PHYTOCHEMICAL, ANTIMICROBIAL AND BIOLOGICAL INVESTIGATIONS OF METHANOLIC EXTRACT OF LEAVRS OF *CORCHORUS CAPSULARIS*

A Dissertation Submitted to the Department of Pharmacy

The University of East West in partial fulfillment of

Requirements for the degree of Bachelor of Pharmacy (B. PHRM)

Submitted By
Jannat Mahal Rume
2010-1-70-048
East West University

DEDICATED

TO

MY PARENTS

Certificate

This is to certify that the thesis In-vitro antioxidant, Biomedical & antimicrobial assay of Methanolic extract of leaves of ***Corchorus capsularis*** submitted to the department of pharmacy, East West University, Aftabnagar, Dhaka, in partial fulfillment of the requirements for the degree of bachelor of pharmacy (B.Phrm) was carried out by Jannat Mahal Rume (ID#2010-1-70-048) under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed in this connection is duly acknowledged.

Dr. Chowdhury Faiz Hossain
Chairperson
Department of Pharmacy
East West University
Aftabnagar, Dhaka.

Certificate

This is to certify that the thesis In-vitro antioxidant, Biomedical & antimicrobial assay of Methanolic extract of leaves of ***Corchorus capsularis*** submitted to the department of pharmacy, East West University,Aftabnagar,Dhaka, in partial fulfillment of the requirements for the degree of bachelor of pharmacy (B.Phrm) was carried out by Jannat Mahal Rume (ID#2010-1-70-048) under our guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information and laboratory facilities availed in this connection is duly acknowledged.

Dr. Repon Kumer Saha
Supervisor
Associate professor, Department of Pharmacy
East West University

Declaration by Research candidate

I, Jannat Mahal Rume, hereby declare that the dissertation entitled "Phytochemical, Antimicrobial and Biological Investigations of Methanolic extract of leaves of *Corchorus Capsularis"* submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of degree of Bachelor of Pharmacy (Honors) is a genuine & authentic record of original research work carried out by me during Spring 2013- Fall 2013 under the supervision and guidance of Dr. Repon Kumer Saha, assistant professor, Department of Pharmacy East West University and it has not formed the basis for the award of any other Degree/Diploma/Fellowship or other similar title to any candidate of any University.

Place: Dhaka

Date: 16.01.2013

Signature of candidate

(Jannat Mahal Rume)

Contents

Corchorus capsularis

Acknowledgement

All praise is for God who has given me the ability to complete my B. PHRM thesis & the opportunity to study in this subject.

It is my pleasure and proud privilege to express my heartiest regards and gratitude to my respected teacher and supervisor Dr. Repon Kumer Saha, Department of Pharmacy, Associate Professor of Pharmacy, East West University; for his mastermind direction, constant supervision and support, optimistic counseling and continuous backup to carry out the research work as well as to prepare this dissertation.

I feel proud to express my heartiest gratitude to my reverend teachers, Dr. Chowdhury Faiz Hossain, Chairperson, Department of Pharmacy, East West University, for extending his helping hands & affectionate attitude whenever I needed.

Finally I would like to extend my thanks to the office staffs of the Faculty of Pharmacy who have cooperated with and all the research students in the lab for their help and assistance, friendly behavior and earnest cooperation, which enabled me to work in a very congenial and comfortable atmosphere.

I remember here the inspiring words of my family members and to all my well-wishers. I say many thanks to them for their wholehearted inspiration during my thesis work.

Abstract

The crude methanolic extracts derived from the ***Corchorus capsularis*** was screened in vitro for possible phytochemical and antibacterial. Under phytochemical analysis, antioxidant test & Chemical screening was done. The antioxidant property found in crude methanolic extracts derived from the ***Corchorus capsularis*** was very good. The crude methanolic extract of leaves of ***Corchorus capsularis*** showed modrtate antibacterial activity against different type of bacterias. The plant species can be a source of antibacterial agent(s) and antioxidant agents.

Different types of phytoconstituents such as sugar, steroid, flavonoids, glycosides , triterpenes, tannins and saponins along with their structure and their applications have also been reported from the leaf, bark, root and seeds of the species. These compounds have significant biological activities like anti-inflammatory activities, cardiac and antinociceptive. The crude methanolic extract of leaves of ***Corchorus capsularis*** also showed to have prevented haemolysis of RBCs.

RATIONALE AND OBJECTIVE OF THE WORK

Higher plants represent a rich source of new molecules with pharmacological properties, which are lead compounds for the development of new drugs. During the last decades, the renewed interest in investigating natural products has led to the advent of several important drugs, such as the anticancer substances vinblastine, vincristine and taxol, or the anti-malarial agent artemisinin. Success in natural products research is conditioned by a careful plant selection, based on various criteria such as chemotaxonomic data, ethnomedical information, field observations or even random collection. One main strategy in the isolation of new leads consists of the so-called bioactivity-guided isolation, in which pharmacological or biological assays are used to target the isolation of bioactive compounds. The work described in this dissertation is an attempt to isolate the chemical constituents of the ***Corchorus capsularis*** and to evaluate the possible pharmacological and microbiological profiles. Although some lectins protein was reported earlier, yet no extensive chemical and biological investigations have been carried out on this plant. There is medical flow about this plant and that is the plant can be used as demulcent, deobstruent, diuretic, lactagogue, purgative, and tonic, tussa jute is a folk remedy for aches and pains, dysentery, enteritis, fever, dysentery, pectoral pains, and tumors. Ayurvedics use the leaves for ascites, pain, piles, and tumors. Elsewhere the leaves are used for cystitis, dysuria, fever, and gonorrhea. The cold infusion is said to restore the appetite and strength.. Therefore, the objective of this work is to explore the possibility of developing new drug candidates from this plant for the treatment of various diseases.

INTRODUCTION

Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date. The therapeutic use of plants certainly goes back to the Sumerian and the Akkadian civilizations in about the third millenium BC. Hippocrates (ca. 460–377 BC), one of the ancient authors who described medicinal natural products of plant and animal origins, listed approximately 400 different plant species for medicinal purposes. Natural products have been an integral part of the ancient traditional medicine systems, e.g. Chinese, Ayurvedic and Egyptian **[1].** Over the years they have assumed a very central stage in modern civilization as natural source of chemotherapy as well as amongst scientist in search for alternative sources of drugs. About 3.4 billion people in the developing world depend on plant-based traditional medicines. This represents about 88 per cent of the world's inhabitants, who rely mainly on traditional medicine for their primary health care. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

According to the World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, condition and therefore contains chemical components that are medically active. These non-nutrient plant chemical compounds or bioactive components are often referred to as phytochemicals ('phyto-'from Greek - *phyto* meaning 'plant') or phytoconstituents and are responsible for protecting the plant against microbial infections or infestations by pests **[2].**

Medicinal plants are increasingly gaining acceptance even among the literates in urban settlements, probably due to the increasing inefficacy of many modern drugs used for the control of many infections such as typhoid fever, gonorrhoea, and tuberculosis as well as increase in resistance by several bacteria to various antibiotics and the increasing cost of prescription drugs, for the maintenance of personal.

Unfortunately, rapid explosion in human population has made it almost impossible for modern health facilities to meet health demands all over the world, thus putting more demands on the use of natural herbal health remedies.

Current problems associated with the use of antibiotics, increased prevalence of multiple-drug resistant (MDR) strains of a number of pathogenic bacteria such as methicillin resistant *Staphylococcus aureus, Helicobacter pylori,* and MDR *Klebsiela pneumonia* has revived the interest in plants with antimicrobial properties **[3].** In addition, the increase in cases of opportunistic infections and the advent of Acquired Immune Deficiency Syndrome (AIDS) patients and individuals on immunosuppressive chemotherapy, toxicity of many antifungal and antiviral drugs has imposed pressure on the scientific community and pharmaceutical companies to search alternative and novel drug sources.

Phytochemistry

The study of natural products on the other hand is called phytochemistry. