Antimicrobial and Cytotoxic Investigations Of Methanolic Extract Of *Dracaena spicata*

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

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Declaration By The Research Candidate

I, Sadiya Afrin, hereby declare that the dissertation entitled"**Antimicrobial and Cytotoxic Investigations of Methanolic extract of *Dracaena spicata***" submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of therequirement for the award of the degree Bachelor of Pharmacy is a completerecord of original research work carried out by me during 2014, under the supervision and guidance of Ms Nazia Hoque, Senior Lecturer, Department ofPharmacy, East West University and the thesis has not formed the basis for theaward of any other degree/diploma/fellowship or other similar title to anycandidate of any university.

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Abstract

The plant *Dracaena spicata* has been used for the general promotion of health and longevity by Asian tribal. It is used as a traditional medicine for the treatment of various diseases like meals, fever, ulcer, stomachaches etc.

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

The antimicrobial activities of methanolic solvent extract of plant weretested against the Gram-positive and Gram-negative bacterial strains and fungi by observing the zone of inhibition. The antimicrobial test was performed by Disc diffusion method. The crude methanolic extract of *Dracaena spicata*plant showed poor antimicrobial activities against Gram-negative bacteria and fungi at concentrations of 400 µg/disc. However, no activity was found against *Salmonela paratyphy* and *Bacillus subtilis.*

The cytotoxic activity of the plant was done by using *Artemia saline* Leach. The LC_{50} was observed approximately as 36.829µg / mL with a R^2 value of 0.9735. Cytotoxicity of methanolic extract of *Dracaena spicata* was not good, LC_{50} value less than 5 µg / mL indicates excellent cytotoxic effect. So, further studies are needed to evaluatethe cytotoxicity of isolated pure compounds.

In conclusion, furtherinvestigations are needed to identify the active constituents and the exact mechanism(s) of action responsible for the reported antimicrobial and cytotoxic properties of *Dracaena spicata*

Key Words:
Dracaena spicata, Antimicrobial, Cytotoxicity.

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1.General Introduction:

Natural environment has been a source of medicinal agents for thousands of years, since healing with plants dates backprobably to the evolution of *Homo sapiens.* Even to date, about 80% of the world's inhabitants relymainly on traditional medicines for their primary health care, while medicinal plants continue to play an important role in the health care systems of the remaining 20%. Partly based on their use in traditional medicine, an impressive number of modern drugs have also been isolated from natural plant species. Remarkably, even today there real definition for this special group of plants that has been accompanying mankind throughout history. Most frequently, 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 notion of aromatic plants is even less definite. The attribute aromatic indicates plants having an aroma; being fragrant or sweet-smelling, while the word aroma is supposed to imply also the taste of the material (aromatic herbs).Spice plants are plants used for seasoning, spicing, flavoring and coloring foods, drinks and different products of the food processing industry, i.e. making a product more enjoyable.(Solecki and Shanidar 1975)

1.1.Medicinal plant:

1.1.1. Background:

Plants have been used in treating human diseases for thousands of years. Some 60,000 years ago, it appears that Neanderthal man valued herbs as medicinal agents; this conclusion is based on a grave in Iran in which pollen grains of eight medicinal plants were found. One of these allegedly ancient medicinal herbs, yarrow, is discussed in this work as a modern medicinal plant.Since prehistoric times, shamans or medicine men and women of Eurasia and the Americas acquired a tremendous knowledge of medicinal plants. All of the native plant species discussed in detail in this work was used by native people in traditional medicine. The fact that hundreds of additional species were also used by First Nations Canadians (Arnason et al. 1981) suggests that many of these also have important pharmacological constituents that could be valuable in modern medicine.

Up until the 18^{th} century, the professions of doctor and botanist were closely linked. Indeed, the first modern botanic gardens, which were founded in 16^{th} century Italy, in Pisa, Padova and Florence, were medicinal plant gardens attached to medical faculties or schools.

The use of medicinal plants is not just a custom of the distant past. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines (Duke 1985). A 1997 survey showed that 23% of Canadians have used herbal medicines. In addition, as much as 25% of modern pharmaceutical drugs contain plant ingredients (Duke 1993)

1.1.2.Importance of medicinal plant:

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 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

However, the pace of re-adopting the use of traditional medicinal plants is by no means uniform in western medicine (Duke 1993, Cox and Balick 1994).

In parts of Europe, especially in Germany, herbal medicine (or phytomedicine) is much more popular than is the case in North America. Some 67,000 different herbal products are available in Germany (Foster 1995). The already well-established medicinal plant trade of Europe is increasing at an annual rate of about 10%.

In Canada, and the US, the regulatory climate has been much less receptive to herbal medicines (Tyler 1993b). This is because lack of proper scientific evaluation, limited regulation, absence of quality control, limited education of many herbal practitioners, and the presence of "snake-oil salesmen" have all combined to give herbal medicine a bad reputation. However, in response to public demand for "alternative" or "complementary" medicine, this situation is changing. At least 20% of Canadians have used some form of alternative therapy, such as herbalism, naturopathy, acupuncture, and homeopathy (Kozyrskyj 1997).

Herbs are the fastest-growing part of the pharmacy industry of North America, with an annual growth variously estimated as 15 to 20%, and thousands of herbal products are now available to Canadians (Carmen-Kasparek 1993). Herbal remedies have been estimated to have a current value of between two and ten billion dollars in North America, depending on how comprehensively the category of medicinal herbs is interpreted (Marles 1997). Foster (1995) predicted that with

appropriate research and regulation, "herbal medicine will regain its rightful status as an important and integral aspect of classical medicine."

1.1.2.1.Nutraceuticalsand functional foods:

Medicinal plants are finding a new, expanding market as herbal components of health foods and preventative medicines, especially under the marketing term "nutraceuticals" (about as frequently spelled nutriceuticals) (Insight Press, 1996a, 1996b; also see Childs 1997). An economic analysis of nutraceuticals in Canada is found in Culhane (1995) and a good general discussion is in Spak (1998). Essentially synonymous phrases include "medical foods," "pharma foods," "phytofoods" and "functional food. All of these terms are applied to substances that may or may not be considered foods or parts of food, but provide health benefits when eaten.

The most widely used of these terms, nutraceutical, was coined by Dr. Stephen DeFelice of the Foundation for Innovative Medicine, a New Jersey based industry group. His definition was "a food derived from naturally occurring substances which can and should be consumed as part of the daily diet, and which serves to regulate or otherwise affect a particular body process when ingested."

The term is now commonly applied to an extremely wide variety of preparations with perceived medicinal value but not necessarily with apparent food value (such as amino acids, essential fats, dietary fibres and fibreenriched foods, plant and animal pigments, antioxidants, vitamins, minerals, sugar and fat substitutes, fatless meat, skim milk, genetically engineered designer foods, herbal products and processed foods such as cereals, soups and beverages). Some have contended that fruits and vegetables should be included in "functional foods" because they are so nutrient-packed, while others would reserve the term for foods fortified in some fashion for health (in this sense, the first functional food seems to have been calcium-fortified orange juice).

The term "phytonutrient," which should be used for plant materials that by definition have nutritional value, has been applied to medicinal plant preparations without apparent food value.

"Phytomedicines" have been defined as therapeutic agents derived from plants or parts of plants, or preparations made from them, but not isolated chemically pure substances, such as menthol from peppermint (Foster 1995). Unlike pharmaceuticals, which are usually potentially toxic medications that can only be prescribed by a medical doctor, nutritional supplements for the most part can be purchased from a health food store, herbal practitioner or independent distributor. Because they are much less expensive than drugs, herbal preparations or extracts, as additions to diet, have been advanced as a new, cost-effective health care system.

Plant-based vitamins and a wide variety of chemical constituents in fruits and vegetables provide many of the benefits of medicinal plants (fruits and vegetables *are* medicinal plants, although rarely thought of as such), and concentrated extracts from them are commonly marketed today as nutriceuticals.

1.1.2.2.Pharmaceutical compounds from plants:

Above, we have discussed the use of medicinal plants in the form of raw herbs and crude extracts. Modern pharmacology, however, relies on refined chemicals - either obtained from plants, or synthesized. The first pure medicinal substance derived from plants was morphine, extracted from the opium poppy at the turn of the 19th century.

Often, chemicals extracted from plants are altered to produce drugs. For example, diosgenin is obtained from various yam (*Dioscorea*) species of South America, and is converted to progesterone, the basis of the oral contraceptive pill. Aspirin-like chemicals were once obtained from willows (*Salix* species) and European meadowsweet (*Filipendulaul maria*), but aspirin is now synthesized in the laboratory.

Numerous medicines in use today are extracted from plants. About 50 to 60% of pharmaceutical drugs are either of natural origin or obtained through use of natural products as starting points in their synthesis (Verlet 1990, Balandrin et al. 1993).

The commercial value of biologically active compounds from plant sources has been estimated to approach $30 billion annually worldwide (Deans and Svoboda 1990). Higher plants have given rise to about 120 commercial drugs and 10-25% of all prescription drugs contain at least one active compound from a higher plant (Duke 1993, Cox and Balick 1994).

The tradition for developing plant-based drugs in modern Western medicine is largely based on a paradigm (model) that there is a single active ingredient in medicinal plants, or at least a primary chemical, that is responsible for the medical effectiveness. However, it may be that many preparations used in traditional herbal medicine are effective because of synergistic (interactive) therapeutic effects of several ingredients.

Certainly many traditional herbal drug preparations are compounded from several plants. Such drug mixtures are not of interest to pharmaceutical firms, because they generally cannot be patented (although under some conditions natural products can secure patent protection).

On the other hand, as a visit to a pharmacy or "health-food" store quickly reveals, numerous companies are marketing plant mixtures as "dietary supplements," which are in fact being utilized as non-prescription drugs, although there is generally limited or no modern research proof of effectiveness. Since the private sector has limited interest in this issue, there is a clear need for public supported (government) research.

