Evaluation of Antimicrobial and Cytotoxic Activity of Dichloromethane (DCM) Extract of *Dracaena spicata*

A Dissertation Submitted to the Department of Pharmacy,
East West University, Bangladesh, in Partial Fulfillment of The
Requirements for the Degree of Bachelor of Pharmacy

Submitted By

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Declaration by the Research Candidate

I, **Nushrat Jahan Choity**, ID: 2013-1-70-068, hereby declare that the dissertation entitled **"Evaluation of Antimicrobial and Cytotoxic Activity of Dichloromethane (DCM) Extract of *Dracaena spicata*** " submitted by me to the Department of Pharmacy, East West University in partial fulfillment of the requirement for the award of the degree of Bachelor of Pharmacy is a record of research work under the supervision and guidance of **Ms. Nazia Hoque**, Assistant Professor, Department of Pharmacy, East West University, Dhaka.

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This is to certify that the thesis entitled "**Evaluation of Antimicrobial and Cytotoxic Activity of Dichloromethane (DCM) Extract of *Dracaena spicata***" submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a bonafide record of original and genuine research work carried out by **Nushrat Jahan Choity**, ID: 2013-1-70-068 in 2016 of her research in the Department of Pharmacy, East West University, under the supervision and guidance of me.

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Certificate by the Chairperson

This is to certify that the thesis entitled "**Evaluation of Antimicrobial and Cytotoxic Activity of Dichloromethane (DCM) Extract of *Dracaena spicata*"** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy is a bonafide record of original and genuine research work carried out by **Nushrat Jahan Choity**, ID: 2013-1-70-068 in 2016.

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Dedication

Dedicated to My Parents and My Family Members

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Abstract

Medicinal plants are defined as feral and/or cultivated plants that, based on tradition and literature records, can be directly or indirectly used for medical purposes. The basis for this use is that these plants contain so called active ingredients (active principles or biologically active principles) that affect physiological (metabolic) processes of living organisms, including human beings. 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 is to evaluate the antimicrobial and cytotoxic activity of dichloromethane (DCM) extract of *Dracaena spicata*. The test samples of *D. spicata* exhibited zone of inhibition ranging from 10 to 30.0 mm against the test organisms. The highest (30.0mm) zone of inhibition was demonstrated against *Vibrio mimicus* and *Salmonella paratyphi*. The brine shrimp lethality bioassay was performed to evaluate the cytotoxic activity of DCM extract of the *Dracaena spicata* by their brine shrimp lethality. From this test, the concentration required for killing 50% of the brine shrimp larva or LC_{50} of the DCM extract of the *Dracaena spicata* was calculated approximately as 29.0976 μg /mL with a R^2 value of 0.8699. So, it is evident that the DCM extract of *Dracaena spicata* is mild to moderate cytotoxic as well as biologically active and also has high antimicrobial potentiality. 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 can be significantly beneficial to mankind.

Key Words: *Dracaena spicata*, Antimicrobial, Cytotoxic, Zone of inhibition, DCM Extract

Chapter 1

Introduction

1.1 General Introduction

Plants and man are inseparable. Plants existed on the earth in the geological past form the early history of the earth. The use of plants to alleviate human suffering is as old as the evolution of human civilization itself. From the early stages of human civilization, plants, especially medicinal plants have played a pioneering role for the welfare of human beings. Recently, dramatic changes have taken place in the primary health care system of world population through the development of science, technology and medical science, but till to day 400 cores of people of the world are totally dependent on herbal medicine. It is revealed that even in the developed countries 25%, of the prescribed drugs come from plant sources and herbal medicines are used by about 75-80% of the world's population for primary health care because of their better cultural acceptability, better compatibility with human body and lesser side effects. WHO consultative body of 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 synthesis of useful drugs*" (Sofowora,1982).

Bangladesh is very rich in Bio-diversity. It has more than 500 medicinal plants species. An alarmingly populous, but size-wise a very small country is rather unique in having diversified genetic resources in a wide range of habitats. Increasing population pressure and multifarious anthropogenic activities on the natural ecosystems are posing severe and serious threats to once dense and rich genetically diversified plant communities of this country. Loss of habitats from the wild forests as well as from the village groves, cultivated plains and wild lands are quite common in this country. A broad genetic base has been replaced by a narrow one, and the old genetic diversity is disappearing both inside and outside of the ancient gene centers. This trend is inevitable with the need for highly efficient and uniform cultivars in advanced and sophisticated farming systems. At present, we have no real protected area for natural genetic resources and also have no specific practical policy on conservation of biodiversity. Although there are several gene banks having limited facilities to preserve some economic crops like rice, jute, wheat, pulses etc in Bangladesh, but there is no centralized organization to maintain germplasms of the wild relatives for agriculture, horticulture, medicinal and economically less important forest species. Bangladesh Agricultural Research Council (BARC) is very worried about this.

However, the rich and diverse heritage of traditional medicinal system in the Indian sub-continent including Bangladesh is increasingly threatened by the interplay of a number of factors such as rapid deforestation and habitat destruction, indiscriminate collection and exploitative trade network.

In Bangladesh there are about 297 Unani, 204 Ayurvedic and 77 Homeopathic drug manufacturing industries where the medicinal plants are extensively used in both raw and semi– processed forms of medicine in various pharmaceutical dose formulations. These plants also serve as important raw materials for many modern medicinal preparations (Ullah *et al*., 2014).

1.2 Contribution of Medicinal Plants to New Era

At the beginning of modern medicine the Muslim physicians had done a great job. The Arabian Muslim physicians, like Al-Razi and IbnSina (9^{th} to 12^{th} century AD), brought about a revolution in the history of medicine by bringing new drugs of plant and mineral origin into general use. Al Razi's important books are: Qitab-al-Mansuri, Al-Hawai, Qitab-al-Muluki, Qitab-al-Judari-wal-Hasabah etc.The famous medical book, Al-Kanun, of IbnSina was the prescribed book of medicine in the schools of western medicine for several centuries (National Health Portal, 2016)

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

1.3 Functions that are provided by medicinal plants

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

The high costs of western pharmaceuticals put modern health care services out of reach of most of the world's population, which relies on traditional medicine and medicinal plants to meet their primary health care needs. Even where modern medical care is available and affordable, many people prefer more traditional practices. This is particularly true for first nations and immigrant populations, who have tended to retain ethnic medical practices.

In the last decade, there has been considerable interest in resurrecting medicinal plants in western medicine, and integrating their use into modern medical systems. The reasons for this interest are varied, and include:

- **Low cost:** herbals are relatively inexpensive and the cost of pharmaceuticals to government 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 (Czygan, 1990).

There are three main reasons for which plants have been found useful in medicine.
First, they may be used directly as teas or in other extracted forms for their natural chemical constituents.
Second, they may be used as agents in the synthesis of drugs.

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

Others

- 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 (Botanical-Online, N.D.).

1.4 Medicinal plants of Bangladesh

Medicinal plants have been used by humans for centuries to soothe and improve discomfort and various health problems. Today, they're the best option for those who prefer a natural treatment for their ailments.

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

Table 1: Some Medicinal Plants of Bangladesh (Rahman, 2013).

Serial	**Common Name**	**Botanical Name**	**Parts Used**
1	Malkunki	*Celustrus paniculatus Wild*	Bark, leaves, seed
2	Hajodi	*Cissusqua drangularis L.*	Whole plant
3	Khira	*Cucumis sativus L.*	Fruit, seed
4	Gudmar	*Gymnema sylvestre Retzx*	Whole plant, leaves
5	Satavar	*Asparagus adscendens Roxb*	Tubers
6	Safedmusli	*Chlorophytum borivilianum*	Tubers
7	Puskarmul	*Inular acemosa Hook*	Roots
8	Sakarkhand	*Manihotes culentacrantz*	Tubers
9	Cockscomb	*Celosia cristala L.*	Inflorescence
10	Red poppy	*Papaver rhoeas*	Flowers
11	Caper spurge	*Euphorbia lathyrus*	Seed latex
12	Kalazira	*Nigella sativa L.*	Seed
13	Afim	*Papaver somniferum L.*	Latex, seed
14	Pipli	*Piper Longum L.*	Fruits, roots
15	Babchi	*Psorale acorylifolia*	Seed, Fruit
16	Bael	*Aeglemar melos L. Corr.*	Roots, leaves, fruit
17	Neerh	*Azaflirachta indica*	Bark leaves, flowers, seed, oil
18	Palas	*Buteamonos sperma (Lam.)*	Bark, leaves, flowers, seed, gum
19	Gugul	*Commiphoram ukul Engh J*	Resinous gum
20	Olive	*Olea europeae*	Leaves, oil
21	Arjun	*Terminalia arjuan Roxb.*	Bark
22	Behela	*Terminalia bellirica Gaertu*	Bark, fruit
23	Hirda	*Terminalia bellirica Gaertu*	Fruits
24	Nagakesar	*Mesua ferrea L.*	Blowers, oil .
25	Markingnut	*Semecarpus anacardium L.*	Fruits

1.4.1 Traditional Medicine

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

Because of their potentialities and close association with the culture and tradition of the people, traditional systems of medicine have assumed a unique position in the health care of the people living in even the remotest areas of the country. Although the use of traditional medicine is so deeply rooted in the cultural heritage of Bangladesh the concept, practice, type and method of application of traditional medicine vary widely among the different ethnic groups. Traditional medical practice among the tribal people is guided by their culture and life style and is mainly based on the use of plant and animal parts (Majumdar *et al*., 2006). Among the largest ethnic group, the bangles on the main land, there are two distinct forms of traditional medicine practice:

One is the old and original form based on old knowledge, experience and belief of the older generations. This includes:

- **Folk medicine,** which uses mainly plant and animal parts and their products as medicines for treating different diseases and also includes treatments like blood-letting, bone-setting, , hot and cold baths, therapeutic fasting and cauterization.
- **Religious medicine**, which includes use of verses from religious books written on papers and given as amulets, religious verses recited and blown on the face or on water to drink or on food to eat, sacrifices and offerings in the name of God and gods, etc.
- **Spiritual medicine,** which utilizes methods like communicating with the supernatural beings, spirits or ancestors through human media, torturous treatment of the patient along with incantations to drive away the imaginary evil spirits and other similar methods.

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

- **Unani-Tibb or Graeco-Arab system,** which has been developed by the Arab and Muslim scholars from the ancient Greek system, and

- **Ayurvedic system,** which is the old Indian system, based on the Vedas the, oldest scriptures of the Hindu saints of the Aryan age (World Health Organization, 2000).

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

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

Table - 2: Some crude drugs used as medicine in Bangladesh (Ali *et al*., 1991)

Scientific Name	**Plant Part(s) Used**	**Treatment**
Callicarpa japonica	Leaf	Dyspepsia, heart burn
Callicarpa macrophylla	Whole plant	Tonic, dermatitis, cancer, antidote.
Clerodendrum indicum	Whole plant	Rheumatoid arthritis, jaundice, skin diseases.
Clerodendrum inerme	Leaf, flower	Night blindness, pneumonia, colic, rheumatoid arthritis.
Clerodendrum trichotomum	Leaf, stem, flower	Heart diseases, rheumatoid arthritis, skin diseases.
Clerodendrum viscosum	Whole plant, leaf	Giddiness, typhus, colic in cattle, diabetes, fever, aphrodisiac.
Lantana camara	Root, flower	Cough, mental diseases, fever.
Stachytarpheta indica	Leaf, stem	Leukorrhea.
Premnain tegrifolia	Leaf, bark, root	Fever, energy stimulant
Lippia alba	Leaf	Cuts and wounds.

1.4.2 Tribal medicine

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

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

Scientific Name	**Tribal Name**	**Disease**
Annona mouricata	Marma , Penchi	Pain in head and leg
Kalanchoe pinnata	Tanchongya, Rockkia	Cough and asthma of children
Leea indica	Chakma, Haskura	Sore, leprosy, eczema, itching
Cortin caudatus	Chakma, Sholokjara	Arthritis, paralysis
Eupatorium odoratum	Naramuk, Rajsthali	Bleeding

1.5 Opportunities of drug development from natural products

Medicinal plants are resources of new drugs. It is estimated there are more than 250,000 flower plant species. Nature has been a source of therapeutic agents and a significant number of modern drugs have been developed from natural sources, many based on their use in traditional medicine. Over the last century, a remarkable number of top selling drugs have been derived from natural products (Vincristine from *Catharanthus roseus*, morphine form *Papaver somniferum*, quinine and quinidine from *Cinchona spp).* Nowadays, approximately 40% of the modern drugs have been developed from natural source. More precisely, 39% of the 520 new approved drugs between 1983 and 1994 were natural products or their derivatives, and 60-80% of antibacterial and anti-cancer drugs were from natural origin. In 2000, approximately 60% of all drugs in clinical trials for the multiplicity of cancer had natural origin. In 2001, eight (simvastatin, pravastatin, amoxicillin, clavulanic acid,azithromycin, ceftriaxone, cyclosporine and paclitaxel) Of the 30 top - selling medicines were natural products or their derivatives (Cragg and Newman, 2013).

In light of all these facts, natural product drug discovery process failed to generate little respect. As drug discovery has emerged into a highly competitive era in which the quality of chemical collections and the time taken from assay to drug development are crucial factors in

the success of a company, combinatorial chemistry has become the darling of the pharmaceutical industry, bringing with it the promise of new level of chemical diversity. But this adoption of new strategy by the pharmaceutical companies gained little momentum. Biotechnology companies working in the fields of combinatorial biosynthesis, genetic engineering and met genomic approaches to identify novel natural product lead molecules have met with limited success. These disappointments have led the pharmaceutical industry to consider whether natural product chemical diversity can or will continue to generate valuable templates for drug development (Katiyar *et al.*, 2012).

Natural products offer a potentially infinite source of chemical diversity unparalleled to any synthetic chemical collection or combinatorial chemistry approach. In addition to that, these potent natural product compounds can have astounding chemical structures that can lead to unexpected, alternative medicinal chemistry programs based on important biological targets. In the past few years, new natural products with a wide variety of chemical classes have been reported in the scientific literatures. Moreover, a total of 19 natural product based drugs were approved for marketing worldwide in between the year 2005 to April 2010, among which 7 being classified as natural products, 10 semi-synthetic natural products and 2 natural product derived drugs (Decorte, 2016).

1.5.1 Approaches to 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.

1.5.2 Steps of drug development from plant sources

Selection of plant species:

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

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

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

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
- 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 (Katiyar *et al.*, 2012).

1.6 Extraction of crude from medicinal plants

1.6.1 Extraction procedures

There are several extraction procedures for obtaining active component from medicinal plants.

Maceration

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.

Infusion

Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs.

Digestion

This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstruum is thereby increased.

Decoction

In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat-stable constituents. This process is typically used in preparation of Ayurvedic extracts called “quath” or “kawath”. The starting ratio of crude drug to water is fixed, e.g. 1:4 or 1:16; the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used as such or processed further

Ultrasound extraction (Sonication)

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of rauwolfi, a root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules (Sasidharan, 2010).

