Biological Evaluation of Three Medicinal Plants *Polygonum hyropiper*, *Hibiscus rosa-sinensis, Clerodendrum viscosum* and a Locally Cultured Fungus, *Auricularia auricular*

This Thesis Paper is Submitted to the Department of Pharmacy, East West University in Conformity with the Requirements for the Degree of Bachelor of Pharmacy

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THIS THESIS PAPER IS DEDICATED TO

MY BELOVED PARENTS

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CERTIFICATE

This research paper, submitted to the Department of Pharmacy, East West University in conformity with the requirements for the Degree of Bachelor of Pharmacy (B. Pharm) was carried out by Antara Deb Mou ID: 2007-1-70-026.

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CERTIFICATE

This is to certify that, the research work on "**Biological Evaluation Of Three Medicinal Plants *Polygonum hyropiper, Hibiscus rosa-sinensis, Clerodendrum viscosum and Locally cultured Fungus Auricularia auricular* "** submitted to the Department of Pharmacy, East West University, 43, Mohakhali, Dhaka, in the partial fulfillment of the requirement for the Degree of Bachelor of Pharmacy (B.Pharm) was carried out by Antara Deb Mou ID# 2007-1-70-013 under my guidance and supervision and that no part of the thesis has been submitted for any other degree.

I further certify that all the resources of the information in this connection are duly acknowledged.

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Professor Chowdhury Faiz Hossain

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ABSTRACT

This study was undertaken to find out the Cytotoxicity , Antioxidant & Antimicrobial activity of the different parts of *Polygonum hydropiper* L, leaf of *Clerodendrum viscosum, Hibiscus rosa-sinensis* & locally cultivated fungus *Auricularia auricular.* Disc diffusion technique was used against one Gram positive and four Gram negative bacteria and two fungus. But they did not show any zone of inhibition against these micro-organisms up to the concentration of 250 µg/µL and 500 µg/Ml. The Brine shrimp lethality bioassay method was used to determine the cytotoxicity activities. By using Biostate 2008 (software) determined the LC_{50} values. On the other hand, the antioxidant activity was investigated by DPPH radical scavenging assay result that from all sample *Polygonum hydropiper* leaf ethyl acetate extract had potent antioxidant activity. Thin layer chormatographic technique was used to get information about the phytochemicals of the plant extracts.

Key word: *Polygonum hydropiper* L, *Clerodendrum viscosum, Hibiscus rosa- sinensis, Auricularia auricular,* Cytotoxicity, Antioxidant, Antimicrobial, Thin layer chromatography.

Table of Contents **Page numbers**

Figures **Page Number**

Table of Graphs **Page number**

Graphs **Page number**

INTRODUCTION

1.Introduction

1.1.Medicinal Plant & Medicinal Fungus

Medicinal plants are various plants thought by some to have medicinal properties, but few plants or their phytochemical constituents have been proven by rigorous science or approved by regulatory agencies such as the United States Food and Drug Administration or European Food Safety Authority to have medicinal effects. Allopathic medicines derived from plants, and pharmacological research results about a plant. Pharmacognosy is the study of medicines derived from natural sources, including plants. The American Society of Pharmacognosy as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources."

Though the advent of modern or allopathic medicine has somehow diminished the role of medicinal plants in favor of synthetic drugs, even now a number of modern drug discoveries have been based on medicinal plants used by indigenous people.[1]In recent years, because of the costs as well as serious side-effects of a number of modern drugs, attention has turned back to medicinal plants as a source for discovery of newer drugs with less cost and side effects. It has been reported that about 64% of the total world population is using traditional medicine to satisfy their health-care needs.[2] The sheer number of papers published in ethnobotanical or traditional medicinal scientific journals attest to the fact that scientists are increasingly turning their attention to ethnobotanical practices of indigenous people as a strategy for discovery of novel drugs against both ancient and newly emerging diseases.[3]Medicinal plants constitute an important natural wealth of a country. Medicinal plants are used as a source of drugs for the treatment of various human and livestock health disorders all over the world from ancient times to the present day. A total of 250,000 species of flowering plants.

are referred to as medicinal plants. The World Health Organization (WHO) enlisted some 21,000 medicinal plant species.[4] They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be

earned by exporting medicinal plants to other countries. In this way indigenous medicinal plants play significant role of an economy of a country.

Medicinal plants are used at the household level by women taking care of their families, at the village level by medicine men or tribal shamans, and by the practitioners of classical traditional systems of medicine such as Ayurveda, Chinese medicine, or the Japanese Kampo system. According to the World Health Organization, over 80% of the world's population, or 4.3 billion people, rely upon such traditional plant-based systems of medicine to provide them with primary health care.

A fungus is a member of a large group of eukaryotic organisms that includes microorganisms such as yeasts and molds (British English: moulds) as well as the more familiar mushrooms. Medicinal mushrooms are mushrooms or extracts from mushrooms that are used or studied as possible treatments for diseases. Research has shown some medicinal mushroom isolates that have promising cardiovascular, anticancer, antiviral, antibacterial, antiparasitic, anti-inflammatory, and antidiabetic properties. Currently, several extracts (polysaccharide-K, polysaccharide peptide,lentinan) have widespread use in Japan, Korea and China, as adjuvants to radiation treatments and chemotherapy.

The concept of a medicinal mushroom has a history spanning millennia in parts of Asia. Only a few mushroom extracts have been extensively tested for efficacy. The available results for most other extracts, are on isolated cell lines, animal research with rodents, or underpowered clinical human trials. Although medicinal molds do not produce mushrooms, these fungi were the original source of penicillin, the first statins, and griseofulvin. auricula-judae was used in folk medicine as recently as the 19th century, for complaints including sore throats, sore eyes and jaundice, and as an astringent. Today, the mushroom is still used for medicinal purposes in China, where soups featuring it are used as a remedy for colds; it is also used in Ghana, as a blood tonic. Modern research into possible medical applications have variously concluded that A. auricula-judae has antitumour, hypoglycemic, anticoagulant and cholesterol-lowering properties.

1.2 History

Desire to consolidate their existence on earth was the prime natural instinct in all living organisms that came into being on earth at one time or the other. Human being was no exception. Staying healthy and free from diseases have been his prime concerns from the very beginning of his existence on this earth.

In an effort to accomplish these concerns human being utilized and made use of any material that he found helpful in his struggle for existence. Plants having medicinal properties were such a material which human being utilized to stay healthy and to fight against diseases right from the beginning of his rational living activities on this earth. Thus the history of the use of medicinal plants for alleviating diseases had its origin in the activities of the most primitive man of the remote past. Illness, physical discomfort, injuries, wounds and fear of death had forced early man to use any natural substance that he could lay his hands on, without any resistance, for relieving the pains and sufferings caused by these abnormal conditions and for preserving his health against disease and death.

From historical records, it is apparent that most of the early peoples like Assyrians, Babylonians, Egyptians and ancient Hebrews, were familiar with the properties of many medicinal plants. As far as records go, it appears that Babylonians (about 3000 BC) were aware of a large number of medicinal plants and their properties. Some of the plants they used then are still in use in almost the same manner and for the same purposes. Historical records of Assyria and Babylonia indicated that by 650 BC there were about 250 plant medicines in use in that region .As evident from the *Papyrus Ebers* (Written about 1500 BC), the ancient Egyptians possessed a good knowledge of the medicinal properties of hundred of plants. Many of the present day important plant drugs like henbane (*Hyoscyamus* spp.), mandrake (*Mandragora officinarum*), opium (latex of the mature but unripe fruits of *Papaver somniferum*), pomegranate (fruits of *Punica granatum),* castor oil (oil of *Ricinus communis* seed), aloe (juice of leaves of Aloe spp.), onion (*Allium cepa*), hemlock (*Conium* spp.), cannabis (*Cannabis sativa*), senna (*Cassia senna*), and many other were in common use in Egypt about 4500 years ago. 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 on the medicinal use of plants in the Indian subcontinent.[6]

The *Susruta Samhita*, probably written before 1000 BC, deals with details of surgery and therapeutics, while the comprehensive Indian Herbal, *Charaka Samhita*, written about the same period, deals mainly with medicinal agents and cites more than 500 medicinal plants with their medicinal properties and uses.[6]

The earliest description of plant medicines used in the *Ayurvedic* system were described about 1200 BC with a list of 127 plants. The earliest Chinese Pharmacopoeia, the Pen Tsao, appeared around 1122 BC. This authoritative work describes the use of Chaulmoogra oil to treat leprosy. It also recorded the medicinal uses of *Ephedra* species for the first time.[7]

The practice of medicine using medicinal plants flourished most during the Greek civilization when historical personalities like Hippocrates (born in 460 BC) and Theophrastus (born in 370 BC) practiced herbal medicine. The material medica of the great Greek physician Hippocrates (460-370 BC) consists of some 300-400 medicinal plants which included opium, mint, rosemary, sage and verbena. The far ranging scientific work of Aristotle (384-322 BC), a Greek philosopher, included an effort to catalogue the properties of the various medicinal herbs known at that time. The encyclopedic work of Dioscorides (1st Century AD), De Materia Medica (Published in 78 AD), was the forerunner of all modern pharmacopoeias and an authoritative text on botanical medicine. This work featured about 600 medicinal plants. Two of the 37 volumes of books written by Pliny De Elder (23-70 AD) were devoted to medical botany and these included a large number of medicinal plants. Galen (131-200 AD), wrote about 500 volumes of books describing hundreds of recipes and formulations containing a large number of medicinal plants and animal products.[11]The Arabian Muslim physicians, like Al-Razi and Ibn Sina (9th to 12th century AD), brought about a revolution in the history of medicine by bringing new drugs of plant and mineral origin into general use.

The use of medicinal plants in Europe in the 13th and 14th centuries was based on the Doctrine of Signatures or Similars developed by Paracelsus (1490-1541 AD), a swiss alchemist and physician.[10]

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 (*Erythroxylum* species) and tobacco (*Nicotiana tabacum*) was common in these countries in the 14th and 15th centuries.

The 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.

The knowledge of the medicinal plants extends to any part of the world where man has traditionally needed these plants to cure his diseases. Thus, mixture of magic and religion, mixture of necessity and chance, test and error, the passage of different cultures has created a knowledge of vegetal remedies that has formed the base of the modern medicine.

On the other hand "Mushrooms have long been valued as highly tasty and nutritional foods by many societies throughout the world. Early civilizations, by trial and error built up a practical knowledge of those suitable to eat and those to be avoided, e.g. poisonous or even psychotropic. However, in the Orient several thousand years ago, there was the recognition that many edible and certain non-edible mushrooms could have valuable health benefits.[11]

Mushrooms with a long record of medicinal use include the edibles *Grifola frondosa* (maitake) and *Lentinula edodes* (shiitake). *Ganoderma lucidum*, used as tea, is known in Chinese as língzhī ("spirit plant") and in Japanese mannentake ("10,000 year mushroom"). In ancient Japan, *Grifola frondosa* was considered medicinal, and was worth it's weight in silver.[12] As early as the sixteenth century, *Inonotus obliquus* (chaga) was used as a folk medicine in Russia and Northern Europe.

The prophet Muhammad said, "Truffles are 'manna' which Allah, sent to the people of Israel through Moses, and its juice is a medicine for the eyes".[13]The ancient Egyptians considered mushrooms food for royalty. Ötzi the Iceman, a mummified human from 3300 BC, was found carrying *Fomes fomentarius* (ice man fungus) and *Piptoporus betulinus* wrapped in a leather string. *Fomes fomentarius* was was used topically in Europe during the 18th and 19th centuries, and was known to Hippocrates as *mykes*.[16] Although medicinal molds do not produce mushrooms, these fungi were the original source of penicillin, lovastatin, griseofulvin and allowed for the development of the statin medications. Genetic testing has shown mushrooms are

much more closely related to animals than plants.[14] Therapeutic value of medicinal plant cannot be describe in word. A list of medicinal plant and their use show below:

Table 1: List of Therapeutic value of medicinal plant

Name of plant	Therapeutic value
Cinchona officinalis, Artemisia annua	Antimalarial
Catharanthus roseus, Taxus baccata	Anticancer
Azadirachta indica, Glycyrrhiza glabra	Antiulcer
Catharanthus roseus, Momordica charantia	Antidiabetic
Allium sativum	Anticholesterol
Curcuma domestica, Desmodium gangeticum	Antiinflammatory
Acacia catechu	Antiviral
Plumbago indica	Antibacterial
Allium sativum	Antifungal
Ailanthus sp., Cephaelis ipecacuanha	Antiprotozoal
Psidium gujava, Curcuma domestica	Antidiarrhoeal
Coleus forskohlii, Alium sativum	Hypotensive
Piper betle, Abrus precatorius	Astringent
Nandina domestica, Scutellaria baicalensis	Antiallergic

1.3 Medicinal plants used in Traditional Systems of Medicine

Traditional medicine is widespread through the world and it comprises of those practices based on beliefs that were in existence, often for hundreds of years, before the development and spread of modern scientific medicines and which are still in use today. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries.[15]

Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject of very intense pharmacological studies. This has been brought about by the acknowledgement of the value of medicinal plants as potential source of new compounds of therapeutic value and as source of new compounds in drug development.

Traditional medicine or ethno-medicine is a set of empirical practices embedded in the knowledge of a social group often transmitted orally from generation to generation with the intent to solve health problems. It is an alternative to Western medicine and is strongly linked to religious beliefs and practices of indigenous cultures. Medicinal plant lore or herbal medicine is a major component of traditional medicine.

The World Health Organization is giving considerable importance to these alternate medicine (as they act as alternative to allopathy) systems to provide Primary Health Care to millions of people in the developing countries. China developed the Chinese system of medicine, which is practiced in China, Singapore, Taiwan, Japan and other countries. In Indonesia, Jamu and in South Africa, Zulu systems of herbal medicine are practiced. The Unani or Tibb system was developed in the Middle Eastern Arab countries and is practiced in India and in many countries.

In India, Ayurveda (developed in North India), Siddha (developed in Tamil Nadu) and Nagarjuna (developed in Andhra Pradesh) systems of medicine were developed.Ayurveda is also practiced in Sri Lanka, Pakistan and Bangladesh also. Herbo-mineral is another traditional system used in India and other neighbouring countries.

Drugs (balms, oils, pills, tonics, paste etc) are manufactured and marketed in traditional systems. The major advantage of these systems is that they are within the reach of the people, particularly rural poor. These systems are claimed to be pollution free, eco-friendly and have minimal or no

harmful side effects. Also the Traditional systems of medicine continue to be widely practiced on many accounts such as population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material. This fact is well documented in the inventory of medicinal plants, listing over 20,000 species.

In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future.

Traditional system claims to cure the cause of the disease rather than the symptoms and is holistic in its approach. In this system a disease is conceived as an imbalance in the systems of the body and the treatment aims at restoring the balance in the various systems of the body.

1.4 Contributions of medicinal plants to modern medicine

The term medicine refers to a preparation or compound containing one or more drug(s) or therapeutic agent(s), which is used in the treatment, cure and mitigation of various diseases and external injuries of man and other animals. The preparation may also contain substances other than the drug(s). The drug(s) within the preparation is (are) the active therapeutic agent(s) that cures (cure) the disease or heals the wound or injury.

Plants have been providing the human being with the basic necessities of life, that is, food, fuel and shelter, from the very beginning of their existence, and for their continued living and sustenance on this earth. In addition, the medicinal plants, the ones that possessed some various

medicinal properties, have been serving them as the major sources of therapeutic agents for maintenance of their health. These medicinal plants were used by the early human beings, as are done now, in variety of forms, such as in the entire form, and as powders, pastes, juices, infusions and decoctions for the treatment of their diseases and ailments. These various converted forms of the medicinal plants may thus be very conveniently and genuinely called medicinal preparations or medicaments. This way, the medicinal plants formed an integral part of the health management practices and constituted imported items of medicines used in the treatment of diseases from the very early days human civilizations. And in the course of their uses as medicinal agents, the medicinal plants have contributed substantially to the gradual development of medicines to their present state.

Medicines that are used today are not definitely the same ones as those that were used in the ancient times or even in the recent past. Modification, improvement, sophistication and newer discoveries are continuously changing the type, quality, presentation and concept of medicinal preparations.

As therapeutic uses of plants continued with the progress of civilization and development of human knowledge, scientists endeavoured to isolate different chemical constituents from plants, put them in biological and pharmacological tests and thus have been able to identify and isolate therapeutically active compounds, which have been used to prepare modern medicines. In course of time, their synthetic analogues have also been prepared. In this way, ancient uses of *Datura* plants have led to the isolation of hyoscine, hyoscyamine, atropine and tigloidine, *Cinchona* bark to quinine and quinidine, *Rauvolfia serpentina* to reserpine and rescinnamine, *Digitalis purpurea* to digitoxin and digoxin, *Opium* to morphine and codeine, *Ergot* to ergotamine and ergometrine, *Senna* to sennosides, *Catharanthus roseus to vinblastine and vincristine*- mention a few.

Isolation of the natural analgesic drug morphine from Opium, the latex of *Papaver somniferum* capsules, in 1804 is probably the first most important example of natural drugs which plants have directly contributed to modern medicine. Isolation of other important plant derived drugs of modern medicine rapidly followed and many useful drugs have since been discovered and introduced into modern medicine. Drugs like strychnine from *Strychnos nuxvomica* (1817),

emetine from *Cephaelis ipecacuanha* (1817), caffeine from *Camellia sinensis* (1819), quinie from *Cinchona spp.* (1820) and colchinine from *Colchicum autumnale* (1820) constitute some example of such early drugs.[7]

The list of the plant derived medicinal substances occurring in modern medicine is very long now. About 100 such drugs of defined structures are in common use today throughout the world and about half of them are accepted as useful drugs in the industrialized counties. It is estimated that more than 25% (currently the figure is claimed to be 36%) of all prescription drugs used in the industrialized countries contain active principles that are still extracted from plants.

These include drugs like atropine, colchicines, deserpidine, digoxin, L-dopa, emetine, ephedrine, ergometrine, ergotamine, hyoscine, papaverinr, hyoscyamine, lanatosides, lobeline, morphine, narcotine, ouabain, papain, physostigmine, picrotoxin, pilocarpine, pseudo-ephedrine,quinidine, quinine, rescinnamine, reserpine, sennosides, sparteine, strophanthin, strychnine, theophylline, theobromine, vinblastine, vincristine, etc. Other plant derived drugs which are used widely but not generally in the western medicine include anabasine, andrographolide, arecoline, berberine, brucine, cannabinol, caphaeline, cocaine, curcumin, glycyrrhizin, hesperidine, hydrastine, nicotine, palmitine, quercetin, rutin, santonin, vincamine, yohimbine, etc.In addition to those, there are other plant derived chemical substances of known structures that are used as drugs or necessary components of many modern medicinal preparations. These include camphor, capaicin, cucalyptol, menthol, minor cardiac glycosides, various volatile oils, etc. These are only a few examples of a vast number of drugs that are derived from plants.

In addition to these natural drugs of modern medicine, plants have also contributed and are still contributing to the development of modern synthetic drugs and medicines in a number of ways as stated bellow.

•Novel structures of biologically active compounds, isolated from plant sources, often prompt the chemists to synthesize similar or biologically more potent semi-synthetic compounds.

•Synthetic drugs with similar or more potent therapeutic activity are often prepared by structural modification of the plant derived compounds with known biological activity.

•Various analogues and derivatives of plant constituents with similar or better pharmacological actions and therapeutic properties are often prepared by the chemists for use as potent drugs.

Surprisingly, this large quantity of modern drugs comes from less than 15% of the plants which are known to have been investigated pharmacologically out of the estimated 500000 species of higher plants growing on earth

Thus it is apparent that whatever progress science might have made in the field of medicine over the years, plants still remain the primary source of many important drugs used in modern medicine. Indeed, the potential of obtaining new drugs from plant sources is so great that thousands of substances of plant origin are now being studied for activity against such formidable foes as heart diseases, cancer and AIDs.[7]

Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Global estimates indicate that 80% of about 4 billion population can not afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material. This fact is well documented in the inventory of medicinal plants, listing over 20,000 species. In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still like drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increased use of plant drugs will be followed in the future.