1.1.2.3.Seeking new drugs from plants:

For several decades, the pharmaceutical industry has debated the relative merits of seeking new drugs by synthesis in the laboratory or by screening and testing chemical constituents of plants. The majority of commercial refined plant-based drugs come from only about a hundred plant species.

On the whole, laboratory-based chemistry has been supplanting the search for natural drugs because testing plants is comparatively labour intensive and random plant testing has been shown to have a

relatively low rate of return. For example, many thousands of plants have been tested for drugs effective for treating cancer, but the success rate of finding an effective chemical or chemical derivative, like taxol from the Pacific yew (discussed in this work), was found to be only one in several hundred.

Worse, the chance that a pharmaceutical company's investment in plant-based drugs will produce a profitable drug has been estimated as perhaps no better than one in several thousand.

In the United States, bringing a new drug to market costs $125 million (Mendelsohn and Balick 1995), so it is no wonder that drug research is undertaken with great caution. Nevertheless, a number of companies have invested in the last several years in the search for plant-based drugs. There are several reasons for this.

First, there is a need to study biodiversity, especially in third world countries, and the traditional (folklore) medicinal knowledge that native peoples have, before advancing civilization destroys both the plant species and knowledge of their use. Ethnobotany is the branch of biology specifically dedicated to researching the economic relationships between plants and so-called "primitive" human societies.

Second, improvements in automation and robotics have facilitated laboratory evaluation of large samples in a short time.

Third, synthetic chemists have proven to need examples of effective natural drugs from plants as structure-function models in order to rationally design analogous drugs on the basis of molecular structure; having a natural example of how a novel plant-derived enzyme functions on human receptors may enable the engineering of analogous synthesized molecules with predicted biological activity.

1.1.2.4. Economic opportunities:

Most of the world's supply of medicinal herbs is obtained by wild collection (often called "wildcrafting"), not by cultivation. Harvesting renewable wild resources is perfectly legitimate so long as this is conducted in a sustainable fashion that does not eliminate populations or degrade the habitat where the plants grow.

There are still many minor medicinal plant species in Canada that are abundant in nature and can be collected in a sustainable fashion.However, because of shrinking wild resources and a strengthening sentiment that biodiversity should be preserved, cultivation is becoming increasingly important.

When a plant is (or becomes) popular medicinally, its commercial value is likely to lead to over collection. Many very important Canadian drug plants grow in the shade of trees (for examples, ginseng, goldenseal, Mayapple, and Pacific yew) and, because they grow very slowly, are especially susceptible to over collecting. Such non-timber forest resources are of importance to the forest industry, which is looking for alternative crops.

Ginseng has been over collected to the point that the wild Canadian reserve has been designated as "threatened." Native supplies of Pacific yew (which furnishes the anti-cancer drug taxol) are decreasing, and can no longer meet market demand. Sometimes cultivation is preferable even when there is a wild supply, because of the advantages of growing certain cultivars (for example, uniform maturation or consistency of chemical concentrations), proximity of supply, or quality considerations (for example, being able to certify that a product has been grown organically).

Cultivation offers the possibility of not only preserving economically important wild plants in their natural habitats, but also of providing farmers with new crops.

Domestic and foreign markets for medicinal plants are growing rapidly and provide important opportunities for the development and diversification of Canadian agriculture. Currently, ginseng dominates the medicinal crops of Canada. Ginseng (including both the American and Asian species) is the world's most widely used medicinal plant, and Canada's most important medicinal crop, contributing about $100 million annually to the Canadian economy.

Canadian farmers, entrepreneurs and pharmaceutical companies have increasingly been searching to exploit additional medicinal plants that can be grown in Canada, but have been limited by the difficulty of acquiring information on the many promising possibilities that exist.

In the following chapters on selected native Canadian medicinal plants, we provide summary information intended to improve the utilization of economically important plants of Canada, by providing a guide to critical and relevant information. Except for ginseng, information resources are limited and often difficult to obtain. Although our primary focus is economic, we have also tried to include information of general interest, since the topic of medicinal plants is both crucial and fascinating.

1.2.Classification of medicinal plant:

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.

Table 1: Classification of medicinal plant

Based on the active constituents	
Aromatic Herbs	**fennel, ginger, garlic, lemon grass**
Nerving Herbs	Ginger, catnip.
Astringent Herbs	Peppermint, red raspberry
Bitter Herbs	Aloe, cascara, liquorice
Diuretic Herbs	Asparagus
Mucilaginous Herbs	Althea, aloe, burdock, comfrey,
Nutritive Herbs	Acerola, apple,

Based on plant part use	
Whole plant	*Boerhaaviadiffusa*
Root	*Dasamula*
Stem	*Tinosporacordifolia*
Bark	*Saracaasoca*
Leaf	*Aloe vera*
Flower	*Biophytumsensityvum, Mimusopselenji*
Fruit	*Solanum species*
Seed	*Daturastramonium*

Based on habit	
Grasses	*Cynodondactylon*
Sedges	*Cyperusrotundus*
Herbs	*Vernoniacineria*
Shrubs	*Solanum species*
Climbers	*Asparagus racemosus*
Tress	*Azadirachtaindica*

Based on habitat	
Tropical	*Andrographispaniculata*
Sub-tropical	*Menthaarvensis*
Temperature	*Atropabelladona*

Based on Therapeutic value	
Antimalarial	*Cinchona officinalis, Artemisia annua*
Anticancer	*Catharanthusroseus, Taxusbaccata*
Antiulcer	*Azadirachtaindica, Glycyrrhizaglabra*
Antidiabetic	*Catharanthusroseus, Momordicacharantia*
Anticholesterol	*Allium sativum*
Antiinflammatory	*Curcuma domestica, Desmodiumgangeticum*
Antiviral	*Acacia catechu*
Antibacterial	*Plumbagoindica*
Antifungal	*Allium sativum*
Antiprotozoal	*Ailanthus sp., Cephaelisipecacuanha*
Antidiarrhoeal	*Psidiumgujava, Curcuma domestica*
Hypotensive	*Coleus forskohlii, Aliumsativum*
Tranquilizing	*Rauvolfiaserpentina*
Anaesthetic	*Erythroxylum coca*

Spasmolytic	*Atropabelladona, Hyoscyamusniger*
Diuretic	*Phyllanthusniruri, Centellaasiatica*
Astringent	*Piper betle, Abrusprecatorius*
Anthelmentic	*Quisqualisindica, Punicagranatum*
Cardiotonic	*Digitalis sp., Thevetia sp.*
Antiallergic	*Nandinadomestica, Scutellariabaicalensis*
Hepatoprotective	*Silybummarianum, Andrographispanicula*

1.2.1. Traditional medicine:

The World Health Organiza&on states:"Traditionalonal medicine refers to health practices,ap proaches, knowledge and beliefs incorporating plant, animal and mineral basedmedicine,spirit ual therapies, manual techniques and exercises, appliedsingularly or in combination to treat, diagnose and prevent illnesses or maintain well-being.

By definition, 'traditional' use of herbal medicines implies substantial historical use and this is certainly true for many products that are available as 'traditional herbal medicines'. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet healthcare needs. Although modern medicine may exist side-by-side with such traditionalpractice, herbal medicines have often maintained their popularity for historical andcultural reasons. Such products have become more widely available commercially, especially in developed countries. In this modern setting, ingredients are sometimes marketedfor uses that were never contemplated in the traditionalhealing systems from which theyemerged. An example is the use of ephedra (= Ma huang) for weight loss or athleticperformance enhancement (Shaw, 1998). While in some countries, herbal medicines aresubject to rigorous manufacturing standards, this is not so everywhere. In Germany, forexample, where herbal products are sold as 'phytomedicines', they are subject to thesame criteria for efficacy, safety and quality as are other drug products. In the USA, bycontrast, most herbal products in the marketplace are marketed and regulated as dietarysupplements, a product category that does not require pre-approval of products on thebasis of any of these criteria.

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:

i) **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 cauterisation.

ii) **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. and

iii) **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:

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

ii) the**Ayurvedic system** which is the old Indian system based on the

Both the Unani and Ayurvedic systems of traditional medicine have firm roots in Bangladesh and are widely practised 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 organised modern health care facilities are available to them. Medicinal preparations, almost all of which are multicomponental, used in these two systems are invariably made from plant materials, sometimes with the addition of some animal products and also some natural or synthetic organic and inorganic chemical substances.

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

1.3.Tribal medicinal plant:

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 tribaluse to treat about 50 diseases (Yusuf et al.2006).

Table 2: Plants used by the Kavirajes and tribal medicinal practitioners of Bangladesh.

Scientific name	Local name(s)	Plant part(s) used	Treatment
Callicarpa japanica	Rakabbory	Leaf	Dyspepsia, heart burn
Callicarpama crophylla	Jama-thoi	Whole plant	Tonic, dermatitis, cancer, antidote.
Clerodendrum indicum	Brahmonhati	Whole plant	rheumatoid arthritis, jaundice, skin diseases,

			edema, sedative
Clerodendrum inerme	Vana-jhai	Leaf, flower	Night blindness, pneumonia, colic, rheumatoid arthritis.
Clerodendrum trichotomum	Chapa-genda	Leaf, stem, flower	Heart diseases, rheumatoid arthritis, skin diseases.
Clerodendrum viscosum	Viti	Whole plant, leaf	Giddiness, typhus, colic in cattle, diabetes, fever, cold, aphrodisiac,
Duranta repens	Kata mehandi	Whole plant, fruit, bark	Insect repellent, itches, infertility, fever, pneumonia.
Lantana camara	Chaturaangi	Root, flower	Cough, mental diseases, fever.
Lippia alba	Khuria	Leaf	Cuts and wounds.
Lippianodi flora	Bhumi-okra	Leaf, stem, bark	Constipation,eczema, stroke,gonorrhea.
Nyctanthe sarbor	Shefali	Whole plant,	Influenza,hypertension,
Premnainte grifolia	Goniari	Leaf, bark, root	Fever,energy stimulant
Stachytarpheta indica	Supang	Leaf, stem	Leukorrhea.

1.4.Selection and identification of plant:

As per WHO guidelines (WHO 2003), the plant selected for collection should be taxonomically same as recommended by the national pharmacopoeia or other related documents. If a new plant is being selected for collection then it should be properly identified and documented. The botanical identity, scientific name including genus, species, subspecies or variety and family of the plant should be recorded. If available, the local name should also be verified. Complete taxonomical identification is an important factor during selection as taxonomy of the plant species can play an important role in their biological activity.In general, the search for the medicinal plants can follow three main routes: random, ethno (including ethnobotanical, ethnomedical and ethnopharmacological) and ecological search. Random search is extremely laborious and the success rate could be very low. Nevertheless,important drugs such as taxol, derivatives of camptothecin and homoharringtonine have been discovered by the National Cancer Institute (NCI) in collaborations with the United States Department of Agriculture (USDA) using this method.
The ethnobotanical, ethnomedical or ethnopharmacological approach uses information obtained from ethnobotanical survey such as the geographical distribution of the plant, its abundance, whether it is threatened or endangered, shrub/fast growing tree, easily cultivable, easily identifiable (with minimum varieties) etc. Information such as the season of collection, parts that are used and whether those parts are seasonal/replenishable and if there is any reported toxicity, are also required. The information can be obtained from traditional medical practitioners and other people such as village elders and local women who are traditional users of medicinal plants

1.5.Collection of plant:

Medicinal plant materials should be collected inthe proper season so as to ensure the best possible quality of both the starting material as well as the finished product. Seasonal variations can affect the chemical composition of the plants and thus its biological activity. In most cases, maximum accumulation of chemical constituents occurs at the time of flowering which then declines at the beginning of the fruiting stage. The time of harvest should also depend on the plant part to be used since it is well known that depending on the plant species the level of biologically active constituents can vary in different parts at different stages of the plant growth and development. For example, Kursar et al. (1999) found that younger leaves of tropical rainforest plants contained secondary metabolites that were either present in very little quantities or totally absent inmatured leaves. The extracts from these younger leaves showed better biological activity when tested for anticancer activity or activity against *Bacillus subtilis* and *Artemiasalina* (brine shrimp). It also applies to other components in the plant material such as the toxic components. Climatic conditions, e.g. light, rainfall, and temperature (including daytime and nighttime temperature differences) also influence the physical, chemical and biological qualities of medicinal plants. The water and temperature stress related increase in the content of active constituents such as the total phenolic compunds was shown by Nacif de Abreu and Mazzafera (2005) in Hypericumbrasilience. Hence the best time of collection should be determined according to the levels of the biologically active constituents rather than the vegetative yield.

1.6.Drying and grinding:

After collection the plant material must first be preserved so that the active compound will remain unchanged. The most common method for preserving plant material is drying. Drying also decreases the risk of external attack by moulds. In general, plant materials should be dried at temperature below 30°C to avoid decomposition of thermolabile compound. When air-dried, the plant material has to be spread out with good ventilation to facilitate drying.

Plants can be dried in a number of ways: in the open air (shaded from direct sunlight); placed in thin layers on drying frames, wire-screened rooms, or in buildings; by direct sunlight, if appropriate; in drying ovens/rooms and solar dryers; by indirect fire; baking; lyophilization; microwave; or infrared devices. Where possible, temperature and humidity should be controlled to avoid damage to the active chemical constituents. The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials. For example, shade drying is preferred to maintain or minimize loss of color of leaves and flowers; and lower temperatures should be employed in the case of medicinal plant materials containing volatile substances. The drying conditions should be recorded. In the case of natural drying in the open air, medicinal plant materials should be spread out in thin layers on drying frames and stirred or turned frequently. In order to secure adequate air circulation, the drying frames should be located at a sufficient height above the ground. Efforts should be made to achieve uniform drying of medicinal plant materials to avoid mold formation. Drying medicinal plant material directly on bare ground should be avoided. If a concrete or cement surface is used, the plant materials should be laid on a tarpaulin or other appropriate cloth or sheeting. Insects, rodents, birds and other pests, and livestock and domestic animals should be kept away from drying sites. For indoor drying, the duration of drying, drying temperature, humidity and other conditions should be determined on the basis of the plant part concerned (root, leaf, stem, bark, flower, etc.) and any volatile natural constituents, such as essential oils. If possible, the source of heat for directs drying (fire) should be limited to butane,

propane or natural gas, and temperatures should be kept below 30 °C. If other sources of fire are used, contact between those materials, smoke, and the medicinal plant material should be avoided. Grinding improves efficiency of extraction by increasing the surface area of plant material.
This decreases the amount of solvent needed for extraction as it allows the plant material to pack more densely. Therefore, it is essential to grind samples into finer size for better extraction results.

1.7.Extraction procedure of medicinal plant:

1.7.1.Meciration:

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.

1.7.2.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.

1.7.3.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.

1.7.4.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.

1.7.5.Percolation:

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used (Figure 1). The solid ingredients are moistened with an appropriate amount of the specified menstruum and allowed to stand for approximately 4 h in a well-closed container, after which the mass is packed and the top of the percola-tor is closed. Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed

percolator for 24 h. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstruum is added as required, until the percolate measures about three-quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting.

Figure-1: Parcolator

1.7.6.Hot continuous extraction (Soxhlet):

In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus (Figure 1). The extracting solvent in flask A is heated, (AN OVERVIEW OF EXTRACTION TECHNIQUES FOR MEDICINAL AND AROMATIC PLANTS)and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into fl ask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously de-scribed methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This effectstremendous economy in terms of time, energy and consequently financial inputs.At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

Figure- 2.:Soxlet apparatus

1.7.7.Aqueous alcoholic extraction by fermentation:

Some medicinal preparations of Ayurveda (like asavaand arista) adopt the technique of fermentation for extracting the active principles. The extraction procedure involves soaking the crude drug, in the form of either a powder or a decoction (kasaya), for a specified period of time, during which it undergoes fermentation and generates alcohol in situ; this facilitates the extraction of the active constituents contained in the plant material. The alcohol thus generated also serves as a preservative. If the fermentation is to be carried out in an earthen vessel, it should not be new: water should first be boiled in the vessel. In large-scale manufacture, wooden vats, porcelain jars or metal vessels are used in place of earthen vessels. Some examples of such preparations are karpurasava, kanakasava, dasmularista. In Ayurveda, this method is not yet standardized but, with the extraordinarily high degree of advancement in fermentation technology,it should not be difficult to standardize this technique of extraction for the production of herbal drug extracts.

1.7.8.Counter-current extraction:

In counter-current extraction (CCE), wet raw material is pulverized using toothed disc disintegrators to produce a fi ne slurry. In this process, the material to be extracted is moved in one direction (generally in the form of a fine slurry) within a cylindrical extractor where it comes in contact with extraction solvent. The further the starting material moves, the more concentrated the extract becomes. Complete extraction is thus possible when the quantities of solvent and material and their flow rates are optimized. The process is highly efficient, requiring little time and posing no risk from high temperature. Finally, sufficiently concentrated extract comes out at one end of the extractor while the marc (practically free of visible solvent) falls out from the other end. This extraction process has significant advantages:

- A unit quantity of the plant material can be extracted with much smaller volume of solvent as compared to other methods like maceration, decoction, percolation.CCE is commonly done at room temperature, which spares the thermolabile constituents from exposure to heat which is employed in most other techniques.
- As the pulverization of the drug is done under wet conditions, the heat generated during comminution is neutralized by water. This again spares the thermolabile constituents from exposure to heat; The extraction procedure has been rated to be more efficient and effective than continuous hot extraction.

1.7.9 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.

1.7.10. Supercritical fluid extraction:

Supercritical fluid extraction (SFE) is an alternative sample preparation method with general goals of reduced use of organic solvents and increased sample throughput. The factors to consider include temperature, pressure, sample volume, analyte collection, modifier (cosolvent) addition, flow and pressure control, and restrictors. Generally, cylindrical extraction vessels are used for SFE and their performance is good beyond any doubt. The collection of the extracted analyte following SFE is another important step: significant analyte loss can occur during this step, leading the analyst to believe that the actual efficiency was poor.There are many advantages to the use of CO as the extracting fluid. In addition to its favorable physical properties, carbon dioxide is inexpensive, safe and abundant. But while carbon dioxide is the preferred fluid for SFE, it possesses several polarity limitations. Solvent polarity is important when extracting polar solutes and when strong analyte matrix interactions are present. Organic solvents are frequently added to the carbon dioxide extracting fluid to alleviate the polarity limitations. Of late, instead of carbon dioxide, argon is being used because it is inexpensive and more inert. The component recovery rates

generallyincrease with increasing pressure or temperature: the highest recovery rates in case of argon are obtained at 500 atm and 150° C.

The extraction procedure possesses distinct advantages:

- The extraction of constituents at low temperature, which
- strictly avoids damage from heat and some organic solvents.No solvent residues.
- Environmentally friendly extraction procedure.
- The largest area of growth in the development of SFE has been the rapid expansion of its applications. SFE finds extensive application in the extraction of pesticides, environmental samples, foods and fragrances, essential oils, polymers and natural products. The major deterrent in the commercial application of the extraction process is its prohibitive capital investment.

1.8.Parameters for selecting an appropriate extraction method:

- Authentication of plant material should be done before performing extraction. Any foreign matter should be completely eliminated.

- Use the right plant part and, for quality control purposes, record the age of plant and the time, season and place of collection.

- Conditions used for drying the plant material largely depend on the nature of its chemical constituents. Hot or cold blowing air flow for drying is generally preferred. If a crude drug with high moisture content is to be used for extraction, suitable weight corrections should be incorporated.