1.6.2 Parameters for selecting an appropriate extraction method

Nature of constituents

- If the therapeutic value lies in non-polar constituents, a non-polar solvent may be used. For example, lupeol is the active constituent of *Crataeva nurvala* and, for its extraction, hexane is generally used. Likewise, for plants like *Bacopam onnieri* and *Centella asiatica*, the active constituents are glycosides and hence a polar solvent like aqueous methanol may be used.
- If the constituents are thermolabile, extraction methods like cold maceration, percolation and CCE are preferred. For thermostable constituents, Soxhlet extraction (if non-aqueous solvents are used) and decoction (if water is the menstruum) are useful.
- Suitable precautions should be taken when dealing with constituents that degrade while being kept in organic solvents, e.g. flavonoids and phenyl propanoids.
- In case of hot extraction, higher than required temperature should be avoided. Some glycosides are likely to break upon continuous exposure to higher temperature.
- Standardization of time of extraction is important, as: Insufficient time means incomplete extraction. If the extraction time is longer, unwanted constituents may also be extracted. For example, if tea is boiled for too long, tannins are extracted which impart astringency to the final preparation.
- The number of extractions required for complete extraction is as important as the duration of each extraction.
- The quality of water or menstrum used should be specified and controlled.
- Concentration and drying procedures should ensure the safety and stability of the active constituents. Drying under reduced pressure is widely used. Lyophilization, although expensive, is increasingly employed.
- The design and material of fabrication of the extractor are also to be taken into consideration.
- Analytical parameters of the final extract, such as TLC and HPLC fingerprints, should be documented to monitor the quality of different batches of the extracts (Sasidharan, 2010).

1.6.3 Solvents used in extraction process

Solvent is very essential for crude extraction. Extraction largely depends on choice of solvent. Solvents are chosen depends on various parameters:

- polarity
- solubility
- density
- nature of plant
- chemical constituent
- miscibility
- dispersion coefficient

Table 5: Common solvents for crude extraction **(**Richter *et al*., 1996)

Water	**Ethanol**	**Metanol**	**Cloroform**	**Ether**	**Acetone**
Anthocyanis	Tannins	Anthocyanins	Terpenoids	Alkaloids	Phenol
Starches	Polyphenols	Terpenoids	Flavonoids	Terpenoids	Flavonols
Tannins	Polyacetylenes	Saponins		Coumarins	
Saponins	Flavonols	Tannins		Fatty acids	
Terpenoids	Terpenoids	Xanthoxyllines			
Polypeptides	Sterols	Totarol			
Lectins	Alkaloids	Quassinoids			

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**:**

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 (Balouiri *et al*., 2016). The primary assay can be performed in vitro by disk diffusion assay method**,** which includes

- Disc/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 (Balouiri *et al*., 2016).

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 (Balouiri *et al*., 2016).

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 (Spellberg *et al*., 2004).

1.8 Cytotoxic assay using brine shrimp

In Vitro cytotoxicity can be evaluated using brine shrimp model. The brine shrimp lethality assay (BSLA) has been routinely used in the primary screening of the crude extracts as well as isolated compounds to assess the toxicity towards brine shrimp (Mentor *et al*., 2014)

1.9 Plant Review

Figure - 1: *Dracaena spicata*

Figure-2: Flower of *Dracaena spicata*

Table 6: Plant review of *Dracaena Spicata* (eMonocot, N.D.)

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

1.9.1 Taxonomy

Kingdom: Plantae

Clade: Angiosperms

Clade: Monocots

Order: Asparagales

Family: Asparagaceae

Subfamily: Nolinoideae

Genus: *Dracaena*

Species: *D. spicata*

1.9.2 Other species

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

- *Dracaena afromontana*
- *Dracaena americana*– Central America dragon tree
- *Dracaena aletriformis* (Haw.) Bos)
- *Dracaena arborea*– tree dracaena
- *Dracaena aubryana* Brongn
- *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
- *Dracaena marmorata*
- *Dracaena ombet*– Gabal Elba dragon tree

- *Dracaena phrynioides*
- *Dracaena reflexa* Lam. – Pleomele dracaena or "Song of India"
- *Dracaena serrulata* Baker – Yemen dragon tree

  Dracaena surculosa Lindl. – Spotted or gold dust dracaena. Formerly *D. godseffiana* (Catalogue of Life, 2016)

Among them, some common species are:

Species Name	**Description**
Dracaena cinnabari	1) Antimicrobial activity of chloroform and methanol extract of *Dracaena cinnabari* resin 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
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 anti-carcinogen. 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 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
Dracaena cochinchinensis	Resin from *Dracaena cochinchinensis* has been produced by infection with Fusarium and Cladosporiumspp. (Wang *et al.,* 1999)

Dracaena fragrans	It 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.
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.
Dracaena marginata (Dragon Tree)	Thin stems are topped by clusters of slender arching leaves with narrow purple margins. The stems often have interesting natural bends. Some people also train bends by setting the plant on its side for some time. Dragon trees can grow up to 10 feet tall. Cut back the stems to force the plant to branch. The dragon tree is widely used in home, office and commercial decor because it tolerates low light.
Dragon Blood	Both Dragon's blood and loureirin B suppressed two types of peak sodium currents depending upon dose. Further, Liu *et al.,* (2006) explored the material basis for efficacy of modulation of Dragon's blood on the tetrodotoxin-resistant (TTX-R) sodium currents in dorsal root ganglion (DRG) neurons. They suggested that analgesic effect of Dragon's blood may be explained on the basis of interference with pain messages caused by the modulation of Dragon's blood on TTX-R sodium currents in DRG neurons and could be due to the synergistic effect of three components cochinchinenin A, cochinchinenin B, and loureirin B. Recently, Chen and Liu (2006) carried out a computer simulation research for the effects of Dragon's blood and its component loureirin B on sodium channel in dorsal root ganglion cells.

1.9.3 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. Calyxnone. corolonepetalled, cylindric divided half way down into three exterior, and three interior slender, linear, equal, straight segments; color pale greenish yellow, as they advance in age the tube becomes twisted. 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 (United States Department of Agriculture, N.D.).