LITERATURE REVIEW

2. Literature review

Auricularia auricula-judae has been the subject of research into possible medicinal applications. Experiments in the 1980s concluded that two glucans isolated from the species showed potent antitumour properties when used on mice artificially implanted with Sarcoma 180 tumours. This was despite the conclusion of earlier research indicating that, while aqueous extracts from several other fungal species had antitumour effects, extracts from *A. auricula-judae* did not. Further, research on genetically diabetic mice showed that a polysaccharide extracted from *A. auricula-judae* had a hypoglycemic effect; mice fed with food including the polysaccharide showed reduced plasma glucose, insulin, urinary glucose and food intake. Another chemical extracted from the species was an acidic polysaccharide (made up of mostly mannose, glucose, glucuronic acid and xylose) which showed anticoagulant properties. The article concluded that "the polysaccharides from these mushrooms may constitute a new source of compounds with action on coagulation, platelet aggregation and, perhaps, on thrombosis". Another study reported that the species may be effective in stopping platelet binding in vitro, with possible uses regarding hypercholesterolemia. Research has shown that *A. auricula-judae* can be used to lower cholesterol levels generally, and, in particular, is one of two fungi shown to reduce the level of bad cholesterol.[17]

A.auricula is primarily of interest as a functional food for the elderly, with polysaccharide extracts showing particular promise and having been developed as functional food additives for bread. Anti-inflammatory - *A. auricula* polysaccharides have anti-inflammatory activity, which correlates with *A. auricula*'s traditional use for soothing irritated or inflamed mucous membranes. Anti-oxidant - *A.auricula* extracts show strong anti-oxidant properties with a positive correlation between levels of phenols and anti-oxidant capacity. Anti-thromobotic - Polysaccharide extracts of *A. auricula* inhibit platelet aggregation and increase clotting times in-vitro and in-vivo. Its anticoagulant activity was due to catalysis of thrombin inhibition by antithrombin but not by heparin cofactor II. Anti-cholesterol - *A. auricula* polysaccharides have been shown to lower blood total cholesterol (TC), triglycerides and LDL levels and enhance the level of blood HDL, as well as HDL /TC and HDL/LDL ratios, at 5% of feed in rats suffering from hyperlipidemia .

Cardioprotective - Together with *A. auricula*'s general anti-oxidant properties, A. auricula polysaccharides shows a strong cardio protective effect, especially in aged mice, enhancing the activity of superoxide dismutase and reducing lipid peroxidation.[18]

On the other hand Water-soluble polysaccharide (AAP I-a) extracted from *Auricularia auricular* with assistance from ultrasonics, and purified by anion-exchange and gel-permeation chromatography. Additional further structural characteristics were determined from high-performance liquid chromatography/gel permeation chromatograph (HPLC/GPC), Fourier transform infrared (FT-IR) spectrometer and gas chromatography–mass spectrophotometer (GC–MS). AAP I-a is composed of l-rhamnose, l-arabinose, d-xylose, d-mannose, d-glucose, and d-galactose in a molar ratio of 0.2:2.6:0.4:3.6:1.0:0.4. After 35 days of AAP I-a oral administration (50, 100 and 200 mg/kg once a day), the AAP I-a significantly decreased the level of malondialdehyde (MDA) and increased superoxide dismutase (SOD) and glutathione (GSH) activities in mice where ageing is induced by d-galactose ($p < 0.05$). In conclusion, results indicated that AAP I-a possessed potent antioxidant activity.[19]

Auricularia auricula and hawthorn are well known for both traditional food and folk medicine. To develop a novel healthy functional diet (FD), the formulation of *A. auricula* polysaccharide (AAP) and hawthorn flavones were postulated in present study. In vitro, FD significantly increased the radical-scavenging effects and inhibited LDL oxidation, compared with AAP. In vivo, the hyperlipidemic mice, induced by cholesterol-enriched diet, were provided either 150, 300, 450 mg/kg day of FD or 300 mg/kg day of AAP for 12 weeks to evaluate their expected hypolipidemic activity. Compared with CED, both FD and AAP groups could lower serum TC and atherogenic index (AI), improve serum and hepatic antioxidant status. Especially, formulating with hawthorn could enhance hepatic antioxidant status in dose-dependent manner, compared with AAP. Thus, it can be concluded that FD may act as a potent hypolipidemic and powerful antioxidant formulation via improving serum lipid profile and inhibiting lipid peroxidation in mice.

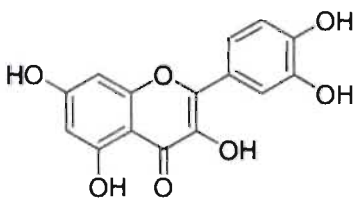
With the development of modern living standard, dyslipidemia has become one of major causes leading to antioxidant stress and atherosclerosis. It is of great significance to maintain the normal body functions by reducing the elevated serum cholesterol to an adequate level. In view of side

effects of recent therapies, it is urgent to produce certain health-promoting functional diet against dyslipidemia. Therefore, we studied on a novel functional formulation diet against hyperlipidemia, using the traditional both edible and medicinal herbs. The antioxidant and hypolipidemic properties are roundly evaluated in vitro and in vivo with the appropriate animal model. Our research also suggests the optimum dosage with potent nutritional property. It could provide the future practical application to produce this functional diet as an adjuvant dietetic food. Evaluation of antioxidative and hypolipidemic properties of a novel functional diet formulation of *Auricularia auricula* and Hawthorn.[20]

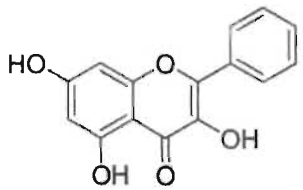
Antitumor activities of two (1 goes to 3)-beta-D-glucans, isolated from the fruiting body of *Auricularia auricula-judae* ("kikurage", an edible mushroom), and other branched polysaccharides containing a backbone chain of (1 goes to 2)-alpha-D-glucosidic or (1 goes to 3)-alpha-D-mannosidic linkage [and their corresponding (1 goes to 3)-D-glycans, derived by mild, Smith degradation] were compared. Among these polysaccharides, a water-soluble, branched (1 goes to 3)-beta-D-glucan (glucan I) of *A. auricula-judae* exhibited potent, inhibitory activity against implanted Sarcoma 180 solid tumor in mice. The alkali-insoluble, branched (1 goes to 3)-beta-D-glucan (glucan II), a major constituent of the fruiting body, showed essentially no inhibitory activity. When the latter glucan, having numerous branches attached, was modified by controlled, periodate oxidation, borohydride reduction, and mild, acid hydrolysis, the resulting, water-soluble, regraded glucan, having covalently linked polyhydroxy groups attached at O-6 of the (1 goes to 3)-linked D-glucosyl residues, exhibited potent antitumor activity. Further investigations using the glucan-polyalcohol indicated that the attachment of the polyhydroxy groups to the (1 goes to 3)-beta-D-glucan backbone may enhance the antitumor potency of the glucan. On the other hand, partial introduction of carboxymethyl groups into glucan II (d.s., 0.47--0.86), which altered the insolubility property, failed to enhance the antitumor activity. The interrelation between the antitumor activity and the structure of the branched (1 goes to 3)-beta-D-glucan is discussed, on the basis of methylation and ^{13}C-n.m.r. studies of the periodate-modified glucans.[21]

Polygonum hydropiper is a common species of wet, sunny sites, and is found to a lesser degree under partial canopy in swamps. Ten flavonoid compounds were isolated from the dried leave of

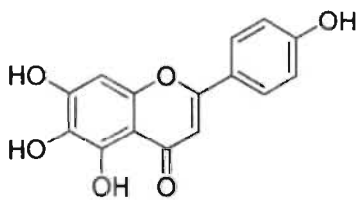
Polygonum hydropiper L. (Laksa leaves), and identified as 3-O-α-L -rhamnopyranosyloxy-3',4',5,7-tetrahydroxyflavone; 3-O-β-D -glucopyranosyloxy-4',5,7-trihydroxyflavone; 6-hydroxyapigenin; 6''-O-(3,4,5-trihydroxybenzoyl) 3-O-β-D -glucopyranosyloxy-3', 4', 5, 7-tetrahydroxyflavone; scutillarein; 6-hydroxyluteolin; 3',4',5,6,7-pentahydroxyflavone; 6-hydroxyluteolin-7-O-β-D -glucopyranoside; quercetin 3-O-β-D -glucuronside; 2''-O-(3,4,5-trihydroxybenzoyl) quercitrin; quercetin.



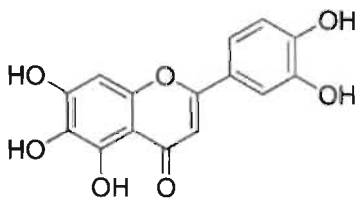
quercetin



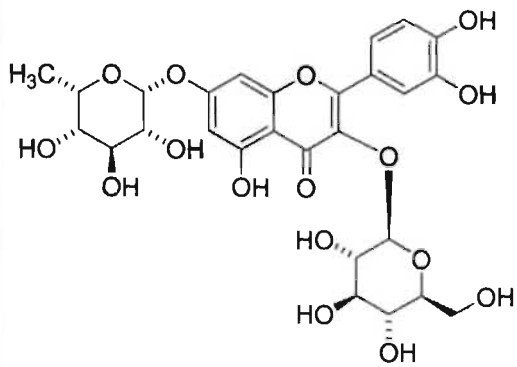
Galangin
(3,5,7-trihydroxyflavone)



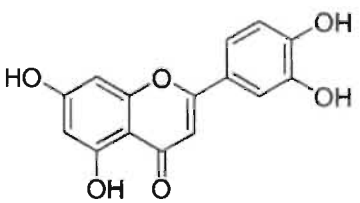
seutillarein



6-hydroxyluteolin



quercetin-3-O-β-glupyranosyl-7-O-α-rhamnopyranoside



3',4',5,7-tetrahydroxyflavone

Figure 1: Structure of quercetin; 3,5,-trihydroxyflavone; seutillarein; 6-hydroxyluteolin, quercetin-3-O-β-glupyranosyle-7-O-α-rhamnopyranoside; 3',4',5,7-tetrahydroxyflavon

Evaluation of the antioxidative activity, conducted in vitro, by using electron spin resonance (ESR) and ultraviolet visible (UV–vis) spectrophotometric assays, showed that these isolated flavonoids possess strong antioxidative capabilities. Measurement of the Trolox equivalent antioxidant capacity (TEAC) values, against ABTS (2,2′-azinobis(3-ethyl-benzo-thiazoline-6-sulphonic acid) radicals and phenyl-tert-butyl nitrone (PBN) azo initiator (AI) also showed strong anti-oxidative activity.

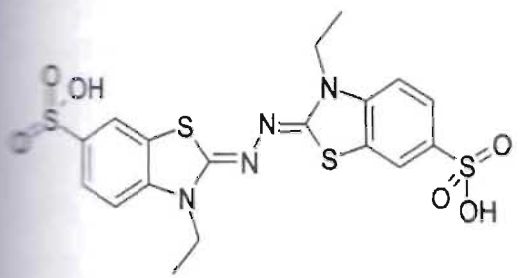


Figure 2 : 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS

The most powerful of the antioxidants was 2″-O-(3,4,5-trihydroxybenzoyl) quercitrin (galloyl quercitrin). A combination of two flavonoid compounds was tested for synergistic anti-oxidative capacity, but no significant improvement was observed.[22]

The antioxidant properties and the effect on nitric oxide (NO) production, in lipopolysaccharide-activated macrophages, of 12 traditional vegetables of the Malaysian Malays, including *Pithecellobium confertum*, *Averrhoa bilimbi*, *Portulaca oleracea*, *Solanum torvum*, *Solanum nigrum*, *Persicaria tenella*, *Cosmos caudatus*, *Pandanus amaryllifolius*, *Curcuma mangga*, *Ocimum basilicum*, *Anacardium occidentale* and *Melicope ptelefolia,* were investigated. Antioxidant activity of the methanolic extracts was evaluated by measuring the production of hydroperoxide and its degradation product (malonaldehyde) resulting from linoleic acid oxidation using ferric thiocyanate and thiobarbituric acid methods, respectively. Radical-scavenging potential was also evaluated using the 1,1-diphenyl-2-picrylhydrazyl radical. Griess assay was used to assess NO-inhibitory activity of the extracts. All species, except *P. confertum*, *S. torvum* and *P. amaryllifolius*, showed antioxidant activity. *M. ptelefolia*, *P. oleracea* and *P. tenella* showed in vitro activity on NO inhibition in murine peritoneal macrophages, whereas other plants showed no significant activity.[23]

Polygonum hydropiper is a widely grown weed in the north-eastern states of India. In the present study, estrogenic effects of the crude root extract (CRE) of Polygonum hydropiper on uterine protein was tested in ovary-intact and ovariectomized (OVX) female albino rats. The methanolic crude extract of Polygonum hydropiper was given to adult ovary-intact and OVX female albino rat in a dose of 1000 mg/kg bodyweight per day. Oral administration of the CRE was carried out for a period of 12 days from the onset of proestrus and for a similar period of time for the OVX female. To study the estrogenic effect, OVX females were subcutaneously injected with 0.1 µg of estradiol-17β (E2). E2 was injected at the interval of 24 h for a total period of 72 h. The uterine protein of ovary-intact and OVX females was studied by single dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis. The results showed similar protein bands in OVX females treated with E2 and CRE. In addition, treatment with CRE stimulated expression of more proteins in the uterus. The results showed that CRE of Polygonum hydropiper mimics the effect of estradiol-17β in the uterine protein profiles of adult female albino rats.[24]

Phenolic compounds, such as phenolic acids, flavonoids, flavonoid glycosides, and resveratrol, were chromatographed on thin-layers of silica in various eluent systems. Systems with the highest selectivity were chosen. Also, reversed-phase HPLC systems were optimized for the separation of investigated compounds and most the selective system was applied for the separation of two phenolic extracts - *Polygonum avicularis* and *Polygonum hydropiper*. Partly separated fractions were evaporated and dissolved in methanol. Fractions with partly separated phenolic compounds were spotted onto the silica layer and developed in an appropriate eluent system. After drying, the plate was derivatised with the Naturstoff reagent and videoscanned. Several phenolic compounds were identified, according to their retention coefficients in both systems.[25]

A study was conducted to determine the antibacterial and antifungal activities of *Polygonum hydropiper (L.)* root extract on chloroform against both bacteria and fungi using the disc diffusion method. The extract showed significant antibacterial activities against four gram-positive (*Bacillus subtilis*, *Bacillus megaterium*, *Stapphylococcus aureus* and *Enterobacter aerogenes*) and four gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella sonnei*) bacteria. The minimum inhibitory concentration (MIC) values against

these bacteria ranged from 16 to 64 µg/ml. The antifungal activities were found strong against six fungi (*Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Mucopus oryzae* and *Tricophyton rubrum*). It can be used in the folk medicine at different parts of the world to treat many diseases including bacterial and fungal infections.[26]

The plant also activities of several medicinal plant species including has some insecticidal properties.[27] The plant also possesses bitter, stimulant, tonic, diuretic, carminative, anthelmintic, emmeragogue, haemostatic and lithotripter properties.

The whole plant, either on its own or mixed with other herbs, is decocted and used in a wide range of ailments including diarrhoea, dyspepsia, itching skin, excessive menstrual bleeding and hemorrhoids . This species is of economic interest of its wide ranging pharmacological activity and one of the major constraints in utilizing natural populations is the existence of plant to plant chemovariability. That is why, there is an increasing awareness in people for the use of this herbal medicinal plant day by day.

Leaves of *Hibiscus rosa-sinensis* are used as emollient, anodyne, and laxative in Ayurveda. In South Asian traditional medicine, variousparts of the plant is used in the preparation of a variety of foods. The flowers have been reported in the ancient Indian medicinal literature to have beneficial effects in heart diseases, mainly in ischemic disease and used in folklore medicine as demulscent, emollient, refrigerant, aphrodisiac, brain tonic and cardio tonic. A decoction of flowers is also useful in bronchial catarrh, menorrhagia, and fertility control. The extracts showed hair growth potential, anticonvulsive activity and hypoglycemic activity. Pharmacopoeial Standardization of Hibiscus rosa- sinensis Linn.

Traditionally bark of the plants is used for ant fertility and is used for the control of dysfunctional citerine bleeding and as an oral contraceptive. The flowers are used as anti asthmatic agents. Many chemical compounds like Cyandin, Quercetin, Hentriacontane, Calcium oxalate, Thiamine, Riboflavin niacin and ascorbic acids have been isolated. The present study has been designed to determine the role of flower and leaf extracts of *Hibiscus rosa- sinensis* was screened for potential antibacterial activity against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtillis*) and two Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*.

H. rosa-sinensis belongs to the family, Malvaceae, the roots are cylindrical, the leaves are simple ovate or ovatelanceolate, the flowers are pedicillate, actinomorphic, pentamerous and complete. As a traditional medicine, the fresh juice of the flower is used to treat gonorrhea, the powdered root is used in the treatment of menorrhagia and the infusion of the petals is used as a refrigerant drink in fever. This work investigates the effect of this plant on tissue lipid profile.[28]

Hibiscus (Malvaceae) is a genus of herbs, shrubs and trees. Its 250 species are widely distributed in tropical and subtropical regions of the world and are reported to posses various medicinal properties viz; antitumor, antihypertensive, antioxidant, antiammonemic .About 40 species are found in India. *Hibiscus rosasinensis Linn* is a native of China and is a potentmedicinal plant. It is a common Indian garden perennial shurb .and often planted as a hedge or fence plant.

Traditionally this drug is attributed to antifertility activity in Ayurvedic literature .The flowers have been reported to posses anti-implantation and antispermatogenic activities .The petroleum ether extracts of the leaves and flowers have been shown to potentiate hair growth in vivo and in vitro.Leaves and flowers also possess hypoglycemic activity .The mucilage of the leaf has anticomplementary activity .The extracts of *Hibiscus rosa-sinensis* have also been shown a protective effect against the tumor promotion stage of cancer development .The anthocynidin from the petals of the plant have protective effect against carbon tetra chloride-induced acute liver damage .The present investigation is the first ever study undertaken to find the unexplored anti-implantation and uterotropic activity of the roots of *Hibiscus rosa- sinensis*, using ethanol, being highly polar, so as to extract the maximum phytoconstituents present in the roots.[29]

The flowers and leaves of *H.rosa-sinensis* contain substantial quantities of flavonoids which are associated with antioxidant, fever-reducing (antipyretic),pain-relieving (analgesic) and spasm-inhibiting (spasmolytic) activities. The decoction of the leaves is used in the treatment of fevers.[30] India and the flower has soothing properties which are used to relieve menstrual cramps and relax spasms and general cramping. Presence of flavonoids in the leaves of *H. rosa-sinensis* is another reason why it is used to treat inflammations.[31] *H.rosa-sinensis* also reduces blood pressure and cholesterol level in blood. This is due to the existence of saponins in *H.rosa-sinensis*. Saponins bind to cholesterol to form insoluble complexes and excreted via the bile. This prevents cholesterol reabsorption and results in a reduction of serum cholesterol. Saponins have

been found to be potentially useful for the treatment of hypercholesterolemia which suggests that saponins might be acting by interfering with intestinal absorption of cholesterol.[32] *H. rosa-sinensis* also aids in wound healing.[33]

and this can be explained due to the existence of tannins and terpenoids which plays important role in promoting wound healing.[34] The presence of terpenoids also contributes to the reason why *H.rosasinensis* also is used to soothe irritated tissues and the mucous membranes that line the respiratory tract, which eases hacking coughs and other respiratory ailments.[31]

Clerodendrum viscosum Linn plant is common throughout the plains of India. Various parts of the plant have been used by tribes in colic, scorpion string, snake bite, tumour and certain skin diseases. also used in Indian folk medicine as in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation,burning sensation and epilepsy. Fresh juice of the leaves has been used as vermifuge and in treatment of malaria.*Clerodendrum infortunatum* leaves on preliminary chemical analysis are found to contain saponin, clerodin (a bitter diterpene).[42] and some enzymes. Leaves also contain a fixed oil which consists of Glycerides of Lenoleic, oleic, stearic and lignoceric acid.[43] Previous phytochemical investigation of the plant revealed the presence of alkyl sterols and 2,- (3,4-dehydroxyphenyl)ethanol-1-O-α-2-rhamnopyranosyl-(1→3)-β-D (4-O-caffeoyl) glycolpyr -anoside (acteoside) in this plant. Traditionally leaves reported to posses tranquilizing effect, insecticidal properties and laid over grain to ward off insects.