- Grinding methods should be specified and techniques that generate heat should be avoided as much as possible.

- Powdered plant material should be passed through suitable sieves to get the required particles of uniform size.

1.8.1.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 *Cratae vanurvala* and, for its extraction, hexane is generally used. Likewise, for plants like *Bacopa monnieri* 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 (e.g. using a Rotavapor) 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.

1.9.Steps involved in the extraction of medicinal plants:

In order to extract medicinal ingredients from plant material, the following sequential steps are involved:

- Size reduction
- Extraction
- Filtration
- Concentration
- Drying

1.9.1. Size reduction:

The dried plant material is disintegrated by feeding it into a hammer mill or a discpulverizer which has built-in sieves. The particle size is controlled by varying the speed of the rotor clearance between the hammers and the lining of the grinder and also by varying the opening of the discharge of the mill. Usually, the plant material is reduced to a size between 30 and 40 mesh, but this can be changed if the need arises. The objective for powdering the plant material is to rupture its organ, tissue and cell structures so that its medicinal ingredients are exposed to the extraction solvent. Furthermore, size reduction maximizes the surface area, which in turn enhances the mass transfer of active principle from plant material to the solvent. The 30-40 mesh size is optimal, while smaller particles may become slimy during extraction and create difficulty during filtration.

1.9.2.Extraction:

Extraction of the plant material is carried out in three ways:

- Cold aqueous percolation
- Hot aqueous extraction (decoction)
- Solvent extraction (cold or hot)

1.9.2.1.Cold aqueous percolation:

The powdered material is macerated with water and then poured into a tall column. Cold water is added until the powdered material is completely immersed. It is allowed to stand for 24 h so that water-soluble ingredients attain equilibrium in the water. The enriched aqueous extract is concentrated in multiple-effect evaporators to a particular concentration. Some diluents and excipients are added to this concentrated extract, which is then ready for medicinal use.

1.9.2.2.Hot aqueous extraction (Decoction):

This is done in an open-type extractor. The extractor is a cylindrical vessel made from type 316 stainless steel and has a diameter (D) greater than the height (H), i.e. the H/D ratio is approximately 0.5. The bottom of the vessel is welded to the dished end and is provided with an inside false bottom with a filter cloth. The outside vessel has a steam jacket and a discharge valve at the bottom.One part powdered plant material and sixteen parts demineralized water are fed into the extractor. Heating is done by injecting steam into the jacket. The material is allowed to boil until the volume of water is reduced to one-fourth its original volume. By this time the medicinal ingredients present in the plant material have been extracted out.

1.9.2.3.Filtration:

The extract so obtained is separated out from the marc (exhausted plant material) by allowing it to trickle into a holding tank through the built-in false bottom of the extractor, which is covered with a filter cloth. The marc is retained at the false bottom, and the extract is received in the holding tank. From the holding tank, the extract is pumped into a sparkler filter to remove fi ne or colloidal particles from the extract.

1.9.2.4.Spray drying:

The filtered extract is subjected to spray drying with a high pressure pump at a controlled feed rate and temperature, to get dry powder. The desired particle size of the product is obtained by controlling the inside. Temperature of the chamber and by varying the pressure of the pump. The dry powder is mixed with suitable diluents or excipients and blended in a double cone mixer to obtain a homogeneous powder that can be straighta way used, for example, for filling in capsules or making tablets.

1.9.2.5.Solvent extraction:

The principle of solid-liquid extraction is that when a solid material comes in contact with a solvent, the soluble components in the solid material move to the solvent. Thus, solvent extraction of plant material results in the mass transfer of soluble active principle (medicinal ingredient) to the solvent, and this takes place in a concentration gradient. The rate of mass transfer decreases as the concentration of active principle in the solvent increases, until equilibrium is reached, i.e. the concentrations of active principle in the solid material and the solvent are the same. Thereafter, there will no longer be a mass transfer of the active principle from plant material to the solvent.Since mass transfer of the active principle also depends on its solubility in the solvent, heating the solvent can enhances the mass transfer.
Moreover, if the solvent in equilibrium with the plant material is replaced with fresh solvent, the concentration gradient is changed. This gives rise to different types of extractions: cold percolation, hot percolation and concentration.

1.9.2.6.Cold percolation:

The extraction of plant material is carried out in a percolator which is a tall cylindrical vessel with a conical bottom and a built-in false bottom with a filter cloth. The percolator is connected to a condenser and a receiver for stripping solvent from the marc.The powdered material is fed into the percolator along with a suitable solvent (ethyl alcohol or another non-polar solvent). The material is left in contact with the solvent until equilibrium of the active principle is achieved. The solvent extract, known as miscella, is taken out from the bottom discharge valve of the percolator. Fresh solvent is added into the percolator and the miscella is drained out after acquiring equilibrium. Overall, the plant material is washed four to five times until it gets exhausted. All washes from the percolator are pooled and concentrated. The solvent in the marc is stripped out by passing steam from the bottom of the percolator. The solvent and steam vapors rise and are condensed in a tubular condenser. The condensate, which is a mixture of alcohol
and water, is collected in a receiver and then subjected to fractional distillation to get 95% pure ethyl alcohol which is again used as a fresh solvent. This type of percolation is not efficient as it takes a long time to reach equilibrium due to the slow mass transfer rate. The mass transfer (AN OVERVIEW OF EXTRACTION TECHNIQUES FOR MEDICINAL AND AROMATIC PLANTS)rate can be enhanced if some sort of movement is created between the particles and the solvent.This can be achieved either by providing inside agitation with a mechanical stirrer or by repeated circulation of the extract back to the percolator. The fi rst method is cumbersome and power intensive whereas the latter has been successful. A circulation pump that continuously circulates the miscella back to the top of the percolator gives a better mass transfer rate and reduces the equilibrium time considerably. Still, this type of percolation is energy-consuming as large amounts of miscella from multiple washes must be concentrated to remove the solvent. To overcome this problem, a battery of percolators can be connected in series. If three washes are required for completion of the extraction, four percolators are connected in series with their respective miscella storage tanks. At a particular time, one percolator is out of circuit, for charging and discharging the material and also for stripping solvent from the marc, whereas the other three percolators are in operation. Material is fed into all the percolators and the solvent is fed into the first percolator. When the equilibrium in the first percolator is reached, the extract from the first percolator is sent to the second percolator. The first

percolator is again filled with fresh solvent. The extract of second percolator is transferred to the third, the extract of first is transferred to second, and fresh solvent is added to the first. The extract of the third percolator is transfered to the fourth percolator. After attaining equilibrium, the extract from the fourth percolator is drained off. The extract of the third percolator goes to fourth, the extract of second goes to third, and the extract of first goes to second percolator. The material of the first percolator, which has received three washes, is completely exhausted. This percolator is taken out of the system for stripping the solvent and discharging the extracted marc. This is again filled with fresh plant material and the sequence is repeated with other percolators. In this way, solvent of each percolator comes in contact three times with solid material and gets fully enriched with active principle. The enriched extract is sent for solvent recovery and concentration. Thus, instead of concentrating three volumes of solvent, only one volume has to be concentrated; this saves energy and the process is efficient.

1.9.2.7.Hot percolation:

Increasing the temperature of the solvent increases the solubility of the active principle, which increases the concentration gradient and therefore enhances the mass transfer of active principle from solid material to the solvent, provided the active principle is not heat sensitive. This is achieved by incorporating a heat exchanger between the circulation pump and the feed inlet of the percolator. The extract is continuously pumped intotubular heat exchanger which is heated by steam. The temperature of the extract in the percolator is controlled by a steam solenoid valve through a temperature indicator controller. This sort of arrangement can be incorporated in single percolators or in a battery of percolators as needed.The percolators that are tall cylindrical towers must be housed in sheds of relatively great height. Tall towers are difficult to operate, especially when charging material and discharging the marc from the top and bottom manholes, which are time-consuming and labor-intensive procedures.Tall towers have been replaced by extractors of smaller height for which the H/D ratio is not more than 1.5.

These extractors have perforated baskets in which the material to be extracted is charged. These perforated baskets, when loaded outside, can be inserted into the extractor with a chain pulley block and, after the extraction, they can be lifted out from the extractor for discharging the marc. Some extractors have an electrical hoist for the charging the material and discharging the marc, which makes the operation less labor-intensive, quick and efficient.The other type of instrument for extraction of medicinal ingredients from plant material is the Soxhlet apparatus, which consists of an extractor, a distillation still, a tubular condenser for the distillation still, a tubular condenser for the recovery of solvent from the marc, a receiver for collecting the condensate from the condenser, and a solvent storage tank. The plant material is fed into the extractor, and solvent is added until it reaches the siphon point of the extractor. Then, the extract is siphoned out into the distillation still, which is heated with steam. The solvent vapors go to the distillation condenser, get condensed and return to the extractor. The level of the solvent in the extractor again rises to the siphon point and the extract is siphoned out into the distillation still. In this way, fresh solvent comes in contact with the plant material a number of times, until the plant material is completely extracted. The final extract in the distillation still, which is rich in active principle, is concentrated and the solvent is recovered.

1.9.2.8.Concentration:

The enriched extract from percolators or extractors, known as miscella, is fed into a wiped fi lm evaporator where it is concentrated under vacuum to produce a thick concentrated extract. The concentrated extract is further fed into a vacuum chamber dryer to produce a solid mass free from solvent. The solvent recovered from the wiped fi lm evaporator and vacuum chamber dryer is recycled back to the percolator or extractor for the next batch of plant material. The solid mass thus obtained is pulverized and used directly for the desired pharmaceutical formulations or further processed for isolation of its phytoconstituents.(EMEA 2006)

1.10.Some extraction procedure:

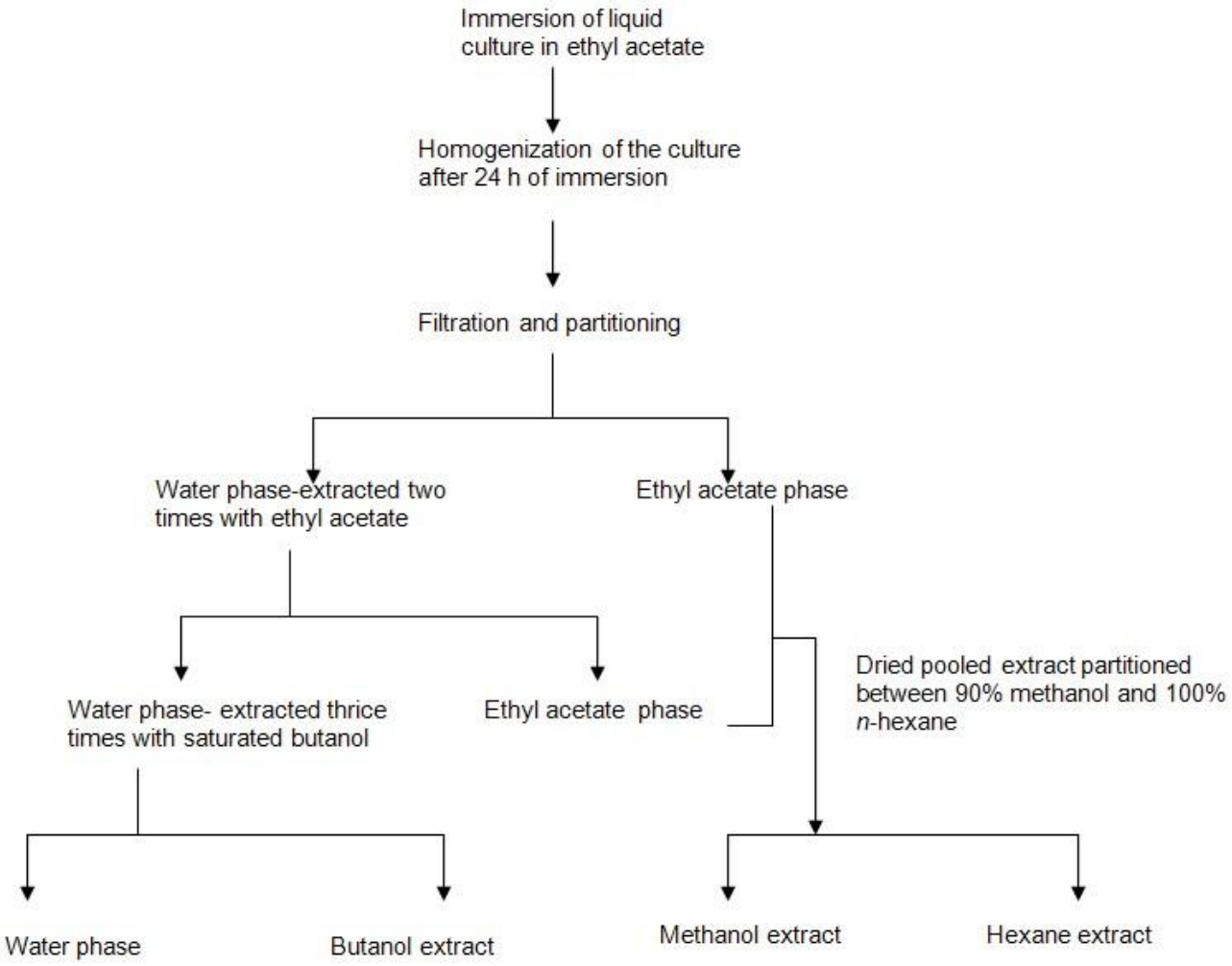

Figure-3: Fow diagram of methanol extract

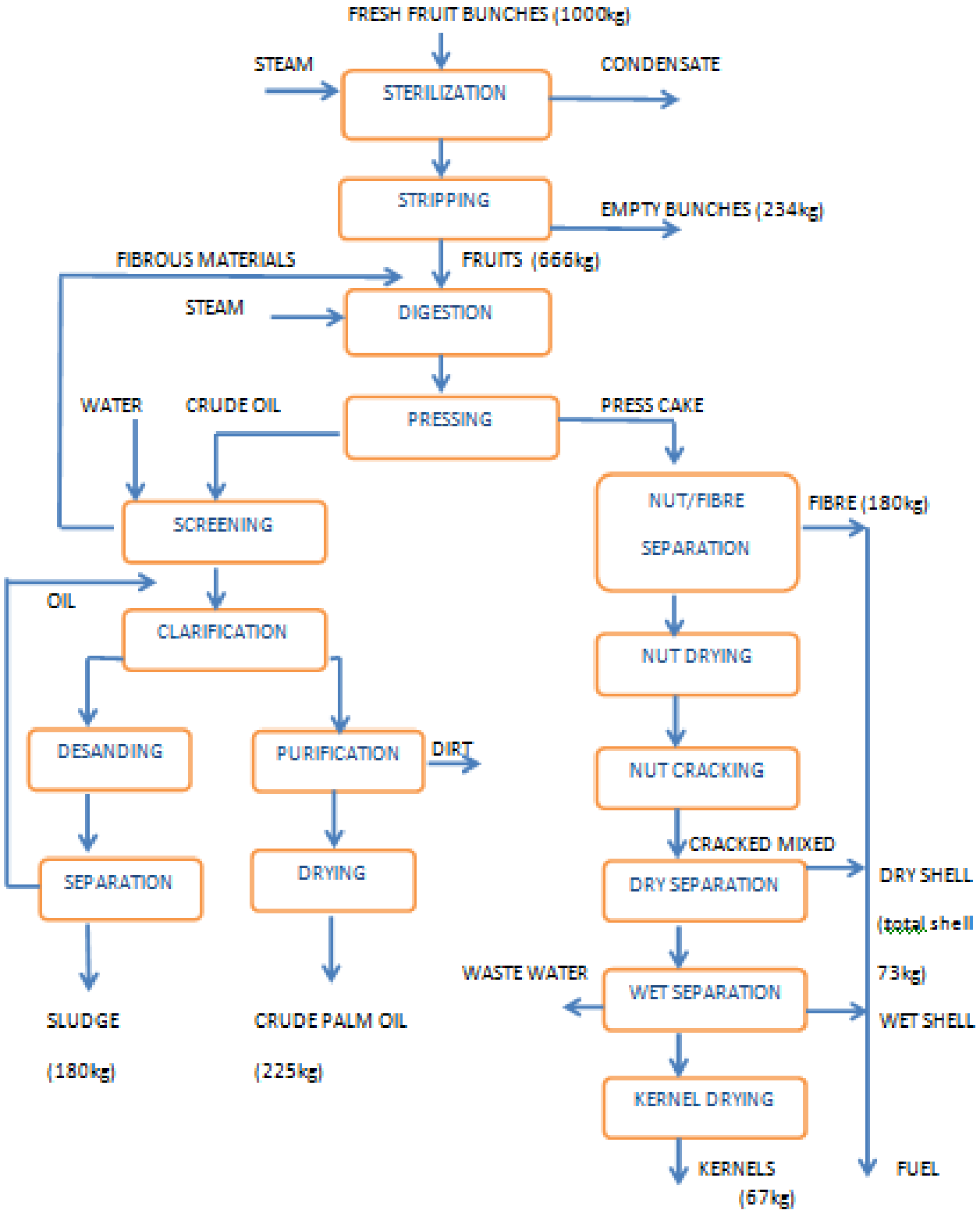

Figure-4: Flow diagram of oil extraction

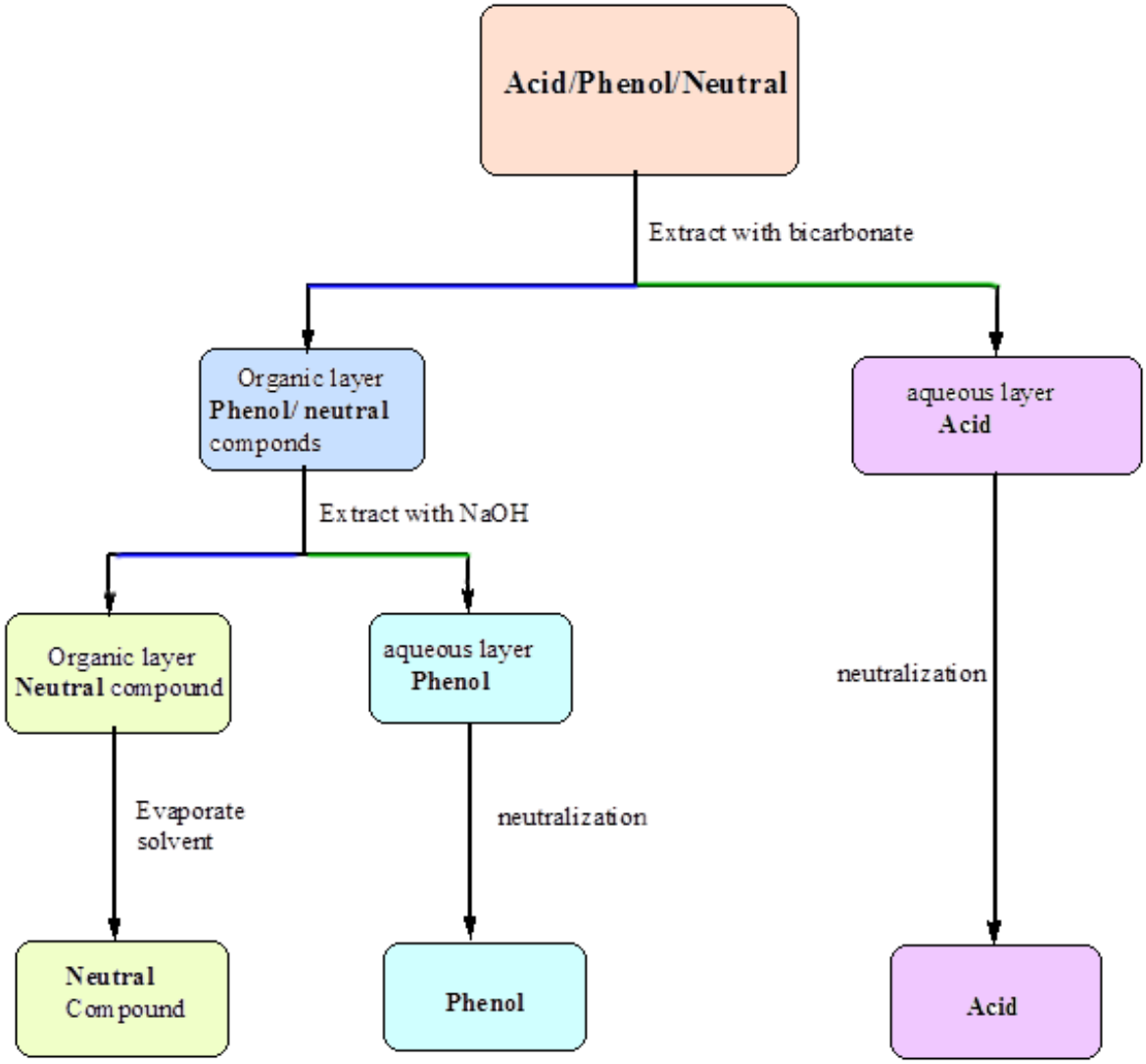

Figure-5: Flow diagram of compound extraction

1.11.Solvent:

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

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

Table3 : Common sovent for crude extraction

water	Ethanol	Metanol	Cloroform	Ether	Acetone
Anthocyanins	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			
		Lactones			
		Flavones			
		Phenones			
		Polyphenols			

1.11.Acute oral toxicity studies:

Acute toxicity studies were conducted using the method described by Lorke. Thirty (30) matured Wistar rats of both sexes were marked with 10% picric acid, weighed and randomly separated into 6 groups (A-F) of 5 rats each. Groups A–E were given varying oral doses (150; 300; 600; 1200 and 1500 mg/kg) investigated plant respectively, while group F (6^{th} group) received an equivalent volume (10 ml/kg) of distilled water. All treatments were given orally by gastric intubation. The rats were observed for signs suggestive of toxicity within 72 h. The animals that survived were further monitored for two weeks for toxic effects. The test was terminated after two weeks and all the animals were humanely sacrificed and postmortem examinations carried out on them.

1.12.Plant review:

Figure-6 :*Dracaena spicata*

1.12.1.Texonomy:

Kingdom: Plantae

Clade: Angiosperms

Clade: Monocots

Order: Asparagales

Family: Asparagaceae

Subfamily: Nolinoideae

Genus: *Dracaena*

Species: *D. spicata*

1.12.2.Plant background:

Dracaena (Dracaena spp.) is grown for its dramatic foliage and carefree nature. This large group of plants includes many species that can grow up to 6 feet tall with long, strap-like leaves, often with red and yellow variegation. Dracaena is an undemanding plant that tolerates low light and low humidity and it will forgive the occasional missed watering. As the plant grows, the lower leaves drop off and the trunk scars over, creating an interesting pattern of markings. D.fragrans, which is

the familiar corn plant and D. marginata, commonly known as the rainbow plant, are two of the more familiar Dracaena species.

1.12.3.Introduction:

Multiple thin, curving stalks with narrow ribbon-like, green leaves edged in purplish-red distinguish this dracaena from its multitude of cousins (Fig. 1). The upright, unbranched stems form a delicate, somewhat abstract silhouette, perfect for accent planting or for low-maintenance night with uplighting.

1.12.4.Other species:

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

- *Dracaena afromontana* – Afromontane dragon tree
- *Dracaena americana* – Central America dragon tree
- *Dracaena aletriformis* (Haw.) Bos)
- *Dracaena arborea* – tree dracaena
- *Dracaena aubryana*Brongn. ex E.Morren (syn.*D. thalioides*)
- *Dracaena bicolor* Hook.
- *Dracaena braunii* Engl. – ribbon dracaena, marketed as "lucky bamboo"
- *Dracaena camerooniana* Baker
- *Dracaena cincta*
- *Dracaena cinnabari*Balf.f. – Socotra dragon tree
- *Dracaena concinna*Kunth
- *Dracaena draco* (L.) L. – Canary Islands dragon tree
- *Dracaena elliptica*
- *Dracaena fragrans* (L.) Ker Gawl. (syn. *D. deremensis*) – striped dracaena, compact dracaena, corn plant, cornstalk dracaena
- *Dracaena goldieana*W.Bull
- *Dracaena hookeriana*
- *Dracaena kaweesakii* Wilkin &Suksathan
- *Dracaena mannii*
- *Dracaena marginata* Lam. – red-edged dracaena or Madagascar dragon tree: see *Dracaena reflexa* var. *angustifolia*
- *Dracaena marmorata*
- *Dracaena ombet* – Gabal Elba dragon tree
- *Dracaena phrynioides*
- *Dracaena reflexa* Lam. – Pleomele dracaena or "Song of India"
- *Dracaena serrulata* Baker – Yemen dragon tree
- *Dracaena surculosa*Lindl. – spotted or gold dust dracaena. Formerly *D. godseffiana*
- *Dracaena tamaranae* – Gran Canaria dragon tree
- *Dracaena umbraculifera* Jacq.

1.12.5.General information:

Scientific name:*Dracaena spicata*

Common name(s): Tribal name: Kadorateg grass

Family: Asparagaceae

Plant type: shrub
Planting month for zone 10 and 11: year round
Origin: native to Mayanmar, Bangladesh,
Availability: generally available in many areas within its hardiness range

1.12.6.Description:

Height: 8 to 15 feet

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

1.12.7.Foliage:

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

Figure-7: Leaf of the *Dracaena spicata*

1.12.8.Flower:

Figure-8 : Flower of *Dracaena spicata*

Flower color: fascicles

Flower characteristic: summer flowering

1.12.9.Fruit:

Figure: Fruit of *Dracaena spicata*

Fruit shape: round

Fruit length: less than .5 inch

Fruit cover: fleshy

Fruit color:orange-red

Fruit characteristic: inconspicuous and not showy

1.12.10.Trunk and Branches:

Trunk/bark/branches: showy; typically multi-trunked or clumping stems

Current year stem/twig color: greenish

Current year stem/twig thickness: not very thick

1.12.11.Culture:

Light requirement: plant grows in part shade/part sun

Soil tolerances: clay; sand; acidic; slightly alkaline; loam

Drought tolerance: high

Soil salt tolerances: poor

Plant spacing: 36 to 60 inches

Light: Bright light. Avoid direct sunlight in summer.

Water: Keep soil lightly moist spring through fall, slightly drier in winter. Do not let soil get waterlogged.

Humidity: Average room humidity. Will tolerate dry air.

Temperature: Normal room temperatures. 60-75°F/16-24°C

Soil: Good-quality, all-purpose potting mix.

Fertilizer: Feed every 2 weeks in spring and summer with a 10-10-10 liquid fertilizer diluted by half.

Propagation: Cut off the cane at any height and root them like stem cuttings.

1.12.12.Other:

Roots: usually not a problem

Winter interest: no special winter interest

Outstanding plant: not particularly outstanding

Invasive potential: not known to be invasive

Pest resistance: long-term health usually not affected by pests

1.13.Use:

- Pills prepared withwarm water twice daily for the treatment of meals by the Chakma
- A root extract of *Dracaena spicata* and *Pandanus foetidus* is taken togather and administered to healthy children during outbreaks of meals by Tanchangya
- Antimicrobial activity
- Atiulcerant activity
- Antithrombolytic
- Antipyretic activity
- Cooked as vegetable.

2.1.Antimicrobial activity test:

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

Result:

The test samples of *D. Spicata* exhibited zone of inhibition ranging from 7.0 to 18.0mm against the test organisms. The highest (18.0mm) zone of inhibition was demonstrated by the aqueous soluble fraction against *Pseudomonas aeruginosa*. Against gram positive bacteria *Staphylococcus aureus*, the carbon tetrachloride and aqueous soluble extractives revealed 15.0mm zone of inhibition.(Yeunuset al., World J Pharm Sci2014)

2.2.Thrombolytic activity:

The thrombolytic activity was evaluated by the method developed by (Prasad et al 2006) by using streptokinase as positive control.

Result:

The crude methanol extracts of aerial parts of leaf of *D. spicata* as well as its hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to screenings for thrombolytic and membrane stabilizing potentials. In order to identify the drugs with the ability to promote lysis of blood clot from natural resources, the extractives *D. spicata* were assessed for thrombolytic activity. Addition of 100 µl streptokinase, a positive control (30,000 I.U.) to the clots of human blood and subsequent incubation for 90 minutes at 37°C showed 66.77 % lysis of clot. On the other hand, distilled water,treated as negative control, revealed a negligible lysis of clot (3.79 %). *D. spicata* extractives showed mild thrombolytic activity and the highest thrombolytic activity was demonstrated by the carbon tetrachloride soluble fraction (21.05±0.23 %).

2.3.Membrane stabilizing activity:

The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit hypotonic solution and heat induced haemolysis of human erythrocytes following the method developed by Omale et al 2008

Result:

The membrane stabilizing activity of *D. spicata* extractives was also determined. The hexane soluble fraction of crude methanol extract of *D. Spicata* demonstrated 64.44±0.68 % & 36.52±0.19

% inhibition of hypotonic solution and heat induced hemolysis, respectively. *D. spicata* exhibited significant membrane stabilizing activity

2.4.Antipyretic activity:

Root extract of the plant possesses antipyretic activity mild. The root extraction of the plant used by the tribal people to recover from fever .

