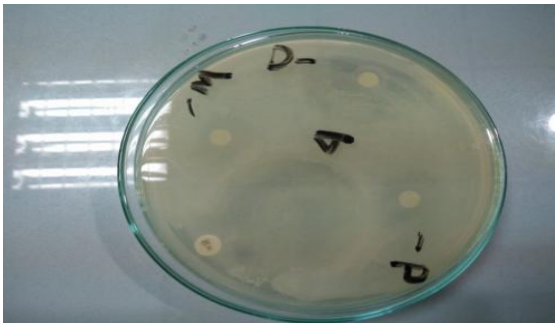

Fig15: test plate 4(*Salmonella paratyphi*)

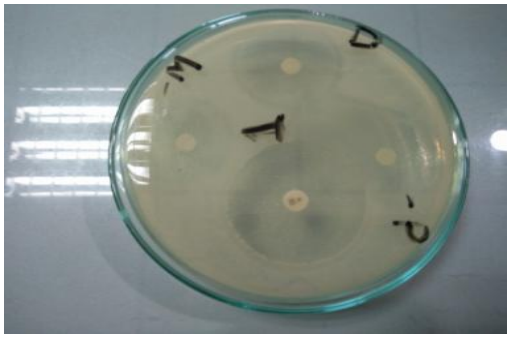

fig16: test plate 1 (*Bacillussereus*)

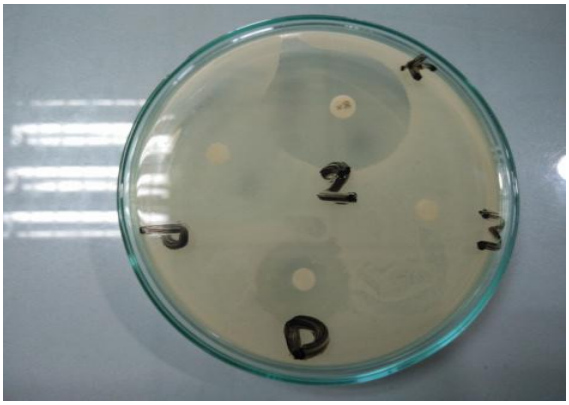

Fig 17: test plate 2 (*Bacillusmegaterium*)

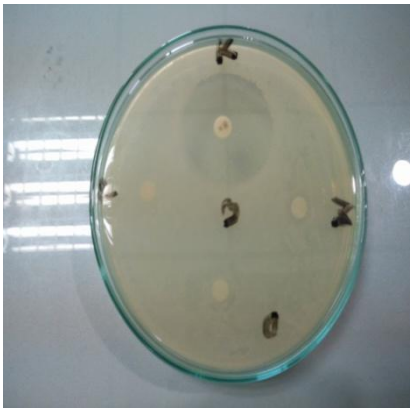

fig18: test plate 3 (*Bacillus subtilis*)

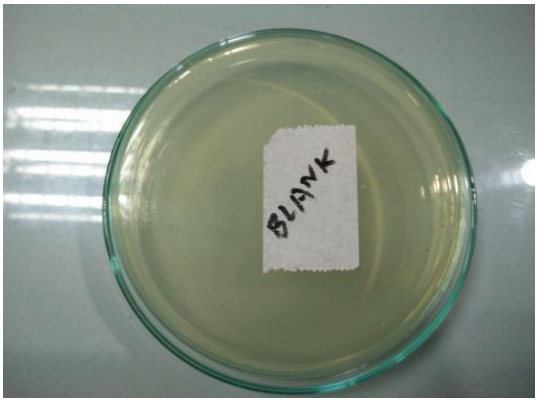

Figure 19: blank

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

4.2 Result and discussion of Cytotoxicity test:

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 methanol extract of *Dracaena spicata* are 46.4729 µg/ml. So, Cytotoxicity of methanolic extract of *Dracaena spicata* was not good, further studies are needed to evaluatethe cytotoxicity of isolated pure compounds.

Percentage of Death (%): (Total naupii - Alive naupii) x 100%/Total naupii

11. Table: Effect of *Dracaena spicata* (methanol extract) on shrimp nauplii

Concentration (µg/ml)	**Log C**	**Number of nauplii taken**	**% motality of methanol extract of *Dracaena spicata***	**Value of x (log LC_{50})**	**LC_{50}**
320	2.50515	10	100	1.6672	46.4729
160	2.20412	10	90		
80	1.90309	10	30		
40	1.60206	10	30		
20	1.30103	10	30		
10	1	10	20		
5	0.69897	10	10		

2.5	0.39794	10	10		
1.25	0.09691	10	10		
0	0	10	0		

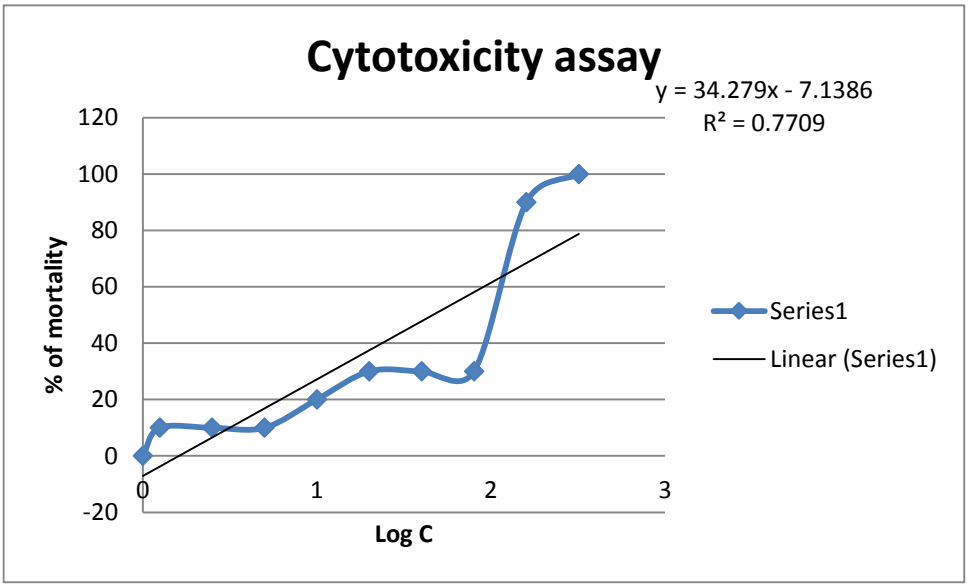

Figure 21: cytotoxicity assay

4.3 Discussion:

Herbal medicines have received high interest as a substitute to clinical treatment, and the demand for herbal remedies has currently increased rapidly. The increase in the number of herbal users as opposed to the insufficiency of scientific evidences on its safety has raised concerns regarding its detrimental effects and related concerns apply to the *Dracaena spicata* in this study.

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 methanol leaf, root/bark extracts of *Dracaena spicata*showed the activity against *Bacillus sereus, Bacillus megaterium, Bacillus subtilis, Vibrio mimicus, Pseudomonas aureaus* and Plant based products have been effectively proven for their utilization as source for antimicrobial compounds. 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). For instance, methanol extracts of *Dracaena spicata* exhibited inhibitory activity against all the strains of *Pseudomonas aureaus.*

Methanol extract was subsequently fractioned and monitored by bioassay leading to the isolation of active fraction by further cytotoxicity analysis. This study presents the toxicity of ethanol extract of *Dracaena spicata,* which should be very useful for any future in vivo or clinical study of this plant extract. Results indicated that bioactive compounds present might account for the plant's pharmacological or toxicological effects. The results somehow support the use of this plant species in traditional medicine. In addition, the present study confirms the utilization of the brine shrimp *(Artemia salina)* bioassay as a reliable, simple, and convenient method in monitoring bioactivity of medicinal plants. From the graph we obtained that, the LC_{50} values of methanol extract of *Dracaena spicata* are 46.4729 μg/ml. So, Cytotoxicity of

methanolic extract of *Dracaena spicata* was not good, further studies are needed to evaluatethe cytotoxicity of isolated pure compounds.

Apart from antimicrobial activities, the plant extracts are also exploited for therapeutic purpose to cure several carcinogenic disorders. 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 methanolic extract of *Dracaena spicata* of the family Asparagaceae tribally used in various disease conditions.

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