Ethanolic extracts of leaves of *Clerodendrum infortunatum Linn, Argyreia nervosa* and *Vitex negundo* were subjected to preliminary screening for antimicrobial activity .All ethanolic extracts exhibited significant anti-microbial activity comparable to the standard drug tetracycline. Ethanolic extract of *Clerodendrum infortunatum* shows more inhibitory zone as compared to ethanolic extracts *Argyreia nervosa* of and *Vitex negundo*. The mixture of all three extracts together in equal concentration shows more inhibitory zone as compared to other extracts.[35]

Three successive extracts of *Clerodendrum infortunatum* L. leaves have been studied for their potential as antioxidants in 1,1-diphenyl-2-picrylhydrazyl (DPPH) model. The scavenging activity of ethanol extract was found to be high when compared to petroleum ether and

chloroform extracts. Hence, it was selected to evaluate the beneficial properties using in vitro and in vivo models. The antioxidant and its protective effects against CCl_4 induced oxidative stress in rats were significantly high. Further, to validate the traditional therapeutic claim, wound healing activity of the plant extracts was also carried out. Among the three extracts tested the petroleum ether and ethanol extracts exhibited a significant response. The presence of high antioxidant and pharmacological properties correlates to the total phenolic contents in the plant Clerodendrum infortunatum L.[36]

Hepatoprotective potential of methanolic extract of *Clerodendrum infortunatum* Linn. (MECI), which was widely used in Indian indigenous system of medicine, was studied against carbon tetrachloride induced hepatotoxicity in rats. Methanol extract at the dose of 100 and 200 mg/kg was administered daily along with carbon tetrachloride once in 72 hours for 14 days. The study was evaluated by assaying the serum biochemical parameters glutamate pyruvate transaminase (ALT), glutamate oxaloacetate transaminase (AST), alkaline phosphatase (ALP), bilirubin and total protein. Malondialdehyde (MDA) level, as well as reduced glutathione (GSH) content and catalase activity (CAT) was determined to explain the possible mechanism of the activity. The substantially elevated serum enzymatic levels of AST, ALT, ALP and total bilirubin were restored towards normalization significantly by the extract. Silymarin was used as standard reference and exhibited significant hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in rats. MDA concentration was decreased, while the liver antioxidative enzyme activity was elevated in all the MECI treated rats. All the results were compared with standard drug silymarin. In addition, histopathology of liver tissue was investigated to observe the morphological changes, showed the reduction of fatty degeneration and liver necrosis. The results of this study revealed that methanol extract of *C. infortunatum* has moderate hepatoprotective activity. This effect may be due to the ability of the extract to inhibit lipid peroxidation and increase in the anti-oxidant enzymatic activity.[37]

INTRODUCTION OF PLANTS

3. Introduction of plants

3.1 *Auricularia auricular*

Auricularia auricular known as the Ear Mushroom is a species of edible auriculariales mushroom found worldwide. Distinguished by its noticeably ear-like shape and brown coloration, it grows upon wood, especially elder. The fruit body of *A. auricular* is normally 3 to 8 centimeters (1.2 to 3.1 in) across, but can be as much as 12 centimeters (4.7 in). Mainly it is seen in late summer and autumn. The species has a tough, gelatinous, elastic texture when fresh, but it dries hard and brittle. The outer surface is a bright reddish-tan-brown with a purplish hint, often covered in tiny, downy hairs of a grey color. It can be smooth, as is typical of younger specimens, or undulating with folds and wrinkles. The color becomes darker with age. The inner surface is a lighter grey-brown in color and smooth. It is sometimes wrinkled, again with folds and wrinkles, and may have "veins", making it appear even more ear-like.

Polysaccharides:20%

Bioavailable: 200mg/g

Proteins : 37.5-50%

Moisture : 5%

Ash : 5%-7%

Remainder : Free sugars, amino acids.

Tot. aerobic count : Less than 10,000 CFU/g

E. Coli : None detectable

Salmonella : None detectable.

Heavy metals :

Pb less than 2.0 ppm Cd less than 1.0 ppm

Hg less than 0.5 ppm

Pesticides : Complying to 90/642/EEC standard.

Figure 3: *Auricularia auricular* in cultivation

Figure 4:*Auricularia auricular* in wood

Etymology:
Latin ***auricularia*** (adj) = earlike, from ***auricula***, diminutive for ***auris***=ear - ***auricula Judae*** = Judas'ear

Synonyms: Auricularia auricula; Hirneola auricula (Latin, from *hirnea* =a vessel for liquids, especially wine)

Common names: Jew's Ear; Wood Ear; Tree Ear.

Botanical Name : *Auricularia auricular*

Family : Auriculariaceae

3.2 *Polygonum hydropiper.L*

Polygonum hydropiper is a common species of wet, sunny sites, and is found to a lesser degree under partial canopy in swamps. Important characters in the key include the bristles along the edge of the ochrea (a sheath at each node) and glands on the tepals.

Figure 5 : *polygonum hydropiper* in wet place.

***Polygonum*:** derived from the Greek words *polys*, "many," and *gonu*, "knee or joint," hence "many joints" because of the thickened joints on the stem.

Hydropiper*: *hydro for "water;" ***piper*** for "pepper".

This plant erect to spreading, annual, 6"-24" tall, mostly smooth, often reddish, peppery tasting forb; stems nodes with short hairs, upper ones swelling with closed flowers; flowers are greenish-pink, 4-or 5-parted, petals and petal-like sepals connected at the base; inflorescence thin, usually unbroken clusters arching or nodding near the tip; blooms at July-Sept. Fruits are brown or black, dull, dry seed. Leaves of this plant alternate, narrowly lance-like, mostly less than 1" wide.

Leaves and stems - raw or cooked. They can also be made into an acid peppery condiment. They are very hot. The leaves contain about 7.5% protein, 1.9% fat, 8% carbohydrate, 2% ash. The leaves are said to contain rutin. Seed - raw or cooked. It is rather small and fiddly to utilize. The seed is used as a condiment - a pepper substitute. The sprouted seeds or young seedlings can be used as a garnish or added to salads, they are commonly sold in Japanese markets. They are very hot.

Botanical name: Polygonum hydropiper

Local name: Bishkatali

Synonyms: Persicaria fastigiatoramosa, Persicaria hydropiper, Polygonum fastigiatoramosum, Polygonum maximowiczii

Family: Polygonaceae.

3.3 *Hibiscus rosa-sinensis*

Hibiscus rosa-sinensis, known colloquially as the Chinese hibiscus, China rose and shoe flower, is an evergreen flowering shrub native to East Asia. It is widely grown as an ornamental plant throughout the tropics and subtropics. The flowers are large, generally red in the original varieties, and firm, but generally lack any scent. Numerous varieties, cultivars, and hybrids are available, with flower colors ranging from white through yellow and orange to scarlet and shades of pink, with both single and double sets of petals. The common traits are: Short-lived showy five petal flowers, most only last for a day or two, with prominent staminal columns and lobed leaves with proper stems.[38]

Figure 6 : *Hibiscus rosa-sinensis*

Hibiscus plants are among the showiest of flowering shrubs, often reaching 30 feet in nature. Glossy foliage varies somewhat in size and texture depending on variety. Normally Blooming Time of hibiscus at Summer and Flowers are 4-8 inches wide, may be single or double. *Hibiscus rosa-sinensis* need a well-drained compost consisting of 2 parts peat moss to 2 parts loam to 1 part sand. The plants need full sun to partial shade with intermediate to warm temperatures. Water the plants freely during the growing season. Fertilize the plants twice monthly with a balanced fertilizer from April through September. To keep mature plants growing vigorously, prune out about 1/3 of the old wood in spring. Pinching out tips of stems in spring and summer

increases flower production. They may be grown outside in zone 7, but should be treated as annuals. *Hibiscus rosa-sinensis* are propagated by cuttings, layering or grafting in spring. They can also be grown from seeds, but usually do not come true from seed.[39]

Botanical name: *Hibicus rosa- sinensis*

Local name: Rakhta jaba

Family : Malvaceae

3.4 ***Clerodendrum viscosum***

Figure 7 : *Clerodendrum viscosum* Linn

Clerodendrum viscosum is a flowering shrub or small tree, and is so named because of its rather ugly leaf. The stem is eresct, 0.5–4 m high, with no branches and produce circular leaves with 6 inch diameter. Leaves are simple, opposite; both surfaces sparsely villous-pubes-cent, elliptic, broadly elliptic, ovate or elongate ovate, 3.5–20 cm wide, 6–25 cm long, dentate, inflorescence in terminal, peduncled, few-flowered cyme; flowers white with purplish pink or dull-purple throat, pubescent. Fruit berry, globose, turned bluish-black or black when ripe, enclosed in the red accrescent fruiting-calyx. The stem is hollow and the leaves are 6-8 inch (15–20 cm) long, borne in whorls of four on very short petioles. The inflorescence is huge, consisting of many tubular snow white flowers in a terminal cluster up to 2 ft (0.6 m) long. The tubes of the flowers are about 4 inch (10 cm) long and droop downward, and the expanded corollas are about 2 inch (5 cm) across. The fruits are attractive dark metallic blue drupes, about a half inch in diameter.

Fruit usually with 4 dry nutlets and the seeds may be with or without endosperm. It flowers from April to August.[40]

The leaves and roots of *C. viscosum* are used as herbal remedy for alopecia, asthma, cough, diarrhoea, rheumatism, fever and skin diseases. It is also known to have hepato-protective and antimicrobial activities. The roots and bark of stem of this plant prepared as decoction and given in the dose of 60-80 ml twice daily for respiratory diseases, fever, periodic fever, cough, bronchial asthma, etc. The leaves are ground well and applied externally to induce ripenning of ulcers and swellings. A paste of leaves and roots are applied externally over skin diseases especially fungal infections and alopecia. Fresh leaves are given for diarrhoea, liver disorders and headache.[4]

Botanical Name*: Cleroderdrum viscosum*

Local Name: Bhait, Bhant in India, Ghentu in Bengali, Bhania in Oriya

Synonym : *Clerodendrum infortunatum* non L.

Family: Verbenaceae

MATERIALS & METHODS

4. Methods And Materials

4.1 Collection of plant material & Extraction

4.1.1 Materials for Extraction

1. Methanol
2. Beaker
3. Funnel
4. Glass rod
5. Ethyl acetate
6. Electric oven
7. Blender machine
8. Filter paper
9. Cotton

4.1.2 Selection of Plants Part

The fresh leaf and stem part of the plant *Polygonum hydropiper* and leaf part of *Hibiscus rosa-sinensis & Clerodendrum viscosum* was selected.

4.1.3 Plant collection and Identification

Ear mushroom (*Auricularia auricular)* was collected from National Mushroom Development & Extension Centre , Sobhanbag, Saver, Dhaka-1340. This organization locally cultivate the mushroom.

Bishkatali (*Polygonum hydropiper*) was collected from wet place like pond side of Haluaghat, Mymensingh, Dhaka. Vaita (*Clerodendrum viscosum)* and Rakhta jaba (*Hibiscus viscosum)* were collected from land side of Haluaghat ,Mymensingh, Dhaka. Bishkatali semi-aquatic in nature. All these plant samples were identified by a taxonomist of Bangladesh National Herbarium. Accession numbers were assigned to these collected plant samples by the Bangladesh National Herbarium.

Bishkatali (*Polygonum hydropiper)* DACB Accession No. 35512, Rakhta jaba (*Hibiscus viscosum)* DACB Accession No. 35481*),* Vaita (*Clerodendrum viscosum)* DACB Accession No. 35484).

4.1.4 Drying and Pulverization

The fresh leaf and stem of the plant of *Polygonum hydropiper* and only fresh leaf of *Clerodendrum viscosum & Hibiscus viscosum* were washed with water to remove adhering dirt and then cut into small pieces, sun dried for 4 days . After complete drying, the entire portions were pulverized into a coarse powder with help of a blender machine and were stored in an airtight container for further use.

4.1.5 Preparation of Plant extract

•Cold extraction

Auricularia auricular was then sun-dried for two weeks. Then the dried part was ground into fine powder by using a blender machine. Then about 80 gram powder was taken in a separate conical flasks and with 100 ml of methanol was added into the each conical flask. It was kept at room temperature for 3 days. After 3 days later, the methanol extract of mushroom part was filtered 3 times by filter paper.

•Hot Extraction

Leaves and bark of *Polygonum hydropiper* were separed from the plant. Leaves and bark were then sun-dried for two weeks. Then the dried leaves and bark of plants were ground into fine powder by using a blender. Then the powder of leaf placed into a nylon bag. 100 mg of leaf powder extract with 300ml of ethyl acetate in soxlet machine. It requires about 3-4 hrs. Then same sample is extract by methanol. In this way bark of the plant also extracted. The amount of the bark was 80 gm.

The filtered solution of mushroom powder-methanol and *Polygonum hydropiper* leaf and stem part of methanol and ethyl acetate solution was placed into a 1000 ml round bottle flask.

Figure 8 : Rotary Evaporator (IKA RV05 basic, Biometra)

Then with the help of rotary evaporator, the methanol extract solution of plant leaves were allowed to evporated to give gummy or semi-solid material about 2.3 gm of *Auricularia auricular*, 9.8 gm of *Polygonum hydropiper* leaf ethyl acetate,7.5 gm of *Polygonum hydropiper* leaf methanol,1gm of *Polygonum hydropiper* stem ethyl acetate and 3.5gm of *Polygonum hydropiper* stem methanol. Their amount were weighed by anlalytical balance.The resulting plant's leaf extracts were mostly semi-solid in nature. They were stored in small beakers for further analysis.

Other two plants *Hibiscus rosa-sinensis & Clerodendrum viscosum* were extract in a small amount about 10 gm. Only leaves of the plants were collected and wash then dried into the sun light. Then the dried leaves of plants were ground into fine powder by using a blender machine. Then about 10 gram powder of each plant leaves were stored in two sparate conical flasks and soaked with 50 ml of methanol was added into the each conical flask. They were kept at room temperature for 3 days. After 3 days later, the methanol extract of plant leaves were filtered 3 times by filter paper. This was done for both plant samples. The filtered solution of plant leaf powder-methanol solution was kept for 7 days. After that the solvent evaporate naturally and get about 0.38g of *Hibiscus rosa-sinensis* leaf and 0.37gm of *Clerodendrum viscosum* leaf extract.

4.2 Brine Shrimp Lethality Bioassay

4.2.1 Introduction

Brine shrimp lethality bioassay is rapid general bioassay for the bioactive compound of the natural and synthetic origin.[42]Bioactive compounds are almost always toxic at high dose. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Brine shrimp lethality bioassay is a bench top bioassay method for evaluating anticancer, anti-microbial and pharmacological activities of natural products and it is a recent development in the bioassay for the bioactive compounds. By this method, natural product extracts, fractions as well as the pure compounds can be tested for their biosphere-activity. Here, in vivo lethality in a simple zoological organism (Brine shrimp nauplii) is used as a convenient monitor for screening and in the discovery of new bioactive natural products. This bioassay is indicative cytotoxicity and a wide range of pharmacological activity of the compounds.[43]Brine shrimp lethality bioassay stands superior to other cytotoxicity testing procedures because it is a rapid method utilizing only 24 hours, inexpensive and requires no special equipment. Unlike other methods, it does not require animal serum. Furthermore, it utilizes a large number of organisms for statistical validation and a relatively small amount of sample.

4.2.2 Principle

Brine shrimp eggs are hatched in simulated sea water to get nauplii. Test samples are prepared by dissolving in DMSO, desired concentration of the test samples are prepared. The nauplii are the counted by visible inspection and are taken in test tubes containing 4 ml of simulated seawater. Then the sample of different concentration are added to the premarked test tubes through a micropipette. The test tubes are the left for 24 hours and nauplii are counted again to find out the cytotoxicity of the sample.[42]

4.2.3 Materials And Reagents

1. Artemia salina leach (brine shrimp eggs)
2. Small tank with perforated dividing dam to hatch the shrimp
3. Sea salt
4. Lamp to attract shrimps
5. Pipettes
6. Micropipettes and vials
7. Magnifying glass
8. Tests Samples of Experimental Plants
9. DMSO (Dimethyl sulfoxide)

4.2.4 Experimental procedures

4.2.4.1 Preparation of simulated seawater

Since the lethality test involves the culture of brine shrimp nauplii, that is the nauplii should be grown in sea water. Sea water contains 3.8% of sodium chloride & hence 3.8% salt solution should be needed for this purpose. Accordingly, 3.8% of sodium chloride was made by dissolving 38 g of NaCl in one liter of sterilized distilled water that contain basic PH and then filtered off to get clear solution.

4.2.4.2 Hatching of brine shrimp eggs

Artemia salina Leach (brine shrimp eggs) collected from the pet shop was used as the test organism. Simulated seawater was taken in the small tank and the shrimp eggs (1.5 g L–1) were added to one side of the tank and this side was covered. The shrimps were allowed for 24 hrs to hatch and matured as nauplii (larvae). Constant oxygen supply was carried out during the hatching time. The hatched shrimps were attracted to the lamp light on the other side of the divided tank through the perforated dam. These nauplii were taken for this bioassay.

4.2.4.3 Preparation of Sample

Seven different concentrations of sample extracts were made, using Dimethyl sulfoxide (DMSO, 1.0ml) in triplicates (1000, 100, 10µg/ml).

4.2.4.4 Preparation of controls

For negative control, 25 µl and 50 µl of DMSO was added to each of three pre marked test tubes containing 4 ml of simulated sea water and 10 shrimp nauplii. This was considered invalid if the negative control showed a rapid mortality rate and therefore conducted again.

4.2.4.5 Application of brine shrimp nauplii

With the help of the Pasteur pipette 10 living nauplii were added to each of the vials containing 3 mL of simulated sea-water. A magnifying glass was used for convenient counting of the nauplii. For the control test of each vial, one vial containing the same volume of DMSO plus sea water up to 4 mL was used and 10 living nauplii were also taken in these control vials. Precautions were taken to avoid eggs during taking the shrimps in the vials.

4.2.4.6 Counting of nauplii

After 24 h of incubation, the test tubes were observed using a magnifying glass and the number of survived nauplii in each vial were counted and recorded. From this data, the percentage of mortality of the nauplii was calculated for each concentration of the sample.

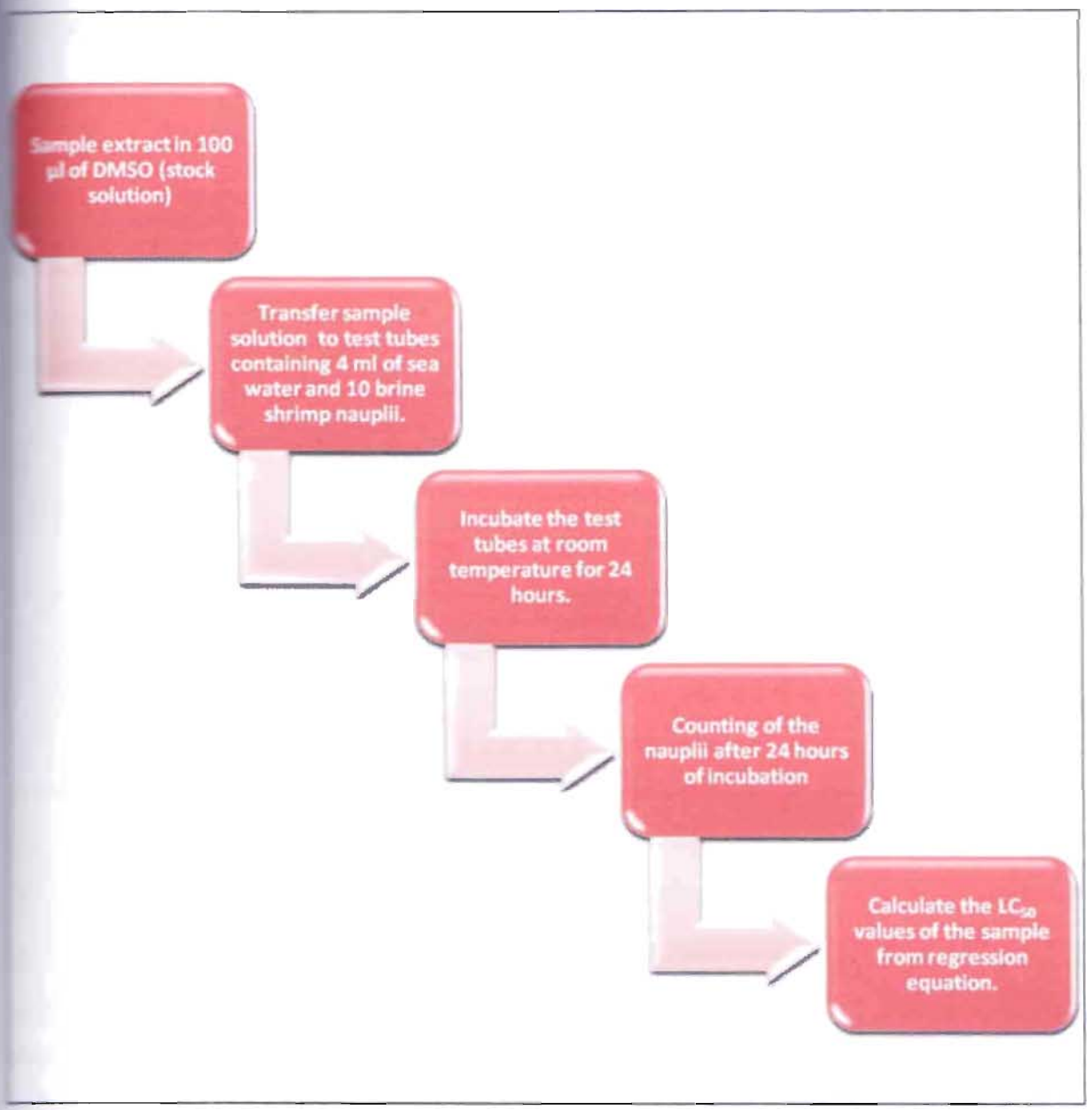
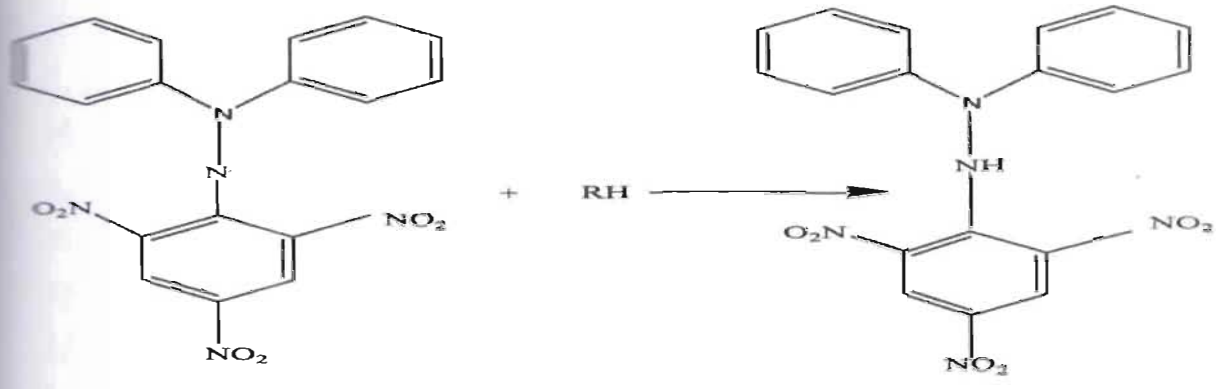


Figure 9 : Schematic diagram representing steps of Lethality Bioassay on Brine Shrimp nauplii.

4.3 DPPH free radical scavenging Method

4.3.1 Introduction

Numerous methods are available for determining the presence and quantification of the degree of antioxidant activity present in the plants extracts. Most of these methods make use of a color reaction and indicator to assess the degree of antioxidant activity. DPPH assay is a qualitative indicator of a free radical scavenging activity. DPPH is reduced from a stable free radical that is purple in color to diphenyl picryl hydrazine that is yellow, in the presence of an antioxidant.



*DPPH (Oxidized form)

DPPH (Reduced form)

4.3.2 Principle

DPPH was used to evaluate the free radical scavenging activity (antioxidant potential) of various compound and medicinal plants. So DPPH is used as reagents. DPPH is a stable free radicals potentially reactive with substance able to donate a Hydrogen atom and thus useful to assess compounds antioxidant activity of specific compounds of extracts. Because of its odd electron, DPPH has a strong absorption band at 517 nm. Since this electron becomes paired in presence of a free radical scavenger, the absorption decreases stoichiometrically with respect to the number of electrons taken up. This change in absorbance produced by this reaction has been widely used to test the ability of several molecules to act as free radical scavengers.

The absorbance is taken by UV-VISIBLE spectrophotometer and methanol is taken as solvent. Ascorbic acid is taken as standard.

4.3.3 Materials

1. DPPH(1,1- diphenyl-2-picrylhydrazyl)
2. n –Hexane
3. Ethyl acetate
4. Dichloromethane
5. Chloroform
6. Ethanol
7. Methanol
8. UV-VISIBLE spectrophotometer (UV-mini SHIMADZU)
9. UV-VISIBLE Lamp
10. Beaker (100 & 250 ml)
11. Test tube
12. Pipette (10 ml)
13. Micropipette (10-100µl)
14. Pasteur pipette
15. Capillary tube
16. Merck TLC Plate precoated si6oF254
17. TLC Tank
18. Filter Paper
19. Amber reagent bottle
20. Electronic balance

4.3.4 Procedure

4.3.4.1 Screening of Antioxidant Activity

Antioxidant activities of different parts of the test plant were determined on the basis of their scavenging potential of the stable DPPH free radical in both qualitative and quantitative assay.

4.3.4.2 Preparation of DPPH solution

DPPH solution was prepared by dissolving 1.6 mg in 4.0 ml of methanol to get 0.04% w\v solution.[44] This solution is kept in dark place.

4.3.4.3 Qualitative Assay

A suitably diluted stock solutions were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of n-Hexane & Ethyl Acetate (1:1) to resolve polar and non-polar components of the extracts. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved band was observed for 10 minutes and the color changes (yellow on purple background) were noted.[45]

4.3.4.4 Quantitative Assay

•Preparation of stock solution

400 mg of extract was weighed accurately and dissolved in 40 ml of methanol. The concentration of the stock solution made is 400µg\ml

•Preparation of test solutions

Five test vial were taken and marked as 20,40,60,80 and 100. Now, 2 ml,4 ml,6ml,8ml , 10 ml of sample solution were taken from stock solution and added to the marked test tubes respectively. After that required quantity of methanol was to each vial to get the final solution of 10 ml. Now, concentrations of the test solutions in different test tubes were 20µg\ml, 40 µg\ml, 60 µg\ml, 80 µg\ml and 100 µg\ml respectively.A blank solution contained only 10 ml of methanol

•Introduction of DPPH solution to the sample solution

100µl of DPPH solution was added to each of the six test tubes in dark place and was shaken by rotation. This mixture was kept at room temperature for 50 minutes in dark place.

Determination of antioxidant activity

After 20 minutes of incubation, absorbance of the reaction mixture was taken at 517 nm using UV-VISIBLE spectrophotometer. 60 Percentage inhibition (%I) was calculated using the following equation:

Percentage inhibition=$\{1-A_{sample}\backslash A_{blank}\}x100$.

Where, A_{sample}= absorbance of the sample at 517nm.

A_{blank}= absorbance of the sample at 517nm.

4.4 Antimicrobial Screening

4.4.1 Introduction

Any chemical substance or biological agent that destroys or suppresses the growth of microorganism is called antimicrobial agent. Antimicrobial screening of a crude extract or pure compound isolated from natural sources is essential to ascertain its activity against various types of pathogenic organisms.

Antimicrobial activity of any plant can be detected by observing the growth response of various microorganisms to the plant extract, which is placed in contact with them. In general, antimicrobial screening is undertaken in two phases:

1. A primary qualitative assay to detect the presence or absence of activity.
2. A secondary assay which quantifies the relative potency, expressed as Minimum inhibitory Concentration (MIC) value of a pure compound, an important method in the development of new antimicrobial compound.

The primary method can be done in three ways as

a) Diffusion method
b) Dilution method
c) Bioautographic method

Among these methods, the disc diffusion method is widely acceptable for the preliminary evaluation of antimicrobial activity. Disc diffusion is essentially a qualitative or semi-qualitative test indicating the sensitivity or resistance of microorganism to the test materials. However, no distinction between bacteriostatic or bacteriosidal activity can be made by this method.[46]

4.4.2 Principle

Diffusion is based on the ability of a drug to diffuse from a confined source through the nutrient agar medium and create concentration gradient. If agar is seeded with a sensitive organism, a zone of inhibition will result where the concentration exceeds the minimum concentration (MIC) for that particular organism.[47]

In this method, measured amount of the test samples are dissolved in definite volumes of solvent to give solutions of known concentration (µg\ml). Then sterile filter paper discs (5 mm in

diameter) are impregnated with known amounts of the test substances and dried. The dried discs are placed on plates (petri dishes) containing suitable medium (nutrient agar) seeded with the test organisms. These plates are then kept at low temperature (4°C) for 24 hours to allow maximum diffusion. A number of events take place simultaneously which include-

- ✓ The dried discs absorb water from agar medium and the material under test is dissolved.
- ✓ The test material diffuses from the discs to the surrounding medium according to the physical law that controls the diffusion of molecules through agar gel.
- ✓ There is a gradual change of test material concentration on the agar surrounding each disc.

The plates are the kept in an incubator (37°C) for 12-18 hours to allow the growth of microorganism. If the test material has antimicrobial activity, it will inhibit the growth of the microorganism, giving a clear, distinct zone called "Zone of Inhibition". The antimicrobial activity of the test agent is determined in term of millimeter by measuring the diameter of the zone of inhibition. The greater the zone of inhibition, the greater the activity of the test material against the test organism.

4.4.3 Materials For Microbiological Investigation

Apparatus and reagents:

- Filter paper discs
- petri dishes
- Inoculating Loop
- Sterile cotton
- Sterile Forceps
- sprit Burner

4.4.4 Collection of Microorganism samples

Salmonella typhi, Pseudomous aureus, Staphylococcus aureus, Escherichia coli, Shigella dysenteriae, Secchromyces cerevisiae, Candida albicans. These seven microorganism samples were collected from the microbiology lab, Department of Pharmacy, East West University. Then I cultured all these bacterial strains into different petri dishes.

4.4.5 Preparation of Bacterial Cultures

At first, all petri dishes were wrapped with paper and placed inside a Autoclave machine for sterilization. After 1 hour, when the sterilization was finished, then Petri dishes were replaced from the autoclave machine.

After that, petri dishes were washed with detergent soap properly. After washing, allowed them to dry. In the mean time, seven tubes for micropipettes, several micropipette tips were washed and properly dried.

4.4.6 Preparation of Agar Solution

A standard rule is, 2.8 gram Nutrient Agar is require for 100 ml Agar Solution.This preparation was kept in a 400 ml glass container.

4.4.7 Sterilization

All the seven Petri dishes were labeled with microorganism names respectively. All the labeled Petri dishes and container of Agar solution was placed inside a autoclave machine for sterilization.

4.4.8 Streaking and Innoculation

After the sterilization by autoclave, sterile Petri dishes and Agar Solution glass container were kept under UV laminar air flow to prevent contamination. The prepared Agar solution was poured into each of the ten Petri dishes in a way so that each Petri dish gets 15-20 ml agar medium. Agar medium was dispensed into each Petri dishes to get 3-4 mm depth of agar media in each Petri dishes. After pouring the agar medium, all Petri dishes were kept in room temperature so that agar medium can become properly solidified.

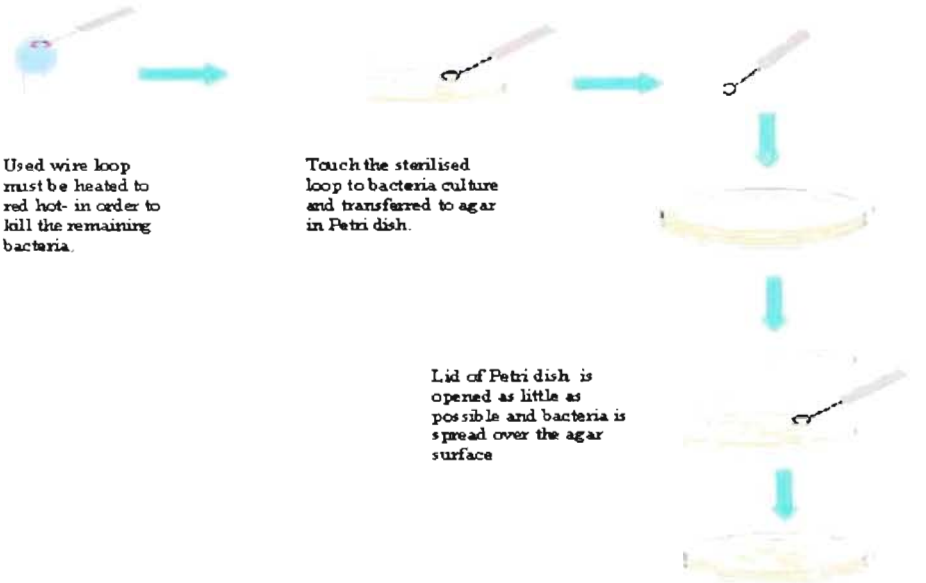


Figure 10 : Inoculation process by sterile loop

The Inoculating loop was soaked in ethanol and also placed under UV laminar air flow. All the sample microorganism were contained in separate closed test tubes. To inoculate the sample bacteria to the agar medium of Petri dish, The bacterial sample test tube cap was opened and the top was exposed to the flame of the gas burner. Before inoculation, the inoculating loop was exposed to the flame of burner to ignite the ethanol in it. The sterile inoculating loop was touched inside the bacterial sample test tube slightly, then the inoculator with the microorganism on it, was streaked across the surface of agar medium of plate leaving the microorganism on the agar medium and a "zigzag" pattern was drawn. In this manner, one by one, all the agar plates were inoculated with microorganism according to their respective labels.

4.4.9 Incubation

Then all the prepared agar plates with respective microorganisms were placed inside a bacteriological incubator for 18 hours to allow the growth of pure fresh culture of microorganism in each of the Petri dishes.

4.4.10 Preservation of cultured microorganisms

After 18 hours incubation, all the petri dishes with respective microorganism cultures were removed from Incubator and then were kept in a refrigerator for further use in in-vitro antimicrobial test.

4.4.11 In-vitro Antimicrobial Screening of Plant's leaf extracts

I had used Kirby Bauer disc diffusion method. This is the most widely used method in a basic research laboratories. By following this method, I had performed the following steps for the antimicrobial test.

4.4.12 Preparation of dried filter paper discs

Whatman filter paper no. 1 was used to prepare discs approximately 6 mm in diameter by a Hole puncher. These discs were also also sterilized by autoclave.

4.4.13 Sterilization

At first seven petri dishes were washed and dried properly. Seven tubes for saline suspension, a glass container containing 0.9% sodium chloride isotonic saline solution, a glass container containing 400ml agar preparation, several tips of micropipette, seven glass rod were placed inside a autoclave machine for sterilization. Before giving them into autoclave machine, dried discs of filter paper, glass rod micropipette tips, tubes were placed into a beaker covered with foil paper. After approximately 1 hour, they were sterilized and then was placed under laminar air flow to prevent any contamination.

Agar preparation was poured into each of the 10 plates so that each plate can achieve 15-20 ml of agar medium. After that, these petri dishes containing agar medium were allowed to get solidified.

4.4.14 Inoculam Preparation

followed Direct colony suspension method. According to this method, the prepared 0.9 % Sodium Chloride saline preparation was poured into 7 tubes in a way that each tube may be able to contain 1ml of saline. These tubes were labeled into the 7 respective sample microorganism names. Then, microorganism culture plates that was stored in refrigerator was brought. With a sterile loop microorganism colonies from the bacterial culture plate was isolated and dipped into tube containing saline suspension. Then the tube cap was closed and the microorganism inoculum was mixed properly by a vortex mixer. The suspension was adjusted to match the 0.5 McFarland turbidity standard, using saline and a vortex mixer. This process was done for each of the 7 test microorganisms.

4.4.15 Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension (tube containing suspension of microorganism), a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This helped to remove excess inoculum from the swab.
2. The dried surface of the agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed. This process was performed for each of the 7 microorganisms. All the plates were labeled with name of microorganisms, dose of the drug disc, standard drug dose at backside.
3. The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks. Extremes in inoculum density was avoided.

4.4.16 Application of plant extract to the prepared filter paper Discs

1. With a spatula, plant extract was taken into very sample amount and weighed by analytical balanced. The weighed amount of extract was placed into a small beaker and required of methanol was added to make a dilute solution of plant extract.
2. A 20 µL micropipette was adjusted to 3 µL so that a single press on the micropipette will deliver a concentration 250 µg of plant leaf extract on a battery of filter paper discs. In the same way that micropipette was also adjusted to 6 µL so that when I press the micropipette was eject a concentration 500 µg of plant leaf extract on a battery of filter paper discs.
3. By this process,7 discs of battery were treated with 250 µg dose of plant leaf extract and another 7 discs of battery were treated with 500 µg dose of plant leaf extract using micropipette.
4. All the treated discs were allowed to dry before application of discs to inoculated agar plates. All these procedures were performed under Laminar Air Flow.

4.4.17 Application of Discs to Inoculated Agar Plates

1. The battery of antimicrobial discs (discs that were treated with plant l extract before) was dispensed onto the surface of the inoculated agar plate by forceps. Each disc was pressed down to ensure complete contact with the agar surface. The discs were dispensed individually.
2. Total 4 discs were dispense in a single agar plate. In a agar plate, two discs containing 250µg and 500 µg plant's extract, one dried disc, one standard Amoxicillin disc was dispensed on the surface of the agar medium. Blank disc was used as a negative control. Standard Amoxicillin disc was used as positive control of this antimicrobial test. All these procedures were performed under Laminar Air Flow to prevent any kind of contamination.
3. The plates were inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied.

4.4.18 Incubation

Ideal incubation period for bacterial growth is 24 hours. The incubator was set to 35°C. After the previously mentioned step, all the plates were placed inside the incubator.

4.4.19 Estimating zone of inhibition and Interpreting Results

After 16 to 18 hours of incubation, each plate was examined. The resulting zones of inhibition was uniformly circular and there was confluent lawn of growth. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding calipers or a ruler, which was held on the back of the inverted petri dish plate. The ruler was sterilized to prevent transmission of bacteria.

Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, was ignored. The result of the test was interpreted by a table or graph.

4.5. Thin layer chromatography

4.5.1 Introduction

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose (blotter paper). This layer of adsorbent is known as the stationary phase.[65] After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved.

Thin layer chromatography can be used to:

- Monitor the progress of a reaction
- Identify compounds present in a given substance
- Determine the purity of a substance

4.5.2 Materials

1. n –Hexane
2. Ethyl acetate
3. Dichloromethane
4. Chloroform
5. Methanol
6. UV-VISIBLE Lamp
7. Beaker
8. Pipette (10 ml)
9. Capillary tube
10. Merck TLC Plate precoated si6oF254
11. TLC Tank
12. Filter Paper

4.5.3 Thin Layer Chromatography

I had performed Normal Phase TLC for TLC of the leaf extract. In case of normal phase TLC, the stationary phase is more polar than mobile phase.

4.5.4 Stationary Phase

I had used commercially available prepared silica gel coated TLC plate

4.5.5 Preparation of mobile phase

Hexane : Ethyl acetate = 3 : 1 this ratio was used to prepare a 20 ml of mobile phase. 10 Hexane, 10 ml Ethyl acetate and was mixed together to prepare a solution termed as mobile phase. A filter paper was cut in size resemble to TLC tank wall size. This filter paper was set to the wall of the TLC tank. So that filter paper can act as support for the TLC plate. Then the prepared mobile was poured into a TLC tank and tank opening was closed. This is very significant to allow the saturation of the environment inside the TLC tank.