2.5. Antiulcerant activity:

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

3.1. Collection:

3.1.1.Plant Material:

Dracaena spicata are 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 March 2014 by the research instructor Nazia Hoque, Senior Lecturer, Department of Pharmacy, East-West University, Dhaka, Bangladesh
The experiments were conducted in Phytochemical Research Laboratory, East West University of Bangladesh in 2014

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 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

Figure-9 : Blender machine

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 aluminium 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 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-10:Rotary evaporator

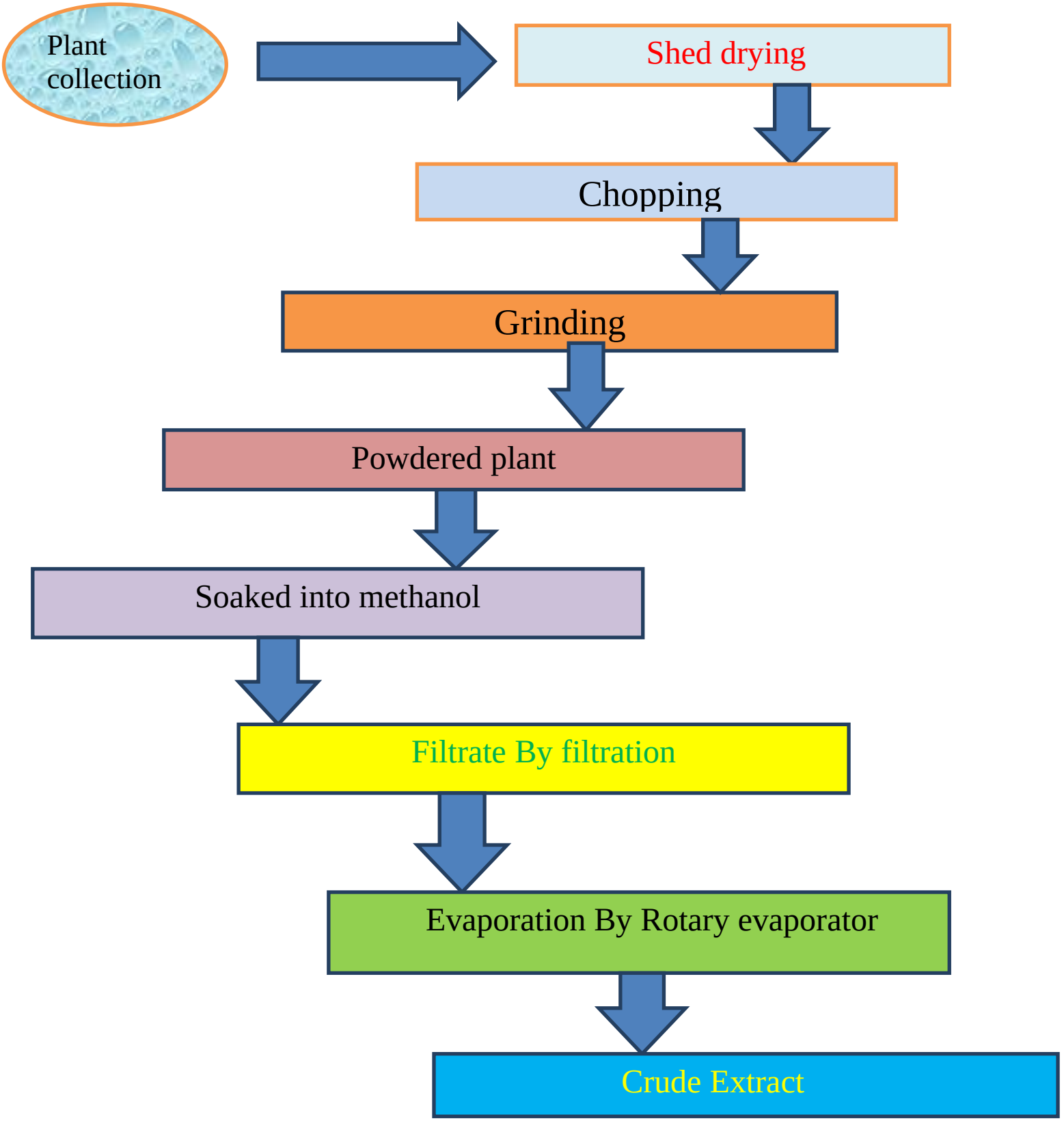

Figure-11 : Schematic presentation of the crude preparation from the plant

3.3.Antimicrobial screening:

The antibacterial assay was performed by disc diffusion technique. Disc diffusion technique is highly effective for rapidly growing microorganisms. In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient(Bauer et al.1988). Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media(Barry1976). 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(Barry1976). 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)

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3.3.1.Test materials used for the study:

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

3.3.2.Reagents:

- Rectified spirit
- Agar purified powder
- Methanol

3.3.3.Apparatus:

Filter paper discs (Sterilized)

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

- Incubator
- Refrigerator
- Autoclave

3.3.4.Test organisms:

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

Table-4: List of test organism

Gram-positivebacteria:
Bacillus subtilis
Salmonella paratyphy
Gram-negative bacteria:
Shigelladysenteriae
E. coli
Aspregillusniger

3.3.5.Culture medium and their composition:

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

Table-5: Ingredient of Nutrient Agar medium

Ingredient	Amount
Bacto peptone	0.5 gm
Sodium chloride	0.5 gm
Bacto yeast extract	1.0 gm
Bacto agar	2.0 gm
Distilled water	100 gm

3.3.6.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.

3.3.7.Sterilization procedure:

In order to avoid any type of contamination and cross contamination bythe 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. Petridishes 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-12 : Laminar hood

3.3.8.Preparation of Subculture:

In an asepticcondition 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-13 : Incubator

3.3.9.Preparation of the test plate:

The test organisms were transferred from the subculture to petridish containing about 10 ml of melted and sterilized agar medium. The bacterial suspension was taken by a loop a mixed with normal saline with the help of vortex machine. Then a sterilized cotton bud was taken and dipped into the bacterial suspension. Then the bacterial sample is applied to the petridish with the help of this cotton bud.

3.3.10.Preparation of disc:

- 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 (400 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.

3.3.11. 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

3.3.12. 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 vernier scale.

Figure-14 : Zone of inhibition

3.3.13.Precaution:

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

3.4.Cytotoxicity assay:

3.4.1. Principle:

Brine shrimp eggs are hatched in simulated sea water to get nauplii. By the addition of calculated amount of Dimethyl sulfoxide (DMSO), desired concentrations of the test sample is prepared. The nauplii are counted by visual inspection and are taken in vials containing 5 ml of simulated sea water. Then samples of different concentrations are added to premarked vials using micropipettes. Then the vials are left for 24 hours. Survivors are counted after 24 hours (Meyer et al.1982).

3.4.2.Materialsrequired:

- *Artemia salina* leach(brine shrimp eggs)
- Sea salt (NaCl)
- Small tank with perforated dividing dam to hatch the shrimp
- Lamp to attract shrimps
- Pipettes, Micropipette
- Glass vials
- Test samples of experimental plants
- DMSO

3.4.3.Procedure:

For cytotoxicity study, Brine shrimp lethality bioassay method was applied for the evaluation of cytotoxic property of methanolic extracts of the *Dracaena spicata*, to calculate the Lethal Concentration at 50% mortality (LC50), the method of which was modified from the original procedure of utilizing the live nauplii of *Artemia salina* by Meyer, et al., (Brine Shrimp: A Convenient General Bioassay for Active Constituents, 1982).

The steps are as followed –At first,

- 38 gm Iodine –free Sodium chloride (NaCl) salt was dissolved into 1L Distilled Water in a transparent glass jar to prepare isotonic solution of NaCl (3.8% NaCl Solution) for simulating the Sea Water in which the *Artemia salina*c ysts hatches. [Reason for using Iodine –free NaCl wasthat the Iodine itself has cytotoxic activity which can modify the actual result. (Vose et al.2000)].
- Then sufficient amount of *Artemia salina* cysts was poured in the solution.

3.4.4.Shrimp serial dilution for cytotoxicity bioassay:

- The solution was then left for 24 hrs for incubation under sufficient light and air flow.
- After 24 hrs of incubation tiny pink or white colored nauplii were visible in stagnant water by turning off the air flow which will attract the nauplii towards the light.
- Then 9 Test Tubes were taken each of which were pre –marked at 5 mL.
- 2 mg of methanolic extract of *Drasaina spicata* was dispersed in 2 mL of isotonic NaCl solutionand the dispersion was sonicated for 10 mins to obtain a homogenous solution in the 1st Test Tube.
- Then 1 mL of the solution from the 1st test tube was transferred to the 2nd test tube already containing 1 mL of Distilled Water.
- The Process was continued up to the 8th test tube by using a Pasteur Pipette.
- 10 nauplii were transferred to each testtube.
- Then the rest of volumes were made up to 5 mL by adding isotonic NaCl solution.
- So, after volume adjustment final concentrations were 400, 200, 100, 50, 25, 1, 2.5, 6.25, 3.125, µg / ml from 1st to 8th test tube respectively.
- A negative control was prepared by adding 10 nauplii in the 9th test tube containing 5ml of DMSO.
- All of the test tubes were left for 24 hrs for incubation.
- After 24 hrs of incubation, Number of the dead naupliies was counted.
- From this data, the Percentage Mortality was calculated for each concentration and Lethal Concentration at 50% Mortality was calculated by using Linear Regression Analysis in Microsoft Excel 2010, (Meyer et al. 1982)

$$\text{Percentage (\%)Mortality} = \frac{\text{No of shrimp died}}{\text{Total No of Shrimp in Each Test}} \times 100$$