1.9.4 Use

- 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 together and administered to healthy children during outbreaks of meals by Tanchangya
- Antimicrobial activity
- Antiulcerant activity
- Antithrombolytic
- Antipyretic activity

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. *D. spicata* extractives showed mild thrombolytic activity. 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. (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).

Significance of this study

For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities, and plant physiological studies would indeed have a major role to play in this burgeoning field. With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. Although active phytochemicals may have been identified, in general, many pathways for the biosynthesis of specific medicinal compounds and the factors (biotic and abiotic) regulating their production remain unclear. At present, a major concern with the use of phytomedicines regards the maintenance of consistent medicinal quality in botanical medicines.

Therefore, plant materials can be potential sources of chemically interesting and biologically important drug entrant. And for this purpose the plant can be further screened against various diseases in order to find out its unexplored efficacy with a gaze to the future with a great deal of expectation DCM extract of *Dracaena spicata* of the family Asparagaceae tribally used in various disease conditions.

In my experiment it shows very strong antimicrobial activity. The plant also shows moderate antimicrobial activity. So in case of anticancer drug preparation this plant extracts may treated as a good candidate as it has notable cytotoxic effect also. 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.

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 is to evaluate the in- vitro pharmacological activities (like antioxidant, antimicrobial) of DCM extract of *Dracaena spicata.* It 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.

Chapter 3

Methodology

3.1 Preparation of plant extract for experiments

3.1.1 Collection

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

3.1.2 Process of powdering

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

3.1.3 Extraction

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

3.1.4 Filtration

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

3.1.5 Evaporation and extract preparation

For evaporating the solvent and collect for reuse I have used rotary evaporator machine with a vacuum pump which helped to reduce the pressure of the inside of glass tube coil, as well

as the whole system. Reduction of pressure causes quick evaporation. On the other part condenser recommenced the solvent so that I could reused it. For this solvent almost 70% solvent get back into liquid form. The extraction was collected from the evaporating flask and the solvent is collected from the receiving flask. Extract transferred into a 50 ml beaker and covered with aluminum foil.

Figure 3: Rotary evaporator

3.2 Antimicrobial Screening

The antimicrobial assay was performed by disc diffusion technique. Disc diffusion technique is highly effective for rapidly growing microorganisms. In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. 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. 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**.** In the present study the crude extract was tested for antimicrobial

activity by disc diffusion method. The experiment is carried out more than once and the mean of the readings is required.

3.3 Test materials used for the study

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

3.3.1 Reagents

- Rectified spirit
- Agar purified powder
- DCM extract

3.3.2 Materials

- Micropipette
- Electric balance(4 digits)
- Nose mask and hand gloves
- Spirit burner and match box
- Laminar air flow unit
- Incubator
- Refrigerator
- Autoclave

3.3.3 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 7: Name of the test organisms

Number	Name of Microorganism	Type of the Microorganism
1	*Bacillus sereus*	Gram Positive
2	*Bacillus megaterium*	Gram Positive
3	*Pseudomonas aureus*	Gram Negative
4	*Salmonella paratyphi*	Gram Negative
5	*Salmonella typhi*	Gram Negative
6	*Vibrio parahemolyticus*	Gram Negative
7	*Vibrio mimicus*	Gram Negative
8	*Bacillus subtilis*	Gram Negative
9	*Escherichia coli*	Gram Negative
10	*Sheigella dysenteriae*	Gram Negative
11	*Staphylococcus aureus*	Gram Positive

3.3.4 Culture Medium and their composition

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

Table 8: Composition of nutrient agar medium

Ingredients	Amount
Bacto peptone	0.5 gm
Sodium chloride	0.5 gm
Bacto yeast extract	1 gm
Bacto agar	2 gm
Distilled water	100 ml

3.3.5 Preparation of the Medium

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

3.3.6 Sterilization Procedure

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

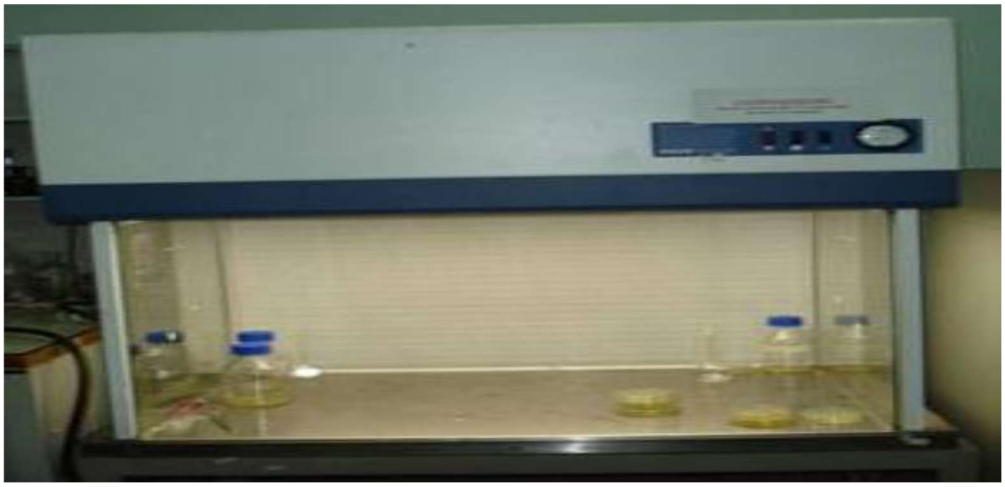


Figure 4: Laminar hood

3.3.7 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 5: Incubator

3.3.8 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.

3.3.9 Preparation of discs

- **Standard discs**

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

- **Sample discs**

Sterilized filter paper discs (6 mm in diameter) were taken by the forceps in the plates. Sample solutions of desired concentrations (300 μg/disc) were applied in the disc with the help of the micropipette in an aseptic condition. These discs were left for a few minutes in aseptic condition for complete removal of the solvent.

3.3.9.1 Diffusion and incubation

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

3.4 Determination of antimicrobial activity by measuring the zone of inhibition

The antimicrobial potency of the test agents were 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.

Precaution

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.

3.5 Cytotoxicity Test: Experimental procedure

3.5.1 Preparation of seawater

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

3.5.2 Hatching of Brine Shrimps

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

3.5.3 Preparation of test solutions

2mg of each measured sample was dissolved in 60µl of DMSO. A series of solutions of lower concentrations were prepared by serial dilution with DMSO. From each of these test solutions 30µg were added to pre-marked glass vials/test tubes containing 5 ml 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 1.25 µg/ml for 9 dilution.

3.5.4 Preparation of Controls

Vincristine sulphate served as the positive control. 0.2 mg of vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 µg/ml from which serial dilutions were made using DMSO to get 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.078125 µg/ml, 0.0390 µg/ml. The control groups containing 10 living Brine Shrimp nauplii in 5 ml simulated seawater received the positive control solutions. As for negative control, 30 µg of DMSO was added to each of the pre-marked test

tubes containing 5 ml of simulated seawater and 10 Shrimp nauplii. The test was considered invalid if the negative control showed a rapid mortality rate and therefore has to conduct again. The test tubes (containing nauplii) were then maintained at room temperature for 24 hrs. under the light for observing the survival rate.

3.6 Counting of nauplii and analysis of data

After 24 hours, the test tubes were inspected using a magnifying glass and the number of survivors was counted. The percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed by using Microsoft Excel. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC50) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

Chapter 4

Result and Discussion

4.1 Results of Antimicrobial Screening

Table 9: Result of antimicrobial activity (*in vitro*) of DCM extract of *Dracaena spicata*

Name of Microorganisms	DCM extract of *Dracaena spicata* (300 µg/disc)	Kanamycine (30 µg/disc)
	Zone of Inhibition in mm	
Bacillus sereus	16	35
Bacillus megaterium	26	37
Bacillus subtilis	20	35
Salmonella paratyphi	30	35
Salmonella typhi	20	34
Vibrio parahemolyticus	16	40
Vibrio mimicus	30	36
Staphylococcus aureaus	10	38
Escherichia coli	0	35
Sheigella dysenteriae	16	34
Pseudomonas aureaus	28	36

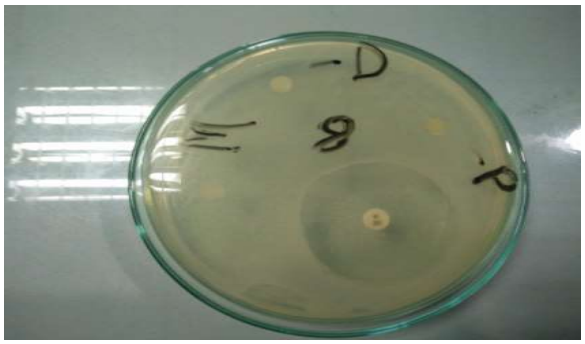


Figure6:Test plate 8(*Staphylococcus aureaus*)

Figure7:Test plate 11(*Pseudumonas aureaus*)

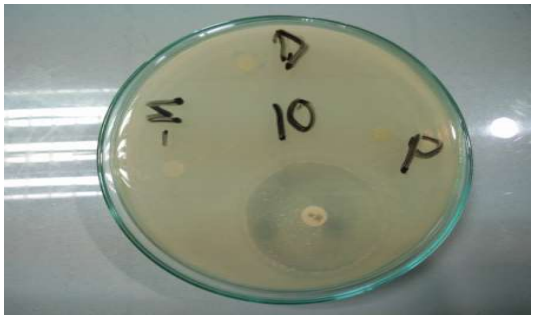


Figure 8: Test plate 10 (*Sheigella dysenteriae*)

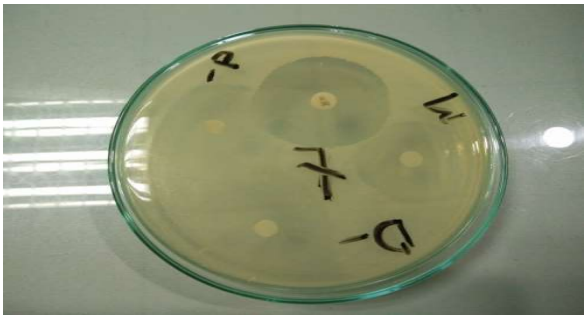


Figure 9: Test plate 7 (*Vibrio mimicus*)

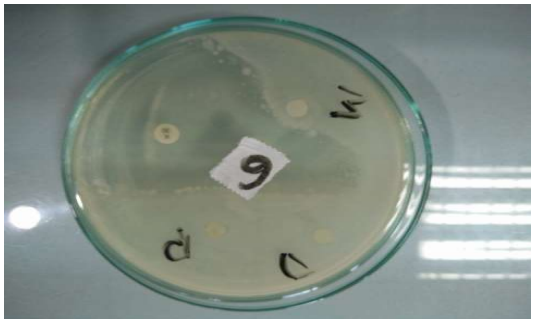


Figure 10:Test plate 6(*Vibrio parahemolyticus*)

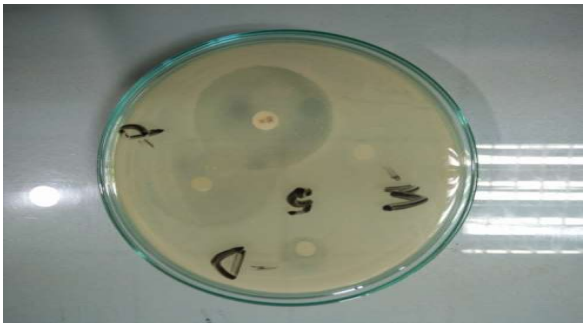


Figure 11: Test plate 5 (*Salmonella typhi*)

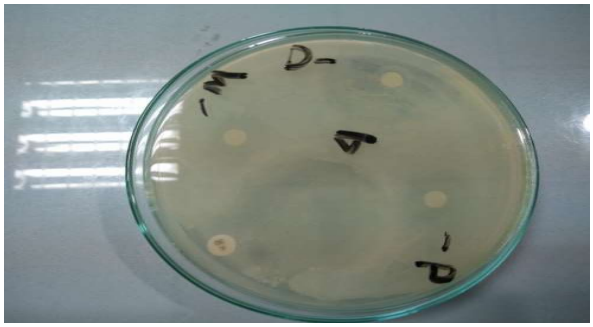


Figure12: Test plate 4(*Salmonella paratyphi*)

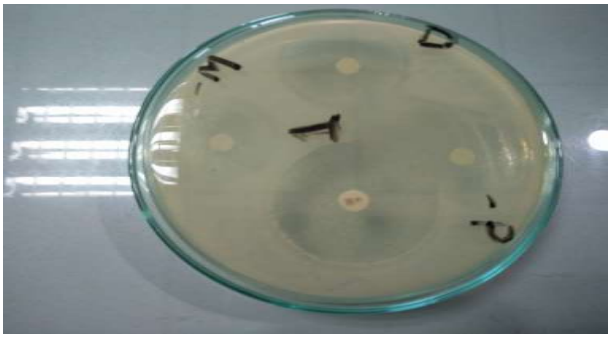


Figure13:Test plate 1 (*Bacillus sereus*)

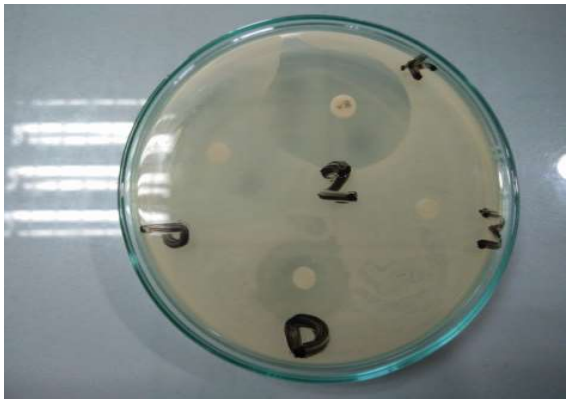


Figure 14: Test plate 2 (*Bacillus megaterium*)

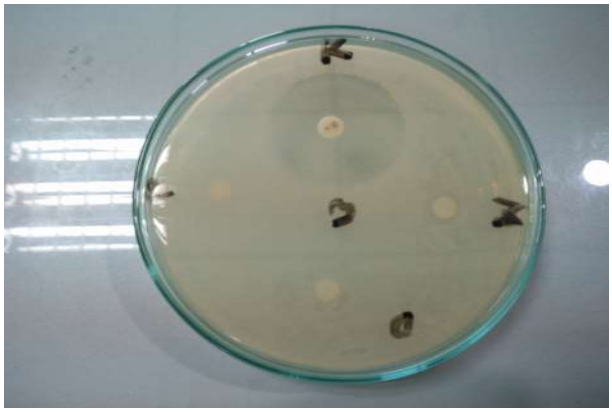


Figure 15: Test plate 3 (*Bacillus subtilis*)