4.5.6 Preparing the chromatogram

A straight line was drawn with pencil which is 0.5-1 cm from the bottom of the plate. Another straight line was drawn just near upper edge of TLC plate.

4.5.7 Spotting the TLC plate

Very little amount of sample was taken then I added 3 drops dichloromethane and 3 drops methanol to make dilute solution of the sample. This prepared dilute solution of sample was taken up by a capillary tube. With the capillary tube containing diluted sample, I just had touched the on a point of the straight line of the TLC plate. This was done 1 times in a same point so that a very sharp and small spot can be obtained.

4.5.8 Developing the plate

After the previously mentioned step, TLC plate was placed inside the TLC tank 30 degree angulary with the tank wall. The tank opening was closed. Care was taken to ensure that the mobile phase did not cross the marked line at lower edge of the plate. Then the sample spot was

moving upward along with the mobile phase. The solvent was allowed to travel from the lower marked straight line to the upper marked straight line of the TLC plate. When the solvent reached the upper line, TLC plate was removed from the tank and dried under normal temperature.

4.5.9 Visualization

TLC plate was then placed under a UV lamp. It is the most widely used method. Under UV light, I had observed fluorescence and different colours of spots. Spots were circled with a pencil for further record.

4.5.10 Detection

45 ml methanol and 5 ml sulfuric acid was mixed to prepare a 10% sulfuric acid solution. The TLC plate was soaked in this 10% sulfuric acid solution. TLC plate was then allowed to dry. I had checked the TLC plate for any change in the colours of the spots.TLC plate was then exposed on a hot plate for charring and observed for any colour change.

RESULTS & DISCUSSION

5 Result And Discussion

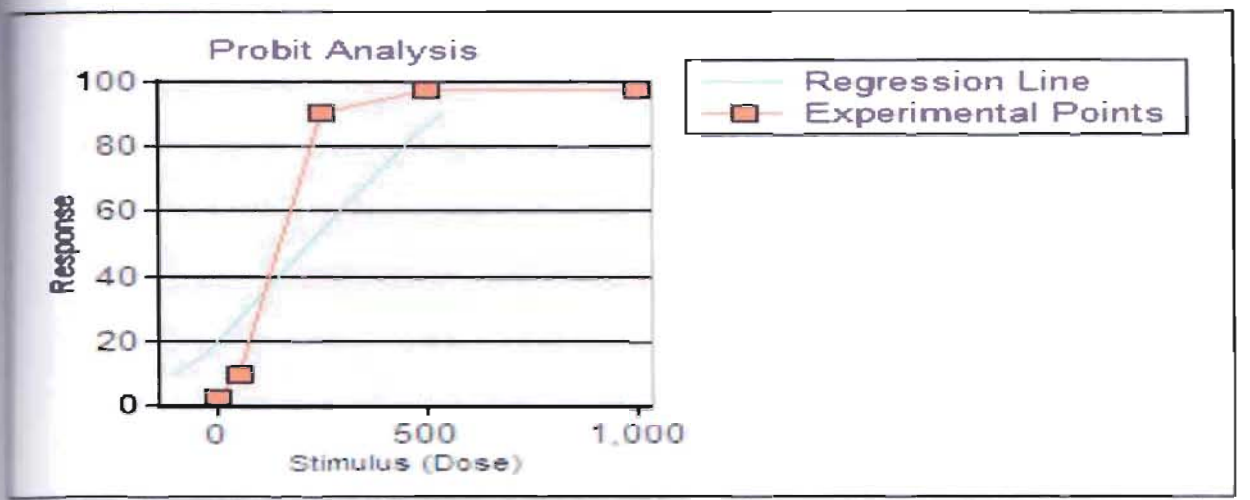
5.1 Result and Discussion of Brine Shrimp Lethality Bioassay

Brine Shrimp Lethality Bioassay is a recent development in the bioassay for bioactive compounds which indicates toxicity as well as a wide range of pharmacological activities (i.e. anticancer, antiviral, insecticidal & pesticidal etc.) of the compounds. Bioactive compounds are almost always toxic in high dose. Pharmacology is simply toxicology at lower doses and toxicology is simply pharmacology at higher doses. Natural plant extracts and pure compounds can be tested for their bioactivity by this method. Here, in-vivo lethality in a simple zoological organism (Brine Shrimp nauplii) is used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products.

The brine shrimp results are interpreted as follows: LC50 <1.0 μ g/ml-highly toxic; LC50 1.0-10 μg/ml toxic; LC50 10-30 μg/ml; moderately toxic; LC50 >30<100 μg/ml mildly toxic and LC50 >100ug/ml as non toxic.[48s]

Table 2: Larvicidal effect of crude methanol extract of *Auricularia auricular* on Artemisia salina

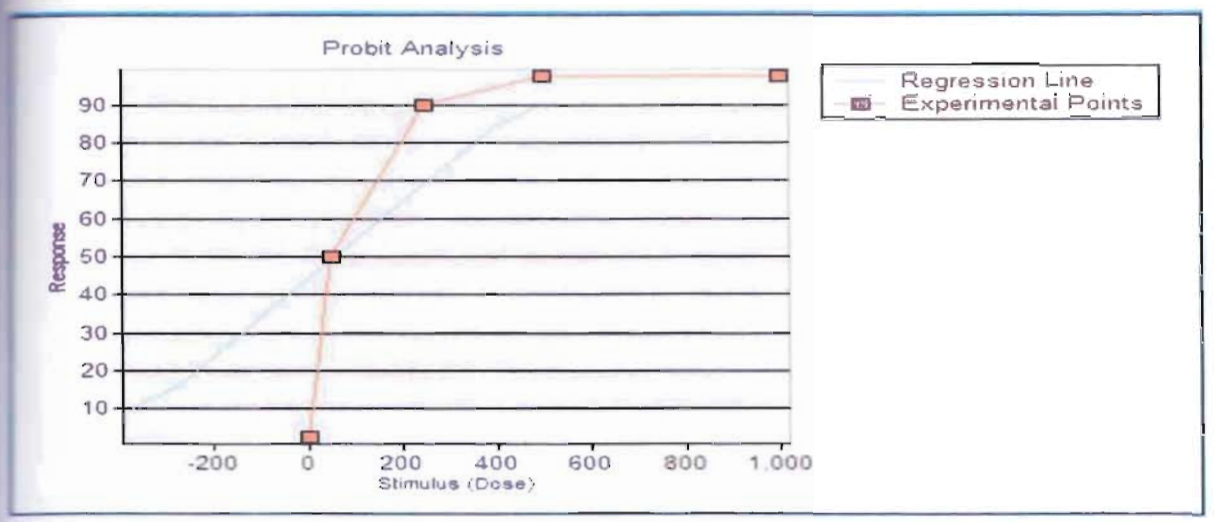
Expt	Conc of extract μg/ml	Initial Num. of Larvae	Total Dead			Total Survivor in			%Mortality	Actual %	Probit	LC_{50} μg/ml
			1	2	3	1	2	3				
control	0	10	0	0	0	10	10	10	0	0.025	3.0396	
1	50	10	2	1	1	8	9	9	1	0.1	3.7183	
2	250	10	9	9	8	1	1	2	9	0.9	6.2817	219.1179
3	500	10	10	10	10	0	0	0	10	0.975	6.9604	
4	1000	10	10	10	10	0	0	0	10	0.975	6.9604	



Graph 1: Larvicidal effect of crude methanol extract of *Auricularia auricular* on Artemisia salina

Table 3: Larvicidal effect of crude ethyl acetate *Polygonum hydropiper* leaf on Artemisia salina

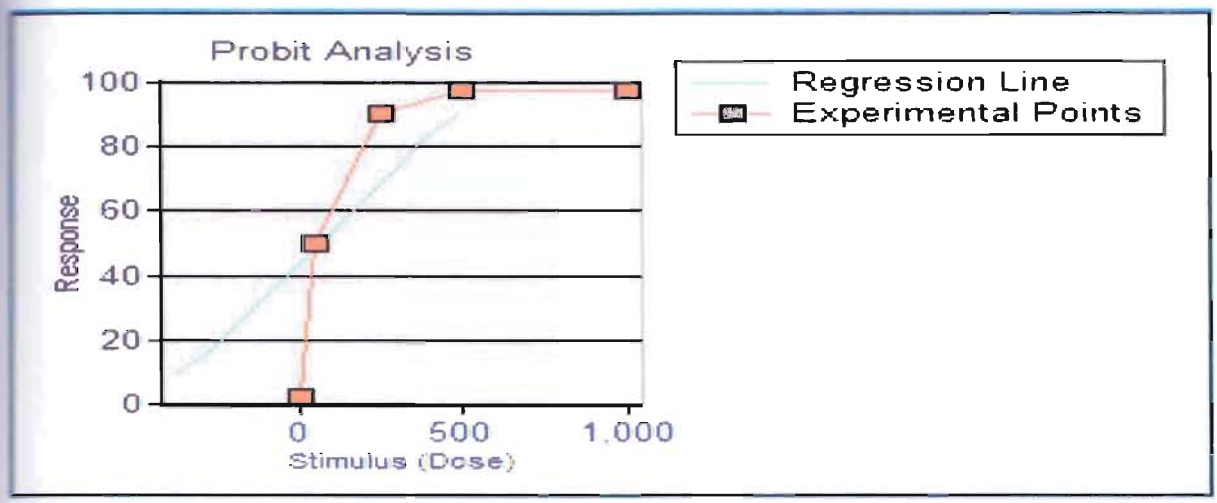
Expt	Conc of extract µg/ml	Initial Num. of Larvae	Total Dead			Total Survivor in			%Mortality	Actual %	Probit	LC_{50} µg/ml
			1	2	3	1	2	3				
control	0	10	0	0	0	10	10	10	0	0.025	3.0396	
1	50	10	6	5	5	4	5	5	5	0.05	5	60.8694
2	250	10	10	9	10	0	1	0	10	0.9	6.2817	
3	500	10	9	10	9	1	o	1	9	0.975	6.9604	
4	1000	10	10	10	10	0	0	0	10	0.975	6.9604	



Ghaph 2 :Larvicidal effect of crude ethyl acetate extract *Polygonum hydropiper* leaf on Artemisia salina

Table 4: Larvicidal effect of crude methanolic extract *Polygonum hydropiper* leaf on Artemisia salina

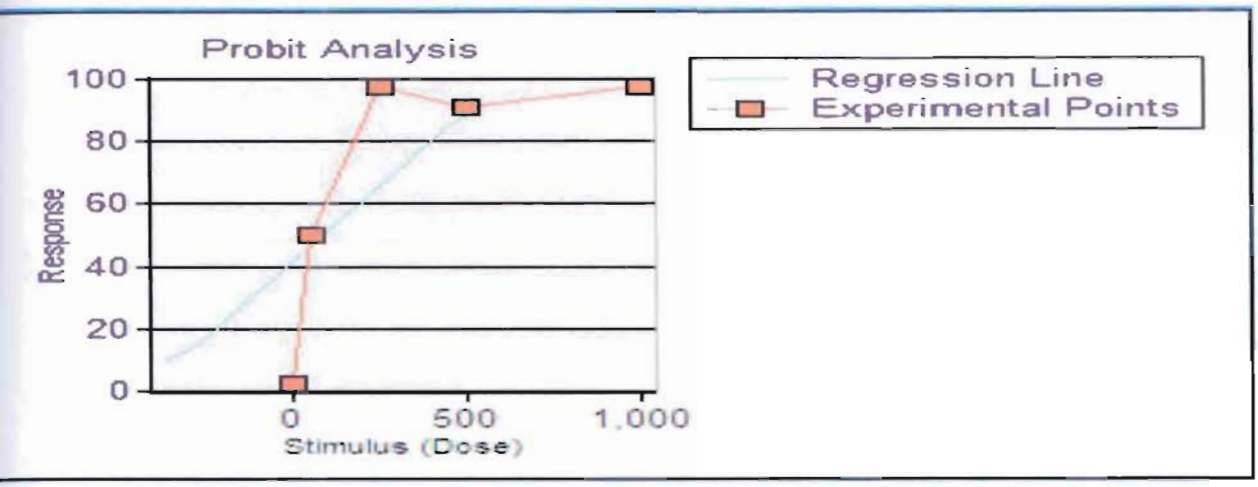
Expt	Conc of extract µg/ml	Initial Num. of Larvae	Total Dead			Total Survivor in			%Mortality	Actual %	Probit	LC_{50} µg/ml
			1	2	3	1	2	3				
control	0	10	0	0	0	10	10	10	0	0.025	3.0396	
1	50	10	6	5	5	4	5	5	5	0.05	5	
2	250	10	9	10	9	1	0	1	9	0.9	6.2817	61.5213
3	500	10	10	10	10	0	0	0	10	0.9722	6.9149	
4	1000	10	10	10	10	0	0	0	10	0.975	6.9604	



Graph 3: Larvicidal effect of crude methanolic extract *Polygonum hydropiper* leaf on Artemisia salina

Table 5: Larvicidal effect of crude ethyl acetate extract *Polygonum hydropiper* stem on Artemisia salina

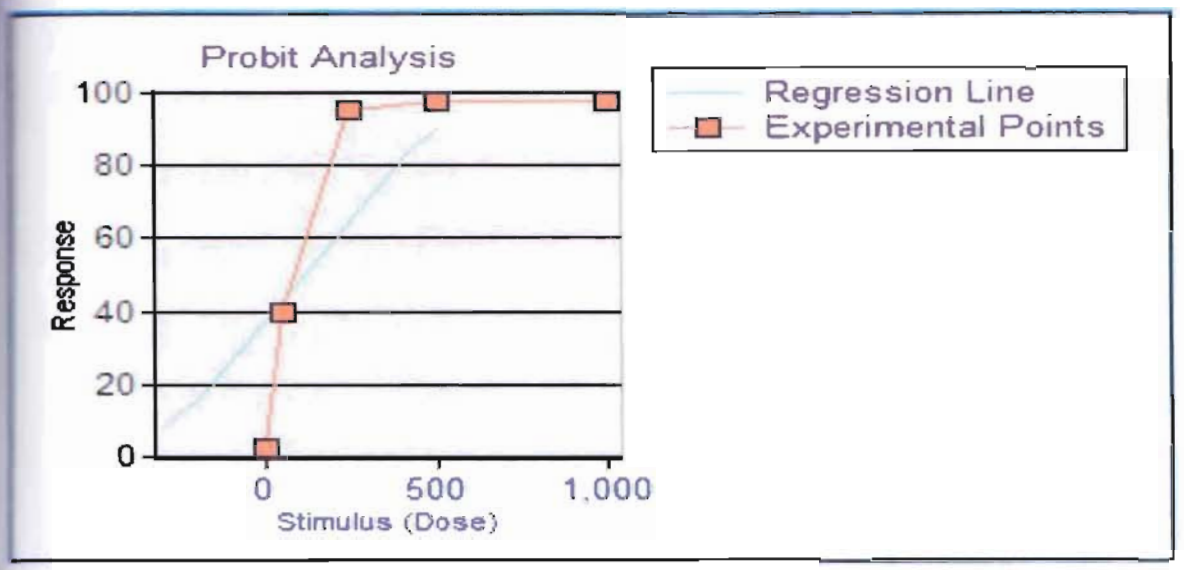
Expt	Conc of extract µg/ml	Initial Num. of Larvae	Total Dead			Total Survivor in			%Mortality	Actual %	Probit	LC_{50} µg/ml
			1	2	3	1	2	3				
control	0	10	0	0	0	10	10	10	0	0.025	3.0396	
1	50	10	5	4	8	5	6	2	5	0.5	5	
2	250	10	10	10	10	0	0	0	10	0.975	6.9604	87.8351
3	500	10	10	10	10	0	0	0	10	0.9091	6.3354	
4	1000	10	10	10	10	0	0	0	10	0.975	6.9604	



Graph 4: Larvicidal effect of crude ethyl acetate extract *Polygonum hydropiper* stem on Artemisia salina

Table 6 :Larvicidal effect of crude methanol extract *Polygonum hydropiper* stem on Artemisia salina

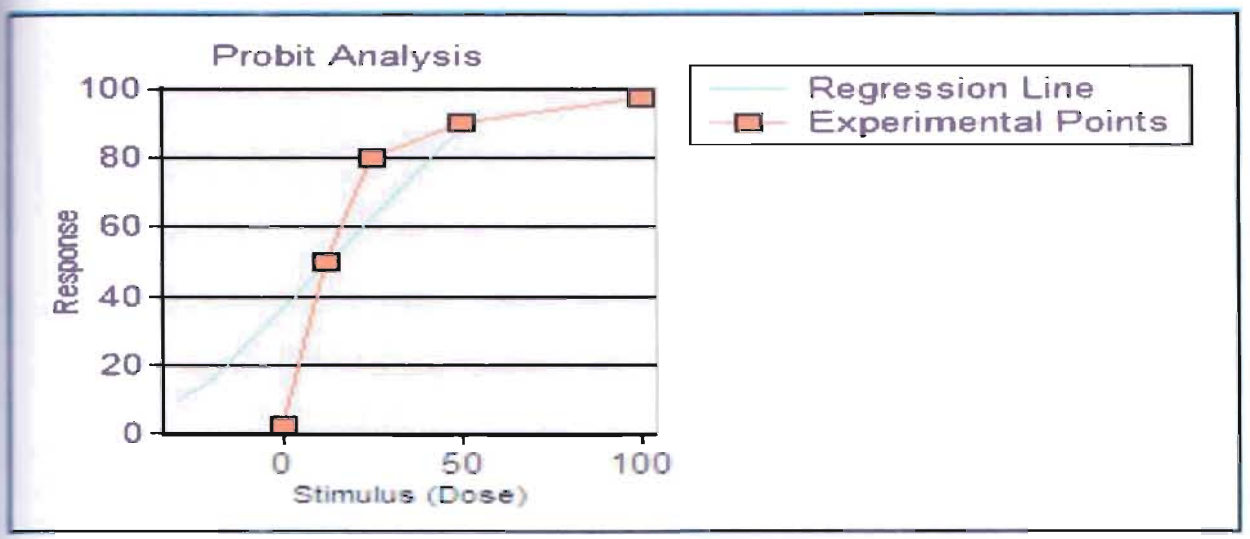
Expt	Conc of extract µg/ml	Initial Num. of Larvae	Total Dead			Total Survivor in			%Mortality	Actual %	Probit	LC_{50} µg/ml
			1	2	3	1	2	3				
control	0	10	0	0	0	10	10	10	0	0.025	3.0396	
1	50	10	5	6	4	5	4	6	5	0.4	4.7471	
2	250	10	5	5	5	5	5	5	5	0.95	6.6451	115.058
3	500	10	10	10	10	0	0	0	10	0.975	6.9604	
4	1000	10	10	10	10	0	0	0	10	0.975	6.9604	



Graph 5: Larvicidal effect of crude methanol extract *Polygonum hydropiper* stem on Artemisia salina

Table 7 : Larvicidal effect of crude methanol extract *Clerodendrum viscosum* leaf on Artemisia salina

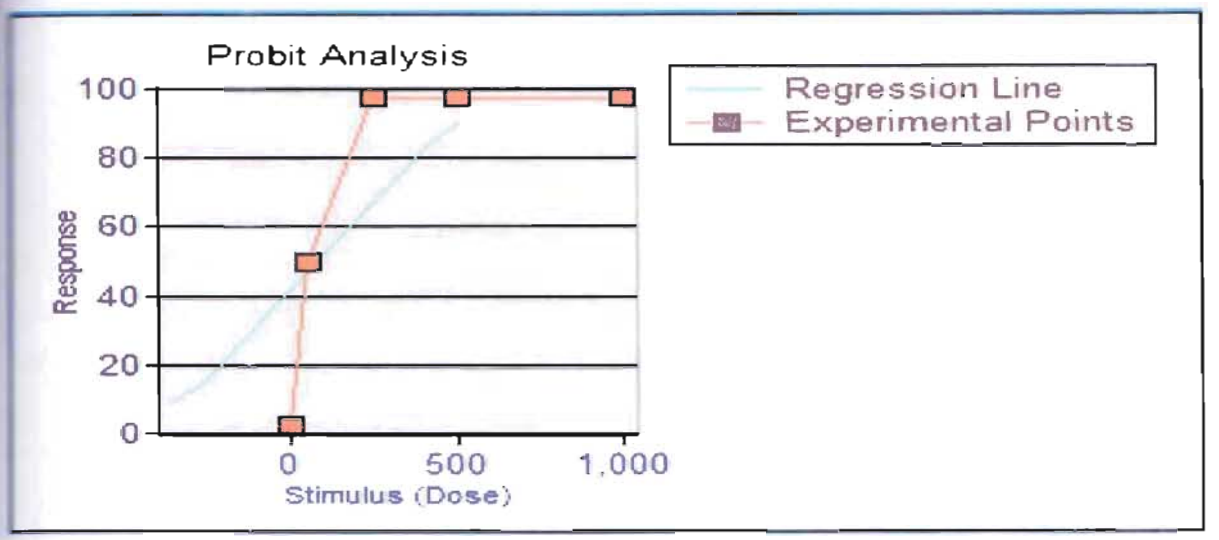
Expt	Conc of extract µg/ml	Initial Num. of Larvae	Total Dead			Total Survivor in			%Mortality	Actual %	Probit	LC_{50} µg/ml
			1	2	3	1	2	3				
control	0	10	0	0	0	10	10	10	0	0.025	3.0396	
1	12.5	10	5	6	4	5	4	6	5	0.5	5	
2	25	10	8	7	8	2	3	2	8	0.8	5.8415	13.1855
3	50	10	10	10	9	0	0	1	10	0.9	6.2817	
4	100	10	10	10	10	0	0	0	10	0.975	6.9604	



Graph 6: Larvicidal effect of crude methanol extract *Clerodendrum viscosum* leaf on Artemisia salina

Table 8 : Larvicidal effect of crude methanol extract *Hibiscus rosa-sinensis* leaf on Artemisia salina

Expt	Conc of extract µg/ml	Initial Num. of Larvae	Total Dead			Total Survivor in			%Mortality	Actual %	Probit	LC_{50} µg/ml
			1	2	3	1	2	3				
control	0	10	0	0	0	10	10	10	0	0.025	3.0396	
1	50	10	5	6	5	5	4	5	5	o.5	5	
2	250	10	10	10	10	0	0	0	10	0.975	6.9604	79.2687
3	500	10	10	10	10	0	0	0	10	0.975	6.9604	
4	1000	10	10	10	10	0	0	0	10	0.975	6.9604	



Graph 7: Larvicidal effect of crude methanol extract *Hibiscus rosa-sinensis* leaf on Artemisia salina.