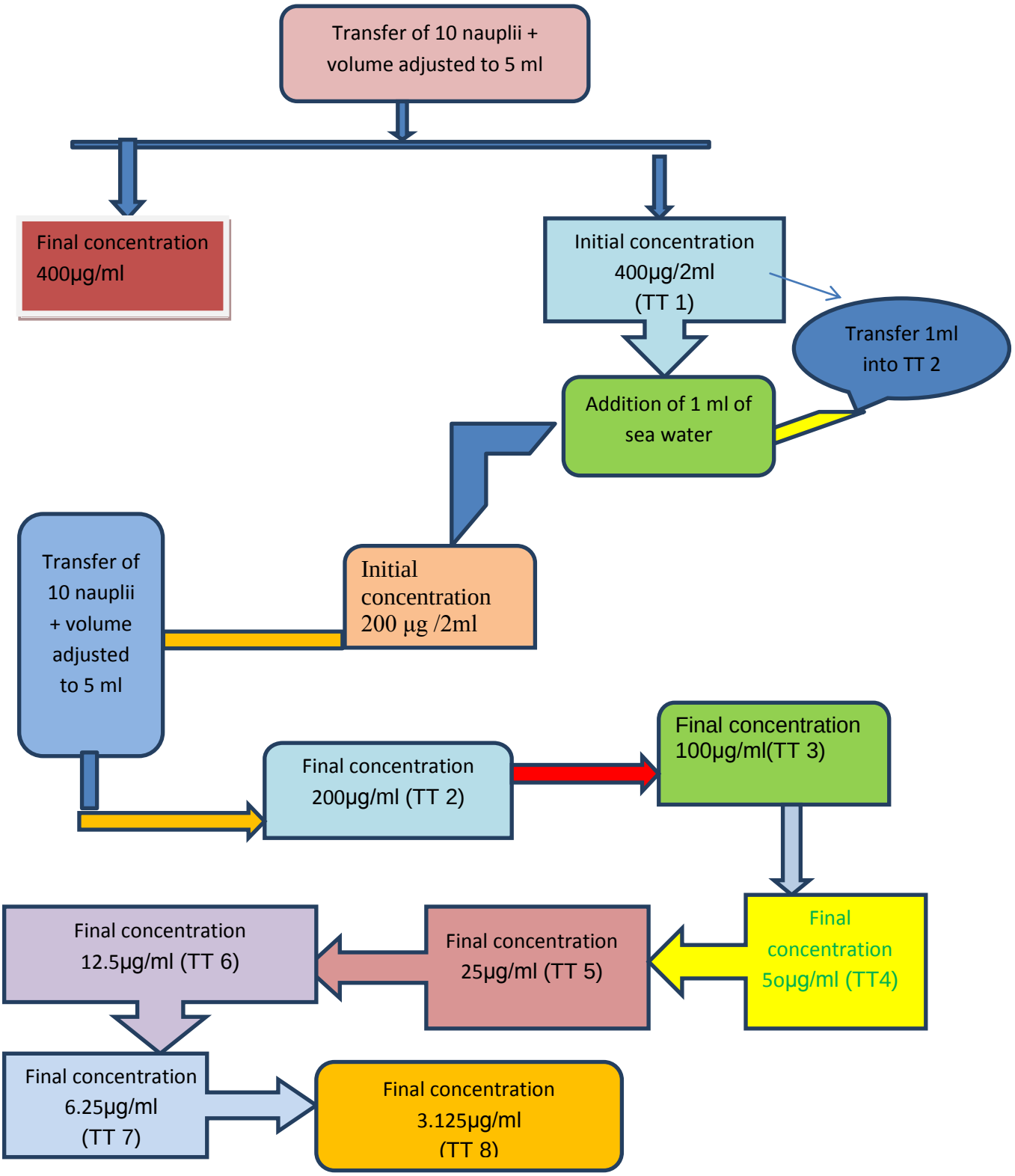

Figure-15 :Schematic representation of experimental procedure of the brine shrimp serial dilution for cytotoxicity bioassay. The arrows indicate that the process between 1st and 2nd test tube was repeated for every successive test tubes. (TT = Test Tube)

4.1.Result of Antimicrobial assay of *Dracaena spicata*:

The methanol extract (400 µg/disc) of *Dracaena spicata* showed antibacterial activity against Gram negative bacteriaand fungi. The methanolic extract of *Dracaena spicata* was active against *E. colli* and *Aspergillus niger*.

Table-6 :Result of Antimicrobial assay of *Dracaena spicata*

Name of the test organism	Diameter of the zone of inhibition(in mm)	
	Methanol extract (400µg/disc)	Kanamycin disc (30µg/disc)
Gram positive bacteria		
Salmonella paratyphy	0	28
Bacillus subtilis	0	27
Gram negative bacteria		
Shigelladysenteriae	0	27
E. coli	8	28
Fungi		
Aspergillusniger	10	26

4.2. Result of cytotoxicity assay of the *Dracaena spicata:*

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

Table-7 : Effect of *Dracaena spicata* (methanol extract) on shrimp nauplii.

Concentration µg/ml	Log C	No of naupli taken	No of dead	% mortality	Value of x (log LC_{50})	LC_{50}
400	2.60206	10	9	90		
200	2.30103	10	7	70		
100	2.00000	10	5	50		
50	1.69897	10	4	40		
25	1.39794	10	4	40	1.586	36.829
12.5	1.09691	10	3	30		
6.25	0.79588	10	1	10		
3.125	0.49485	10	0	0		

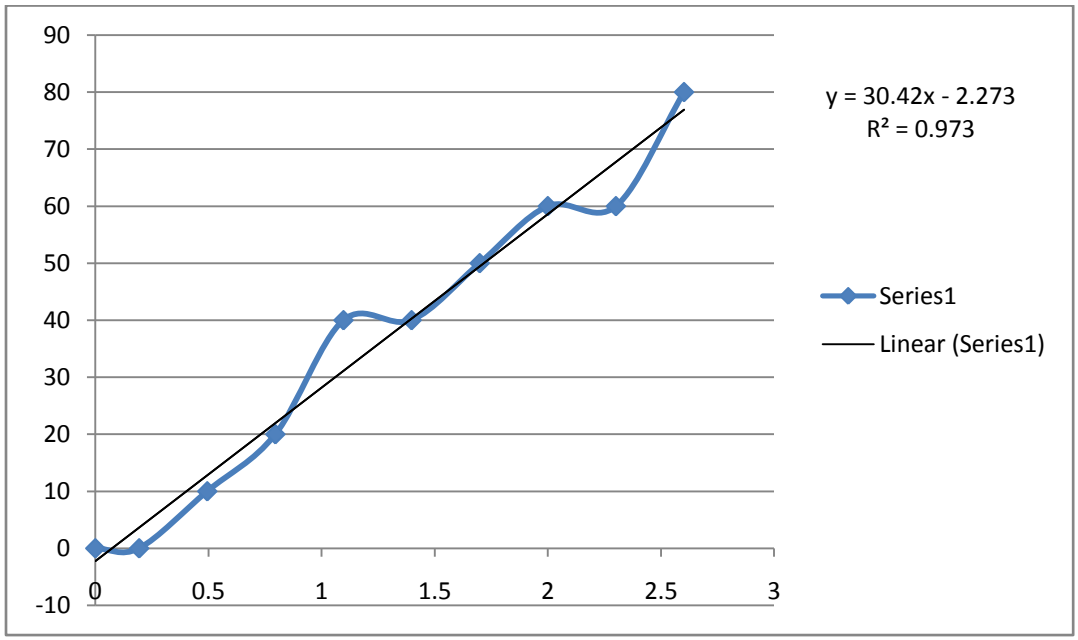

Figure-15:% mortality and predicted regression line of *Dracaena spicata*

5.1.Antimicrobial assay ofthe methanolic extract of *Dracaena spicata*:

The antimicrobial screening using a sensitive in-vitrodiscs diffusion method. The methanolic extract (400µg/disc) of *Dracaena spicata* showed poor antibacterial activity against Gram negative bacteria and fungi.The Antimicrobial activity of the methanoli cextract of the *Dracaena spicata* was tested against 2 Gram positive bacteria and 2 gram negative bacteria and fungi. The antibacterial activity was shown against gram negative bacteria *E.coli*, the diameter of Zone of Inhibition was 8 mm. In case of gram positive bacteria the Zone of inhibition of *Dracaena spicata* was 0 mm. The zone of inhibition against fungi, *Aspregillus niger* was 10 mm. *Dracaena spicata* showed poor antimicrobial activity against the selected microorganisms and thus further studies must be conducted to isolate the pure compounds and to evaluate their antimicrobial activity by using more advanced methods

5.2.Cytotoxicity assay of the methanolic extract of *Dracaena spicata:*

The brine shrimp lethality bioassay was performed to evaluate the cytotoxicity activity of the Methanolic 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 methanolicm extract of the *Dracaena spicata* was calculated approximately as 36.829µg /mL with a R^2 value of 0.9735. Cytotoxicity of methanolic extract of *Dracaena spicata* was not good, since according to Wattanapiromsakul, Wangsintaweekul, Sangprapan, Itharat, & Keawpradub (2005) LC_{50} value less than 5 µg / mL indicates excellent cytotoxic effect. So, further studies are needed to evaluatethe cytotoxicity of isolated pure compounds.

5.1.Conclution:

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 compoundsand 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. The plant also shows moderate antimicrobial activity. The antimicrobial activity of the plant extracts were tested against eleven potentially bacterial pathogenic by using disc diffusion method at different concentrations of the extracts of *Dracaena spicata* to understand the most effective activity.In case of anticancer drug preparation this plant extracts may treated as a good candidate as it has notable cytotoxic effect.The plant possesses cytotoxic activity. It was observed that brime shrimp was died at different concentrations such as-25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and, 400 µg/ml. There are some established research reports regarding the antimicrobial and pharmacological properties of this plant.This is only a preliminary study but the plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates.Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.

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