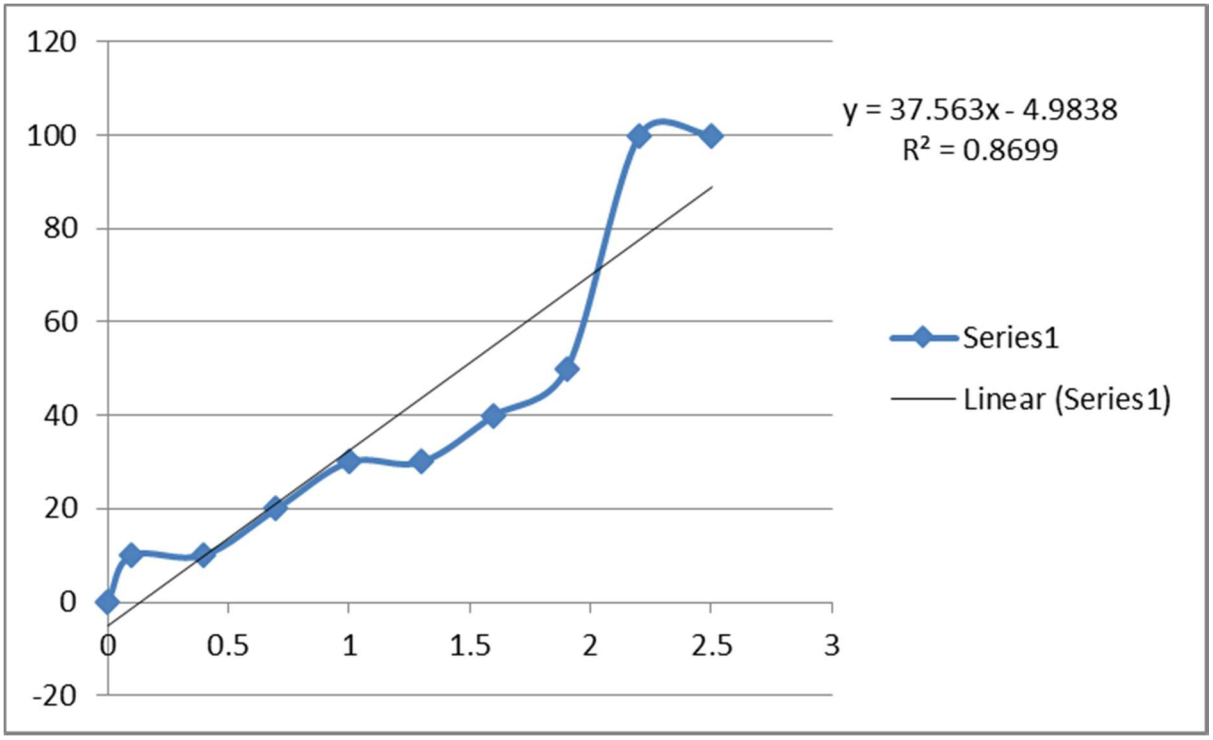
Figure 16: Blank

The DCM extract of *D. spicata* exhibited moderate to strong antimicrobial activity. The test samples of *D. spicata* exhibited zone of inhibition ranging from 10 to 30.0 mm against the test organisms. The highest (30.0mm) zone of inhibition was demonstrated against *Vibrio mimicus* and *Salmonella paratyphi.*

4.2 Result of Cytotoxicity Assay of *Dracaena spicata*

Table 10: Effect of *Dracaena spicata* (DCM extract) on shrimp nauplii

Concentration (µg/ml)	Log C	No of nauplii taken	% mortality of the test sample	Value of x (log LC_{50})	LC_{50}
320	2.50515	10	100	1.46377	29.0976
160	2.20412	10	100		
80	1.90309	10	50		
40	1.60206	10	40		
20	1.30103	10	30		
10	1	10	30		
5	0.69897	10	20		
2.5	0.39794	10	10		
1.25	0.09691	10	10		
0	0	10	0		



4.3 Discussion

For the plant physiologist, work on medicinal plants opens up a wide range of research possibilities, and plant physiological studies would indeed have a major role to play in this burgeoning field. With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. Although active phytochemicals may have been identified, in general, many pathways for the biosynthesis of specific medicinal compounds and the factors (biotic and abiotic) regulating their production remain unclear. At present, a major concern with the use of phytomedicines regards the maintenance of consistent medicinal quality in botanical medicines.

Therefore, plant materials can be potential sources of chemically interesting and biologically important drug entrant. And for this purpose the plant can be further screened against various diseases in order to find out its unexplored efficacy with a gaze to the future with a great deal of expectation methanolic extract of *Dracaena spicata* of the family Asparagaceae tribally used in various disease conditions. In my experiment it shows very positive result for antimicrobial activity and also cytotoxic activity.

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 Chakmaetc.The aim of the present study was to evaluate the antimicrobial activity and cytotoxic activity of dichloromethane extract of *Dracaena spicata.*

Due to its huge therapeutic use by the tribal I get interested to do experiment on this plant. The therapeutic value of medicinal plants lies in the various chemical constituents in it.The test was run using 11 different microorganisms. They are: *Bacillus sereus,Bacillus megaterium, Bacillus subtilis, Salmonella paratyphi, Salmonella typhi, Vibrio parahemolyticus, Vibrio mimicus, Staphylococcus aureaus, E. coli, Sheigell adysenteriae* and *Pseudomonas aureaus*.

The test samples of *D. spicata* exhibited zone of *inhibition* ranging from 10 to 30.0 mm 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 brine shrimp lethality bioassay was performed to evaluate the cytotoxicity activity of the DCM extract of the *Dracaena spicata* by their brine shrimp lethality. From this test, the concentration required for killing 50% percent of the brine shrimp test larva or LC_{50} of the DCM extract of the *Dracaena spicata* was calculated approximately as 29.09 µg /mL with a R^2 value of 0.8699. So, it is evident that the DCM extract of *Dracaena spicata* was cytotoxic as well as biologically active.

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 5

Conclusion

Conclusion

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

With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems. In my experiment it shows very positive result for cytotoxic and antimicrobial activity. The antimicrobial activity of the plant extracts were tested against some potentially bacterial pathogenic by using disc diffusion method at different concentrations of the extracts of *Dracaena spicata* to understand the most effective activity. There are some established research reports regarding the phytochemical and pharmacological properties of this plant. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.

Chapter 6

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