By using Biostate 2008 software I measured the value of LC50. My test sample were *A*uricularia auricular, *Polygonum hydropiper, Clerodendrum viscosum & Hibiscus rosa-sinensis*. LC50 value of *A*uricularia auricular, *Polygonum hydropiper* leaf ethyl acetate, *Polygonum hydropiper* leaf methanol, *Polygonum hydropiper* stem ethyl acetate, *Polygonum hydropiper* stem methanol, *Clerodendrum viscosum & Hibiscus rosa-sinensis* were 219µg/ml, 60.869µg/ml, 61.5213µg/ml, 87.835µg/ml, 115.058µg/ml, 13.1855µg/ml & 79.2687µg/ml respectively.

From my test result I can determined that *Clerodendrum viscosum* is moderately toxic, *Polygonum hydropiper* leaf ethyl acetate, *Polygonum hydropiper* leaf methanol & *Hibiscus rosa-sinensis* are mildly toxic. *A*uricularia auricular & *Polygonum hydropiper* stem methanol are non toxic.

5.2 Results of DPPH Free Radical Scavenging Method

It is well known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. DPPH analysis is one of the tests used to prove the ability of the components of the ginger extract to act as donors of hydrogen atoms 68 The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance.

5.2.1 Results of Qualitative assay

The color changes (yellow on purple background) on the TLC plates were observed due to the bleaching of DPPH by the resolved bands.

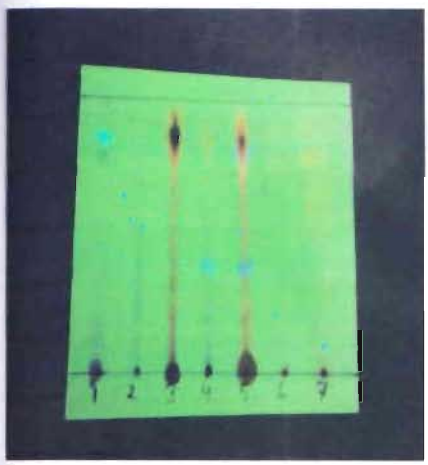


Figure 11 : Under UV light

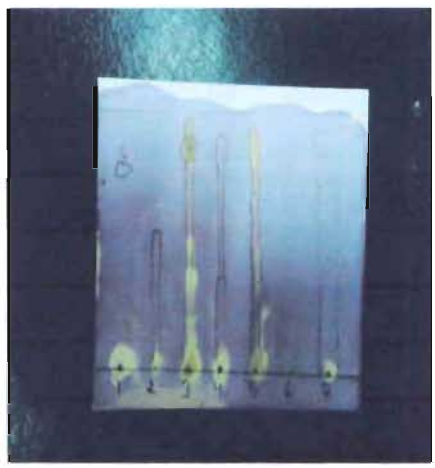


Figure 12 : After applying DPPH

My total sample extract was about seven. During spotting the samples in TLC plate I used number for their identification. These were that-

1. *Auricularia Auricular* methanol extract
2. *Polygonum hydropiper* leaf methanol extract
3. *Polygonum hydropiper* leaf ethyl acetate extract

4.*Polygonum hyropiper* stem methanol extract

5.*Polygonum hydropiper* stem ethyl acetate extract

6.*Hibiscus rosa-sinensis* leaf methanol extract

7.*Clerodendrum viscosum* leaf methanol extract

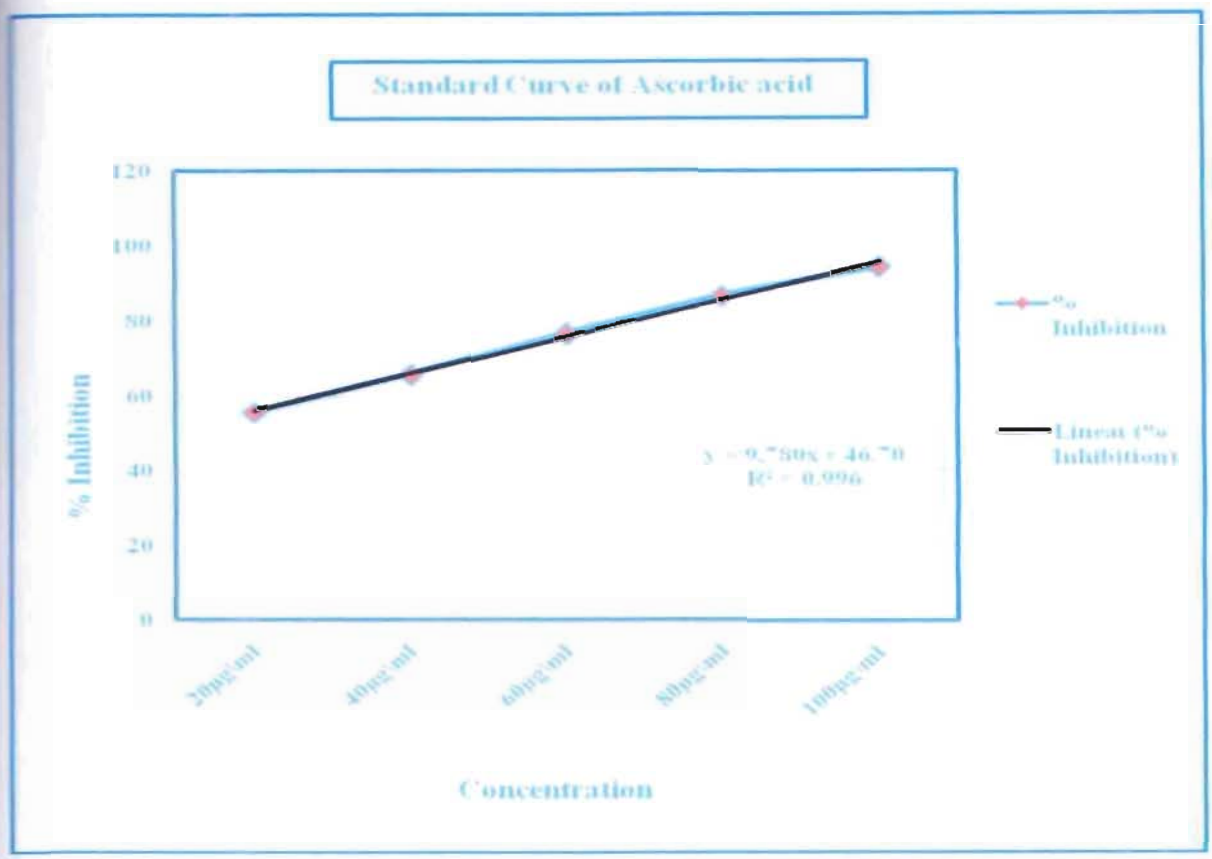
Under UV light I observed the spot with different color. I marked all the spot with pencil. After applying DPPH I waited for ten minute and observed that number 1,2,3,4,5 &7 change their color to yellow from purple. From qualitative assay I can determined that 1,2,4,5,7 contains antioxidant property on its non-polor portion. Number 3 contains antioxidant property in both polor and non polar portion. Only number 6 have no anti oxidant property.

5.2.2 Results of Quantitative Assay

The results quantitative assay of DPPH Free radical scavenging method are summarized in tables (8-14) with corresponding IC_{50} values, Regression equation and R^2 values with Graphs(8-14).

Table 9: IC_{50} value of Ascorbic acid

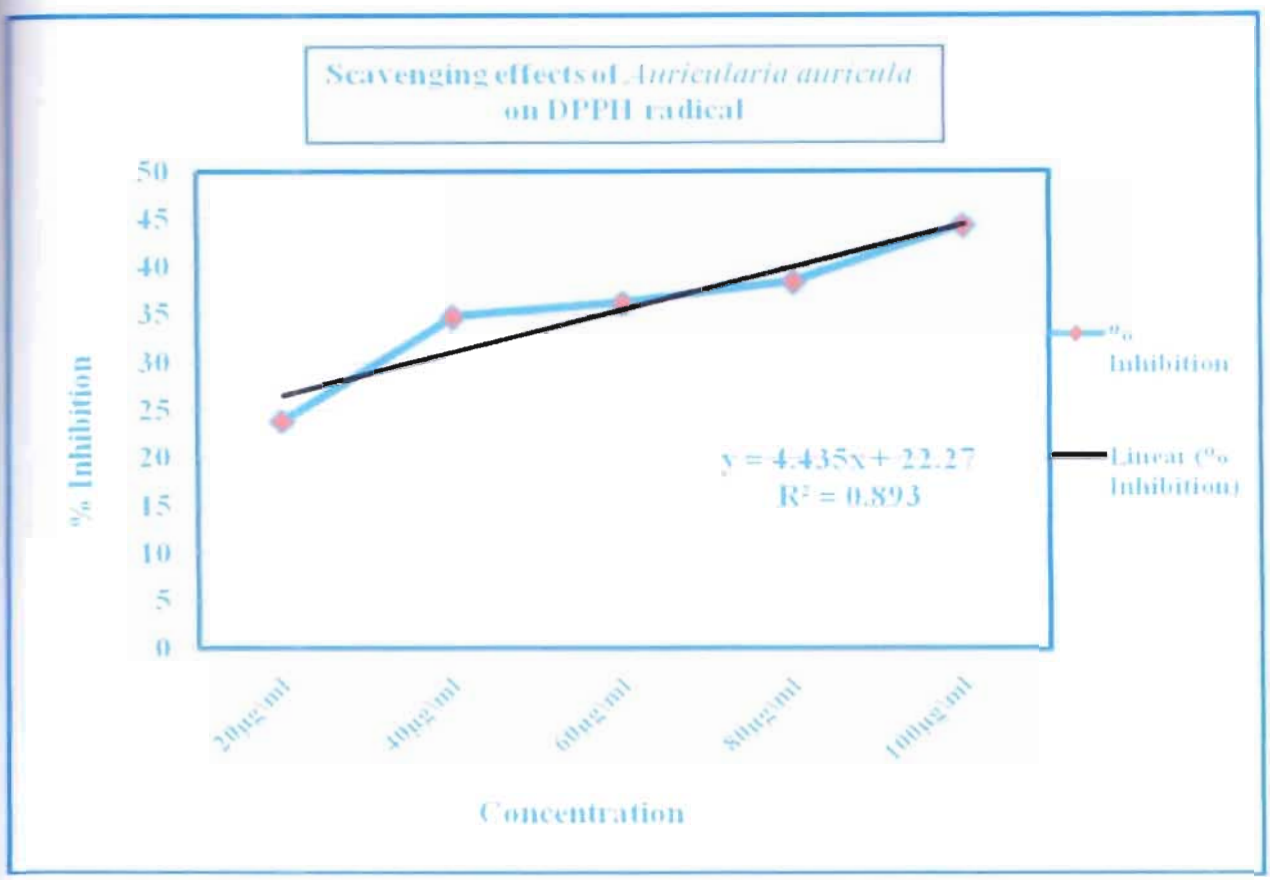
Serial No	Concentration (µg\ml)	Sample Absorbance	Blank Absorbance	% Inhibition	IC_{50}(µg\ml)
1	20	0.2	0.455	56.04395604	2.687
2	40	0.155	0.455	65.93406593	
3	60	0.105	0.455	76.92307692	
4	80	0.06	0.455	86.81318681	
5	100	0.025	0.455	94.50549451	



Graph 8: Standard curve of Ascorbic acid

Table 10 : IC_{50} value of *Auricularia auricular*

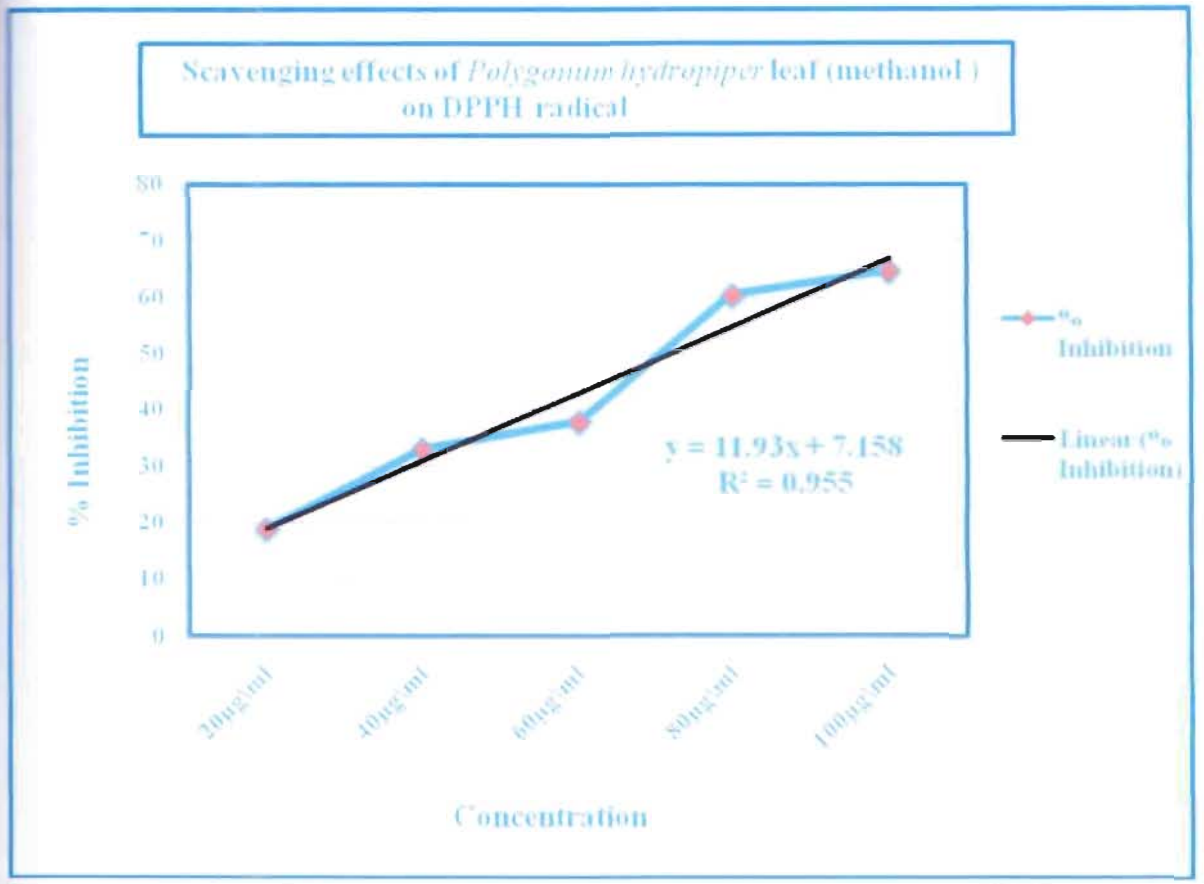
Serial No	Volume of Methanol (ml)	Volume of Extract (ml)	Conc. of Extract (µg\ml)	Sample Absorbance	Blank Absorbance	% Inhibition	IC_{50}(µg\ml)
1	8	2	20	0.74	0.974	24.02464066	6.25253664
2	6	4	40	0.635	0.974	34.80492813	
3	4	6	60	0.621	0.974	36.24229979	
4	2	8	80	0.599	0.974	38.50102669	
5	0	10	100	0.542	0.974	44.35318275	



Graph 9 :Scavenging effects of *Auricularia auricular* on DPPH radical

Table11 : IC_{50} value of *Polygonum hydropiper* leaf methanol

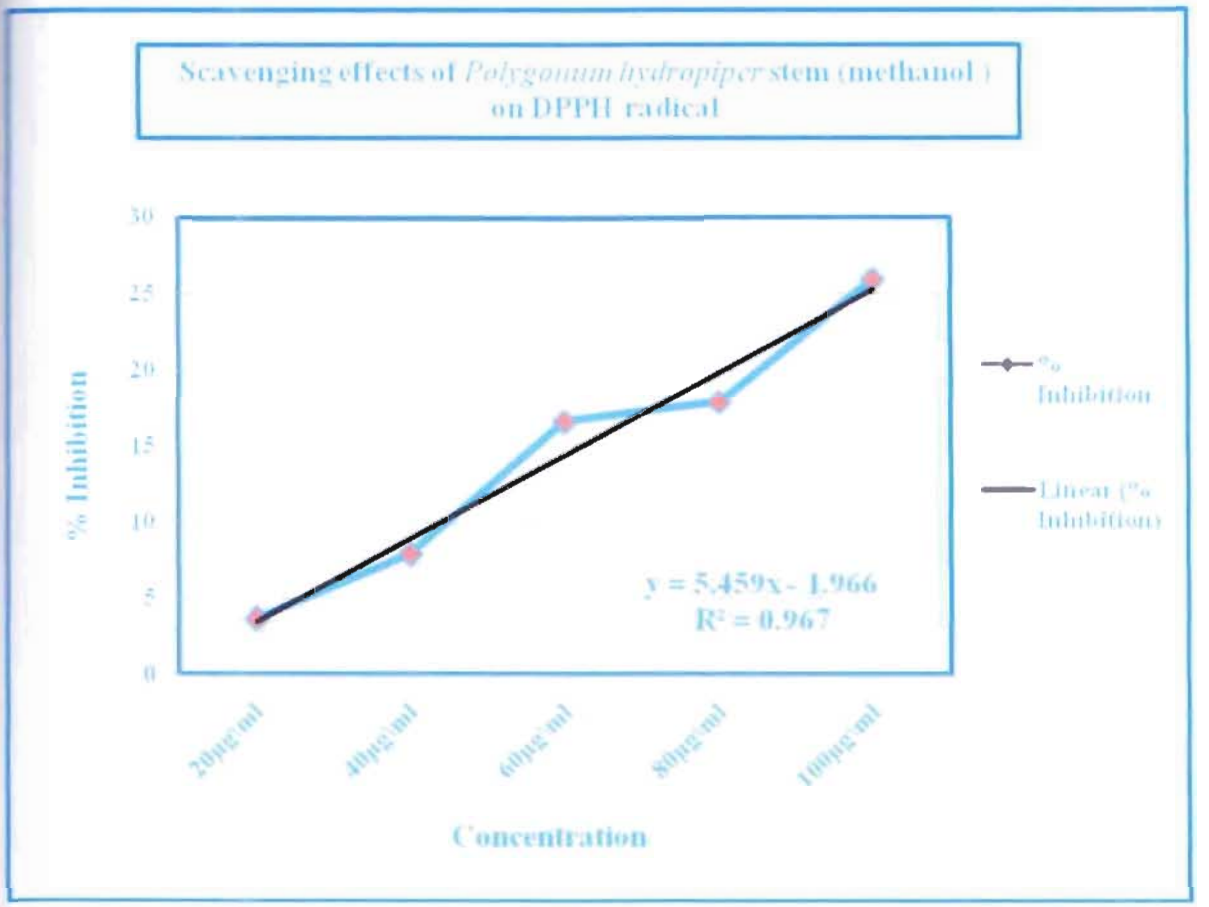
Serial No	Volume of Methanol (ml)	Volume of Extract (ml)	Conc. of Extract (µg\ml)	Sample Absorbance	Blank Absorbance	% Inhibition	IC_{50}(µg\ml)
1	8	2	20	0.803	0.989	18.80687563	
2	6	4	40	0.663	0.989	32.96258847	
3	4	6	60	0.615	0.989	37.81597573	3.59111484
4	2	8	80	0.391	0.989	60.46511628	
5	0	10	100	0.349	0.989	64.71183013	



Graph10 : Scavenging effects of *Polygonum hydropiper* leaf methanol on DPPH radical.

Table12 : IC_{50} value of *Polygonum hydropiper* stem methanol

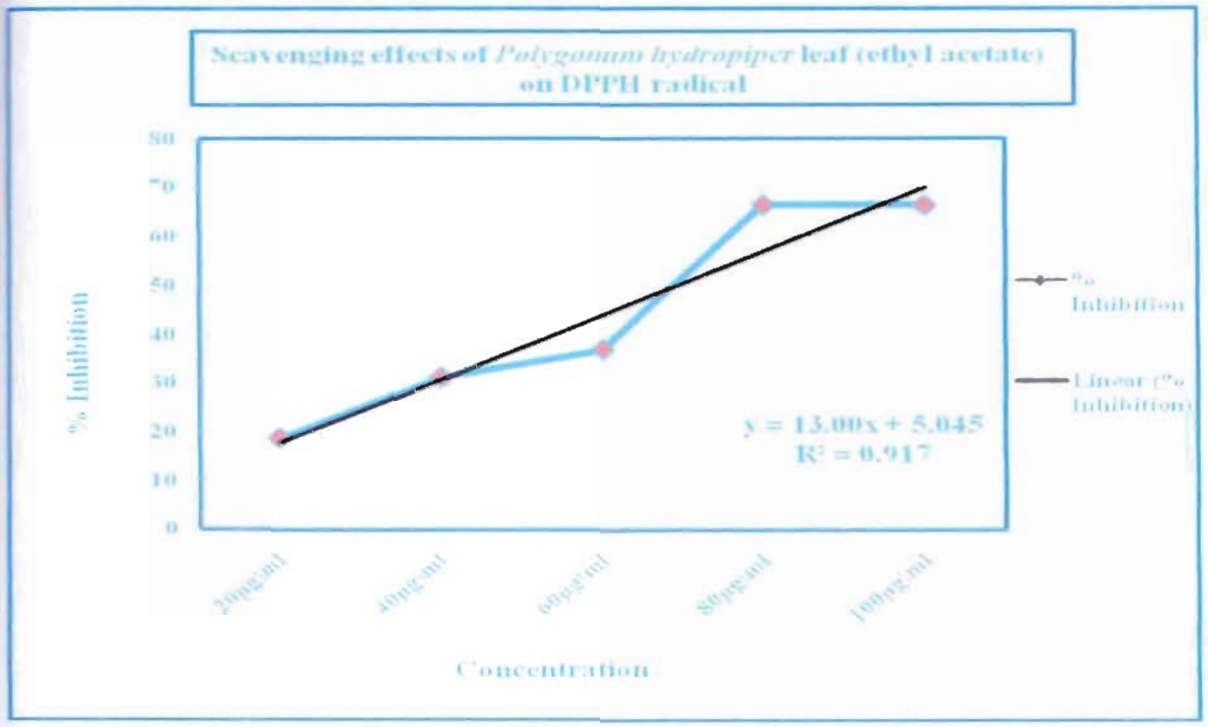
Serial No	Volume of Methanol (ml)	Volume of Extract (ml)	Conc. of Extract (µg\ml)	Sample Absorbance	Blank Absorbance	% Inhibition	IC_{50}(µg\ml)
1	8	2	20	2.096	2.176	3.676470588	9.519326
2	6	4	40	2.004	2.176	7.904411765	
3	4	6	60	1.815	2.176	16.59007353	
4	2	8	80	1.786	2.176	17.92279412	
5	0	10	100	1.611	2.176	25.96507353	



Graph 11: Scavenging effects of *Polygonum hydropiper* stem methanol on DPPH radical.

Table 13: IC_{50} value of *Polygonum hydropiper* leaf ethyl aceate

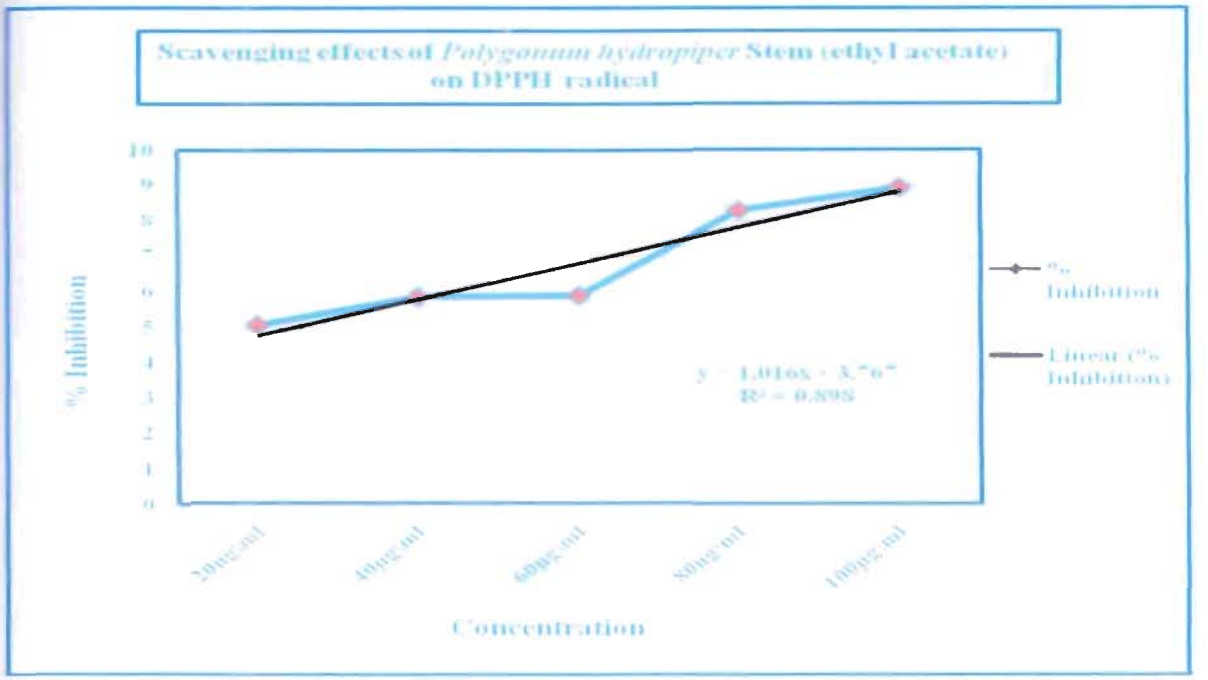
Serial No	Volume of Methanol (ml)	Volume of Extract (ml)	Conc. of Extract (µg\ml)	Sample Absorbance	Blank Absorbance	% Inhibition	IC_{50}(µg\ml)
1	8	2	20	0.806	0.993	18.83182276	
2	6	4	40	0.68	0.993	31.52064451	
3	4	6	60	0.625	0.993	37.05941591	3.458077
4	2	8	80	0.333	0.993	66.4652568	
5	0	10	100	0.334	0.993	66.36455186	



Graph 12:Scavenging effects of *Polygonum hydropiper* leaf ethyl aceate on DPPH radical.

Table 14: IC_{50} value of *Polygonum hydropiper* stem ethyl aceate

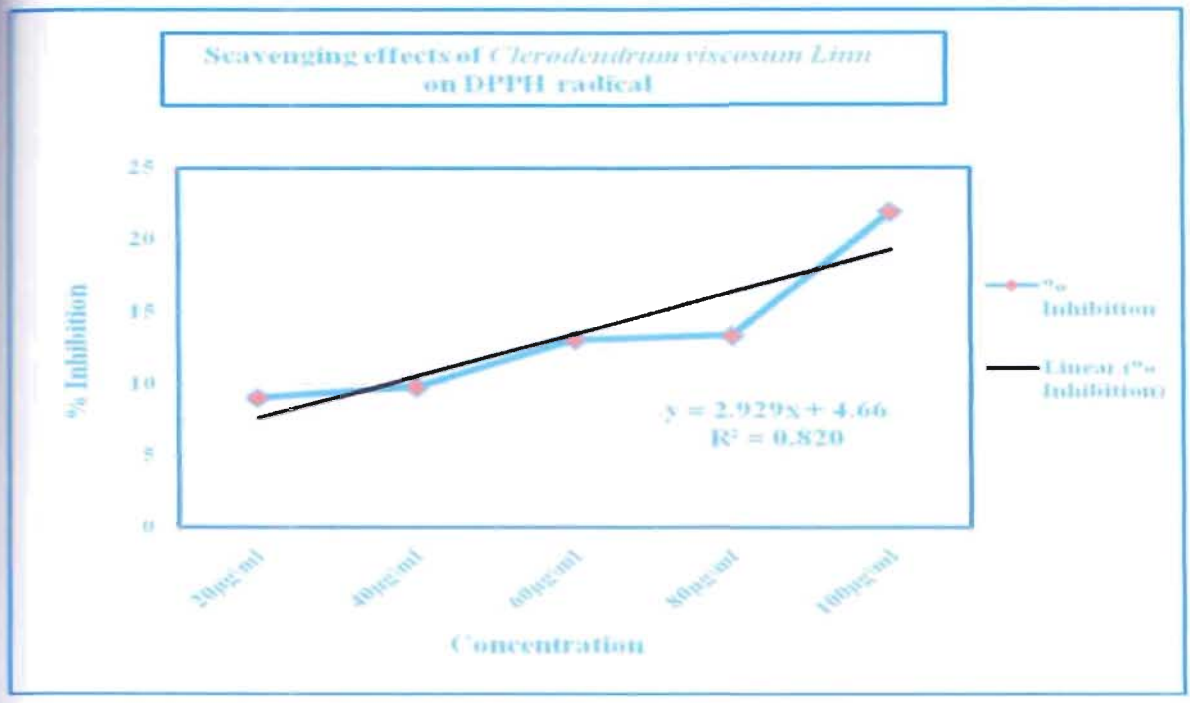
Serial No	Volume of Methanol (ml)	Volume of Extract (ml)	Conc. of Extract (µg\ml)	Sample Absorbance	Blank Absorbance	% Inhibition	IC_{50}(µg\ml)
1	8	2	20	2.091	2.203	5.083976396	45.5049213
2	6	4	40	2.074	2.203	5.855651384	
3	4	6	60	2.073	2.203	5.901044031	
4	2	8	80	2.02	2.203	8.30685429	
5	0	10	100	2.006	2.203	8.942351339	



Graph 13:Scavenging effects of *Polygonum hydropiper* stem ethyl aceate on DPPH radical

Table 15 : IC_{50} value of *Clerodendrum viscosum Linn*

Serial No	Volume of Methanol (ml)	Volume of Extract (ml)	Conc. of Extract (µg\ml)	Sample Absorbance	Blank Absorbance	% Inhibition	IC_{50}(µg\ml)
1	8	2	20	2.26	2.485	9.054325956	15.4796859
2	6	4	40	2.241	2.485	9.818913481	
3	4	6	60	2.16	2.485	13.07847082	
4	2	8	80	2.153	2.485	13.36016097	
5	0	10	100	1.94	2.485	21.93158954	



Graph 14: Scavenging effects of *Clerodendrum viscosum Linn* on DPPH radical

The sample *Auricularia auricular, Polygunum hydropiper* leaf methanol, *Polygunum hydropiper* stem methanol, *Polygunum hydropiper* leaf ethyl acetate, *Polygunum hydropiper* stem ethyl acetate & *Clerodendrum viscosum* had IC_{50} values of 6.25 (µg\ml), 3.59 (µg\ml), 9.51 (µg\ml), 3.45 (µg\ml),45.50 (µg\ml) & 15.47 (µg\ml) respectively while Ascorbic acid has IC_{50} value of 2.68 (µg\ml) indicating potential candidates of antioxidant compounds. Among all this values *Polygunum hydropiper* leaf ethyl acetate (PHLE) shows lowest IC_{50} value 3.45 (µg\ml) & *Polygunum hydropiper* stem ethyl acetate (PHSE) shows highest IC_{50} value 45.50 (µg\ml). It seems that PHLE had highly potent antioxidant capacity & PHSE had less antioxidant capacity. *Polygunum hydropiper* leaf methanol & *Polygunum hydropiper* leaf ethyl acetate had highly potent antioxidant capacity. *Auricularia auricular, Polygunum hydropiper* stem methanol & *Clerodendrum viscosum* had moderate antioxidant capacity. *Polygunum hydropiper* stem ethyl acetate had less antioxidant capacity. On the other hand *Hibiscus-rosa sinensis* had no antioxidant effect.

5.3. Result and Discussion of Antimicrobial Screening

Seven extract of different plant were tested for antimicrobial activities against a number of both gram positive and gram negative bacteria as well as a number of fungi. Standard Amoxicillin discs (30µg\disc) were used for comparison purpose. The result of antimicrobial activity was measured in terms of zone of inhibition (mm), have been shown in the table. The sample discs were used in a concentration of 250 µg/µL and 500 µg/µL.

Table 16: Result of in vitro Antimicrobial Test of *Auriculeria auricular* extract

S.L.	Name of Microorganism	Zone of Inhibition at 250 µg/µL	Zone of Inhibition at 500 µg/µL	Zone of Inhibition of Amoxicillin	Blank Disc
Gram positive Bacteria					
1.	*Streptococcus aureus*	N/A	N/A	18 mm	N/A
Gram Negative Bacteria					
2.	*Escherichia coli*	N/A	N/A	20 mm	N/A
3.	*Pseudomonas aureus*	N/A	N/A	21 mm	N/A
4.	*Salmonella typhi*	N/A	N/A	17 mm	N/A
5.	*Shigella dysenteriae*	N/A	N/A	16 mm	N/A
Fungus					
6.	*Candida albicans*	N/A	N/A	20 mm	N/A
7.	*Saccharomyces cerevisiae*	N/A	N/A	20 mm	N/A

Table 17 : Result of in vitro Antimicrobial Test of *Polygonum hydropiper* leaf methanol extract

S.L.	Name of Microorganism	Zone of Inhibition at 250 µg/µL	Zone of Inhibition at 500 µg/µL	Zone of Inhibition of Amoxicillin	Blank Disc
Gram positive Bacteria					
1.	*Streptococcus aureus*	N/A	N/A	15 mm	N/A
Gram Negative Bacteria					
2.	*Escherichia coli*	N/A	N/A	19 mm	N/A
3.	*Pseudomonas aureus*	N/A	N/A	16 mm	N/A
4.	*Salmonella typhi*	N/A	N/A	18 mm	N/A
5.	*Shigella dysenteriae*	N/A	N/A	16 mm	N/A
Fungus					
6.	*Candida albicans*	N/A	N/A	18 mm	N/A
7.	*Saccharomyces cerevisiae*	N/A	N/A	20 mm	N/A

Table 18 : Result of in vitro Antimicrobial Test of *Polygonum hydropiper* leaf ethyl acetate extract

S.L.	Name of Microorganism	Zone of Inhibition at 250 µg/µL	Zone of Inhibition at 500 µg/µL	Zone of Inhibition of Amoxicillin	Blank Disc
Gram positive Bacteria					
1.	*Streptococcus aureus*	N/A	N/A	10 mm	N/A
Gram Negative Bacteria					
2.	*Escherichia coli*	N/A	N/A	15 mm	N/A
3.	*Pseudomonas aureus*	N/A	N/A	12 mm	N/A
4.	*Salmonella typhi*	N/A	N/A	18 mm	N/A
5.	*Shigella dysenteriae*	N/A	N/A	16 mm	N/A
Fungus					
6.	*Candida albicans*	N/A	N/A	16 mm	N/A
7.	*Saccharomyces cerevisiae*	N/A	N/A	12 mm	N/A

Table 19 : Result of in vitro Antimicrobial Test of *Polygonum hydropiper* stem methanol extract

S.L.	Name of Microorganism	Zone of Inhibition at 250 µg/µL	Zone of Inhibition at 500 µg/µL	Zone of Inhibition of Amoxicillin	Blank Disc
Gram positive Bacteria					
1.	*Streptococcus aureus*	N/A	N/A	13 mm	N/A
Gram Negative Bacteria					
2.	*Escherichia coli*	N/A	N/A	14 mm	N/A
3.	*Pseudomonas aureus*	N/A	N/A	16 mm	N/A
4.	*Salmonella typhi*	N/A	N/A	15 mm	N/A
5.	*Shigella dysenteriae*	N/A	N/A	10 mm	N/A
Fungus					
6.	*Candida albicans*	N/A	N/A	16 mm	N/A
7.	*Saccharomyces cerevisiae*	N/A	N/A	13 mm	N/A

Table 20: Result of in vitro Antimicrobial Test of *Polygonum hydropiper* stem ethyl acetate extract

S.L.	Name of Microorganism	Zone of Inhibition at 250 µg/µL	Zone of Inhibition at 500 µg/µL	Zone of Inhibition of Amoxicillin	Blank Disc
Gram positive Bacteria					
1.	*Streptococcus aureus*	N/A	N/A	15 mm	N/A
Gram Negative Bacteria					
2.	*Escherichia coli*	N/A	N/A	15 mm	N/A
3.	*Pseudomonas aureus*	N/A	N/A	16 mm	N/A
4.	*Salmonella typhi*	N/A	N/A	13 mm	N/A
5.	*Shigella dysenteriae*	N/A	N/A	12 mm	N/A
Fungus					
6.	*Candida albicans*	N/A	N/A	15 mm	N/A
7.	*Saccharomyces cerevisiae*	N/A	N/A	12 mm	N/A

Table 21: Result of in vitro Antimicrobial Test of *Clerodendrum viscosum* leaf methanol extract

S.L.	Name of Microorganism	Zone of Inhibition at 250 µg/µL	Zone of Inhibition at 500 µg/µL	Zone of Inhibition of Amoxicillin	Blank Disc
Gram positive Bacteria					
1.	*Streptococcus aureus*	N/A	N/A	15 mm	N/A
Gram Negative Bacteria					
2.	*Escherichia coli*	N/A	N/A	19 mm	N/A
3.	*Pseudomonas aureus*	N/A	N/A	16 mm	N/A
4.	*Salmonella typhi*	N/A	N/A	18 mm	N/A
5.	*Shigella dysenteriae*	N/A	N/A	16 mm	N/A
Fungus					
6.	*Candida albicans*	N/A	N/A	18 mm	N/A
7.	*Saccharomyces cerevisiae*	N/A	N/A	20 mm	N/A

Table 22: Result of in vitro Antimicrobial Test of *Hibiscus rosa-sinensis* leaf methanol extract

S.L.	Name of Microorganism	Zone of Inhibition at 250 µg/µL	Zone of Inhibition at 500 µg/µL	Zone of Inhibition of Amoxicillin	Blank Disc
Gram positive Bacteria					
1.	*Streptococcus aureus*	N/A	N/A	16 mm	N/A
Gram Negative Bacteria					
2.	*Escherichia coli*	N/A	N/A	19 mm	N/A
3.	*Pseudomonas aureus*	N/A	N/A	16 mm	N/A
4.	*Salmonella typhi*	N/A	N/A	17 mm	N/A
5.	*Shigella dysenteriae*	N/A	N/A	16 mm	N/A
Fungus					
6.	*Candida albicans*	N/A	N/A	18 mm	N/A
7.	*Saccharomyces cerevisiae*	N/A	N/A	19 mm	N/A

In this antimicrobial test, I used Amoxicillin as a positive control. Standard Amoxicillin show the inhibition zone against one gram positive bacteria , four gram negative bacteria & two fungus. I have used seven extract such as *auricularia auricular* (methanol),*Polygonum hydropiper* leaf (methanol & ethyl acetate),*Polygonum hydropiper* stem (methanol & ethyl acetate),*Clerodendrum viscosum* leaf (methanol) & *Hibiscus rosa-sinensis* leaf (methanol). Any sample does not show any inhibition. From literature review showed *Polygonum hydropiper, Clerodendrum viscosum & Hibiscus rosa-sinensis* have antimicrobial action. But in my test I can

not get any effect. It may be occurred due to the geographical change. I had collected all the sample from Bangladesh.

5.4 Thin Layer Chromatography

solvent system n Hexane: ethyl Acetate (3:1)

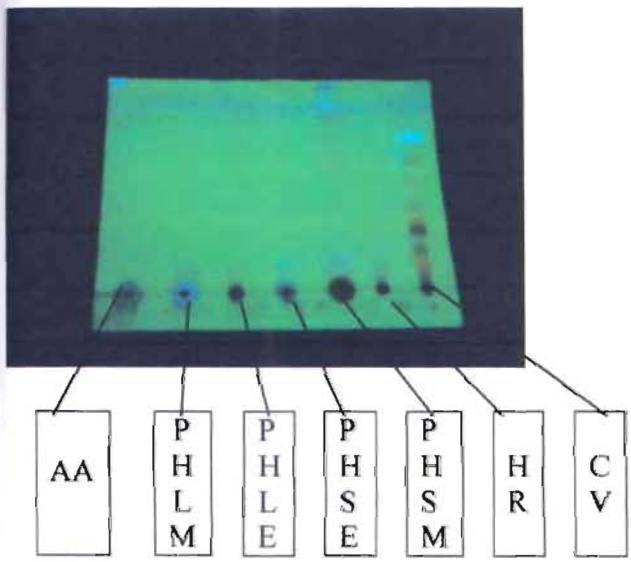


Fig 15 :TLC plate under UV Light

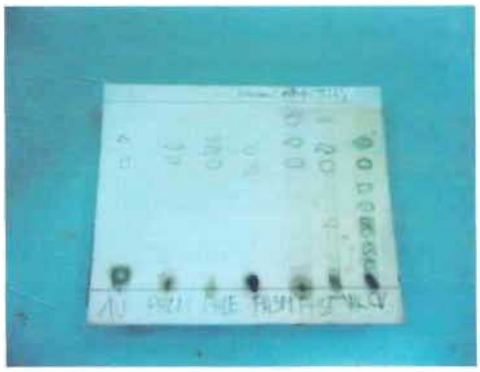


Fig 16:TLC plate after charring

Here I used solvent system n hexane and ethyl acetate (3:1). All sample extract were spotted in the same plate. I marked the sample as AA,PHLM,PHLE,PHSM,PHSE,HR,CV.

Here ,

AA = *Auricularia auricular*

PHLM = *Polygonum hydropiper* leaf methanol extract

PHLE= *Polygonum hydropiper* leaf ethyl acetate extract

PHSM = *Polygonum hydropiper* stem methanol extract

PHSE= *Polygonum hydropiper* stem ethyl acetate extract

HR = *Hibiscus rosa-sinensis*

CV= *Clerodendrum viscosum*

For *Auricularia auricular,*

$$R_f \text{value} = \frac{\text{distance traveled by compound}}{\text{distance traveled by solvent front}}$$

R_f value for one spot of AA = 3.7/4.6

= 0.804

Under UV-light I observed only one spot indicates that the extract possess fluorescent compounds. Looking at the distance traveled by the spot, it can assumed that these spots was weakly polar compounds. It run very quickly on the TLC plate. After charring no color change found. Upon heating on hot plate, spot fades to some extent. R_f values of the spots were calculated. were visible under UV light.

For *Polygonum hydropiper* leaf methanol extract,

$$R_f \text{value} = \frac{\text{distance traveled by compound}}{\text{distance traveled by solvent front}}$$

R_f value for lower spot of PHLM = 3.2/4.6

= 0.695

R_f value for upper spot of PHLM = 3.5/4.6

= 0.760

Under UV-light I observed different types of cluster of spots with different colour . I marked two spots, lower spot indicate violet color and upper spot indicate light brown color . Looking at the distance traveled by the spot, it can assumed that these spots moderately polar compounds.They run slowly on the TLC plate. After charring no color change found. Upon heating on hot plate, spot fades to some extent. R_f values of the spots were calculated. were visible under UV light.

For *Polygonum hydropiper* leaf ethyl acetate extract

$$R_f \text{ value} = \frac{\text{distance traveled by compound}}{\text{distance traveled by solvent front}}$$

R_f value for lower spot of PHLE =3/4.6

= 0.652

R_f value for middle spot of PHLE = 3.4/4.6

=0.739

R_f value for upper spot of PHLE = 3.7/4.6

= 0.804

Under UV-light I observed different types of cluster of spots with different colour . I marked three spots, lower spot indicate light violet color and middle spot indicate light brown color and upper spot indicate dark brown color. Looking at the distance traveled by the spot, it can assumed that these spots moderately polar compounds. They run slowly on the TLC plate. After charring no color change found. Upon heating on hot plate, spot fades to some extent. R_f values of the spots were calculated. were visible under UV light.

For *Polygonum hydropiper* stem ethyl acetate extract.

$$R_f \text{ value} = \frac{\text{distance traveled by compound}}{\text{distance traveled by solvent front}}$$

R_f value for lower spot of PHSE = 3.1/4.6

= 0.673

R_f value for middle spot of PHSE = 3.6/4.6

=0.782

R_f value for upper spot of PHSE = 4.3/4.6

= 0.934

Under UV-light I observed different types of cluster of spots with different colour . I marked three spots, lower spot indicated dark color and middle spot indicate violet color and upper spot indicate dark color. Looking at the distance traveled by the spot, it can assumed that this sample contains both moderate polar and weakly polar compound. One spot run very quickly and other two spots run slowlyin the TLC plate. After charring no color change found. Upon heating on hot plate, spot fades to some extent. R_f values of the spots were calculated. were visible under UV light.

For *Polygonum hydropiper* stem methanol extract

$$R_f \text{ value} = \frac{\text{distance traveled by compound}}{\text{distance traveled by solvent front}}$$

R_f value for spot of PHSM = 1.3/4.6

= 0.282

Under UV-light I observed only one spot indicates that the extract possess fluorescent compounds. Looking at the distance traveled by the spot, it can assumed that these spots was polar compounds. It run very quickly on the TLC plate. After charring no color change found. But one new spot found. Upon heating on hot plate, spot fades to some extent. R_f values of the spots were calculated. were visible under UV- light.

For *Hibiscus rosa-sinensis*

$$R_f \text{ value} = \frac{\text{distance traveled by compound}}{\text{distance traveled by solvent front}}$$

R_f value for lower spot of HR = 1.5/4.6

= 0.326

R_f value for lower middle spot of HR = 3.0/4.6

= 0.652

R_f value for upper middle spot of HR= 3.5/4.6

= 0.76

R_f value for upper spot of HR= 4.2/4.6

= 0.913

Under UV-light I observed different types of cluster of spots with different color . I marked four spots, lower spot indicated dark pink color and middle lower spot indicated light blue color and upper middle pink color and upper spot indicate dark color. Looking at the distance traveled by the spot, it can assumed that this sample contains both moderate polar and weakly polar compound. One spot run very slowly and other two spots run slowly and One spot run very quickly in the TLC plate. After charring no color change found. Upon heating on hot plate, spot fades to some extent. R_f values of the spots were calculated. were visible under UV- light.

For *Clerodendrum viscosum*

$$R_f \text{value} = \frac{\text{distance traveled by compound}}{\text{distance traveled by solvent front}}$$

In this sample I found seven spot. I had marked all sample as alphabetic way from lower to upper. so

R_f value for lower spot A of CV =0.5/4.6

=0.108

R_f value for upper spot B of CV = 1.1/4.6

=0.239

R_f value for upper spot C of CV =1.3/4.6

=0.282

R_f value for upper spot D of CV = 2/4.6

= 0.434

R_f value for upper spot E of CV = 2.4/4.6

= 0.521

R_f value for upper spot F of CV = 3.1/4.6

= 0.673

R_f value for upper spot G of CV = 3.8/4.6

= 0.826

Under UV-light I observed different types of cluster of spots with different color . I marked seven spots, from lower to upper spot I marked these spot as A,B,C,D,E,F,G. Spot A indicated orange color and spot B indicated dark color and spot C indicate dark green color and spot D indicate light yellow color, spot E light spot, spot F indicate dark color and spot G indicate fluorescent. Looking at the distance traveled by the spot, it can assumed that this sample contains more polar, moderate polar and weakly polar compound. After charring no color change found. Upon heating on hot plate, spot fades to some extent. R_f values of the spots were calculated. were visible under UV light.

CONCLUSION

6. Conclusion

Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 per cent of people still rely mainly on traditional remedies such as herbs for their medicines. Medicinal plants are the most important source of life saving drugs for the majority of the world's population.

Polygonum hydropiper , *Hibiscus rosa-sinensis* ,*Clerodendrum viscosum* are medicinal plants and *Auricularia auricular* is locally cultivated fungus in Bangladesh. By using Biostate 2008 software I measured the value of LC50. From this study resulted that *Clerodendrum viscosum* is moderately toxic, *Polygonum hydropiper* leaf ethyl acetate, *Polygonum hydropiper* leaf methanol & *Hibiscus rosa-sinensis* are mildly toxic. *A*uricularia auricular & *Polygonum hydropiper* stem methanol are non toxic. Without *Hibiscus rosa-sinensis* rest of the plants sample and fungus shows good antioxidant property. In these sample, *Polygonum hydropiper* leaf (methanol and ethyl acetate) extract have more antioxidant property compare with Ascorbic acid used as a standard. *Polygonum hydropiper* stem part is less active than leaf part. *Clerodendrum viscosum* & *Auricularia auricular* moderate antioxidant activity. All the sample did not show any action on microorganism. So these plants have some medicinal property. That's why these are important. It is necessary to discover the safe & efficacious drugs in systematical order from medicinal plants for the welfare of humanity.

REFERENCES

References

1. Balick, J.M. and P.A. Cox. Plants, People and Culture,The Science of Ethnobotany, Scientific American Library, New York, **1996**, 228.
2. Cotton, C.M. Ethnobotany, Principle and Application, John Wiley and Sons, New York. Council of Scientific and Industrial Research (Ed.), The Wealth of India: Raw Materials, Council of Scientific and Industrial Research, New Delhi,**1962, 1996,** *6*,425–429.
3. Rahmatullah. M.; Das. A. K.; Mollik. M.A.H.; Jahan. R.; Khan. M.; Rahman.T.; Chowdhury M H. An Ethnomedicinal Survey of Dhamrai Sub-district in Dhaka District, Bangladesh, *American-Eurasian Journal of Sustainable Agriculture*, **2009**, *3*, 881-888.
4. Persoone, G. Procending of the International Symposium on Brine Shrimp, **1980.**
5. Hill; A.F. Economic Botany, TMH edition, *Tata McGraw-Hill Publishing Co., New Delhi, India,* **1972**, 242-43.
6. Obianwu; H.O. Zaria. Nigeria Traditional Medicine and Drug Development in Nigeria, **30 May, 1984.**
7. Ghani A. Medicinal Plants of Bangladesh, chemical constituents and uses, 2nd edition, **2003**, 1-16, 46-47, 185.
8. Claus.; E.P. & Tyler.; V.E.,Jr. Pharmacognosy, 5th edition, **1965** ,9- 10,177.
9. Murray, M.T. The Healing Power of Herbs, 2nd Edition, *Prima Publishing, California, USA*, **1995** , *8*,62,294.
10. Smith; John E.; Rowan; Neil J.; Sullivan; Richard."Introduction". Medicinal mushrooms: their therapeutic properties and current medical usage with special emphasis on cancer treatments, *Cancer Research UK*, **May 2002.**
11. "Maitake Mushroom". Complementary and Alternative Medicine,Diet and Nutrition. *American Cancer Society*, **2008.**
12. *Hadith*; Sahih Muslim. "Book 23, Chapter 27", 5084–9.
13. Molitoris .; H.P. Mushrooms in medicine, *Folia Microbiologica*, **1994**, *39*, 91–8.
14. Lentinan. About herbs, *Memorial Sloan–Kettering Cancer Center*, **2009.**

15. Zakaria, M. Isolation and characterization of active compounds from medicinal plants, *Asia Pacific J. Pharmacol*, **1989**, *6*, 15-20.

16. Farnsworth.; N.R. & Morris,R.N. Higher plants- the Sleeping giant of drug development, *American Journal of Pharmacy*, **1976** ,*147(2)*, 46-52.

17. Hua Zhang.; Zhen-Yu Wang.; Zhi Zhang.; Xue Wang. Purified *Auricularia auricular-judae* polysaccharide (AAP I-a) prevents oxidative stress in an ageing mouse model, *Carbohydrate Polymers*, **11 February 2011**, *84(1)*, 638-648.

18. Yangchao Luo.; Gang Chen.; Bo L.; Baoping Ji .; Yi Guo.; Fang Tian. Evaluation of antioxidative and hypolipidemic properties of a novel functional diet formulation of *Auricularia auricula* and Hawthorn, *Innovative Food Science & Emerging Technologies*, **April 2009**,*10(2)*, , 215-221.

19. Misaki A.; Kakuta M.; Sasaki T.; Tanaka M.; Miyaji H. Studies on interrelation of structure and antitumor effects of polysaccharides, antitumor action of periodate-modified, branched (1 goes to 3)-beta-D-glucan of Auricularia auricula-judae, and other polysaccharides containing (1 goes to 3)-glycosidic linkages, *U.S. National Library of Medicine ,National Institutes of Health,Carbohydr Res.* **1981 May 18**, *92(1)*, 115-29.

20. Zhao Feng Peng.; Dieter Strack.; Alfred Baumert.; Ramanathan Subramaniam.; Ngoh Khang Goh.; Tet Fatt Chia.; Swee Ngin Tan and Lian Sai Chia. Antioxidant flavonoids from leaves of Polygonum hydropiper L.

21. Faridah Abas.; Nordin H. Lajis.; D.A. Israf.; S. Khozirah and Y. Umi Kalsom. Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables.

22. Ajit hazarika.; Hirendra n. sarma. Polygonum hydropiper crude root extract mimics estrogenic properties in females, Evidence of uterine protein profiles studied by sodium dodecyl sulfate polyacrylamide gel electrophoresis **19 may 2006**.

23. Hawrył.; Mirosław A.; Waksmundzka-Hajnos.; Monika.; Makar.; Janusz. Separation of Selected Flavonoids by use of RP-HPLC/NP-HPTLC Coupled Methods, *Journal of Liquid Chromatography & Related Technologies*, **September 2007**, *30*, 2253-2265.

24. M. F. Hasan.; R. Das, Alam Khan.; M.S. Hossain and M. Rahman. The Determination of Antibacterial and Antifungal Activities of *Polygonum hydropiper* (L.) Root Extract, *Advances in Biological Research*, **2009,** 53-56.

25. Kundu.; B.R.; R. Ara.; M.M. Begum.; Z.I. Sarkar. Effect of Bishkatali, *Polygonum hydropipe* L. plant extracts against the red flour beetle, *Tribolium castaneum Herbest. University J. Zool. Rajshahi University,* **2007**, *26*, 93-97.

26. Sharma.; R. Medicinal plants of India, encyclopedia. *Daya Publishing House,Delhi-110035*, **2003**, 46-47.

27. Chevallier. A. The Encyclopedia of Medicinal, *Dorling Kindersley. London,* **1996** ,185.

28. Chopra RN.; Nayar SL. Chopra IC Glossary of Indian Medicinal Plants (Including the Supplement). *Council of Scientific and IndustrialResearch, New Delhi*, **1986**.

29. Sotheeswaran S.; Doyle M.; Aalbersberg W. Medicinal Plants in the South Pacfific. Western Pacific Series, *WHO Regional Publica-tions.Manial, Philippines,* **1998.**

30. Olaleye MT. Cytotoxicity and antibacterial activity of Methanolic extract of *Hibiscus sabdariffa. J. Med. Plants Res,* **2007**, *1(1),* 009-013.

31. Nayak BS.; Raju SS.; Orette FA.; Rao AVC. Effects of *Hibiscus rosa sinensis L (Malvaceae)* on Wound Healing Activity, *A Preclinical Study in a Sprague Dawley Rat. Int. J. Low Extrem.* Wounds, **2007**, *6(2),* 76- 81.

32. Okwu DE.; Josiah C. Evaluation of the chemical composition of two Nigerian medicinal plants, *Afri. J.Biotechnol*, **2006**, *5(4),* 357-361.

33. Chopra.; SL Nayer.; & IC Chopra. The Glossary of Indian medicinal Plants, 1st edition *Publication and information Directorate, CSIR*, **1992.**

34. LD Kapoor. Handbook of Ayurvedic Medicinal Plants, 1st edition CRC press, New Delhi, **2001**, 124-125.

35. Santanu Sannigrahi.; Upal Kanti Mazumder.; Dilipkumar Pal.; Silpi Lipsa Mishra. Hepatoprotective potential of methanol extract of *Clerodendrum infortunatum* Linn. against CCl_4 induced hepatotoxicity in rats, **2009**, *5(20),* 394-399.

36. Sterry.; Paul.; Hughes.; Barry.; HarperCollins. Complete Guide to British Mushrooms & Toadstools, **2009**, 290.

37. Kidd. The use of mushroom glucans and proteoglycans in cancer therapy, alternative medicine review, **2000**, *5*, 4-27.

38. *Hibiscus rosa-sinensis* - Tropical Hibiscus, Malvaceae, Plant of the Week, **2000**.

39. Khare.; CP. Indian Medicinal Plants, *An Illustrated Dictionary Springer-verlag*, **2008**, 160.

40. Meyer.; B.N., Ferrigni.; N.R.; Putnam J.E.; Jacobsen L.B.; Nichols D.E.; McLaughlin J.L. Brine shrimp a convenient general bioassay for active plant constituents, *Journal of Medicinal Plant Research*, **1982**, *45*, 31–34.

41. Persoone.; G. McLaughlin, J.L. Procending of the International Symposium on Brine Shrimp, *Bench-top Bioassay for the discovery of Active Compound in Higher plants*, **1992**, *29*.

42. Nusrat S.; Alam, M. A.; Ahmed, F.; Awal, M. A.; Nahar L.; Sarker S.D. In vitro antioxidant property of the extract of Excoecaria agallocha (Euphorbiaceae), **2008**, *16(3)*.

43. Sadhu S K.; Okuyama E.; Fujimoto H.; Ishibashi M. Separation of *Leucas aspera,* a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities, *Chemical & Pharmaceutical Bulletin*, **2003**, *51*, 595-598.

44. Roland. Antibiotics, *An IntroductionF. Hoffman La roche and co., Basle, Switzerland*, **1982**.

45. Colee J.D. Applied Medicinal Microbiology, *London Science pub*, **1967**, 93.

46. Barry.; A.L. Principle and Practice of Microbiology, *Lea and Fabager, Philadelphia*, **1976**, 21-27.

47. Laurence M. Harwood.; Christopher J. Moody. *Experimental organic chemistry Principles and Practice* (Illustrated edition ed.) 159–173.

48. Stoilova.I.; Krastanov. A.; Stoyanova. A.; Denev. P.; Gargova, S. Antioxidant activity of a ginger extract (Zingiber officinale), *Food Chemistry*, **2007**, 764–770.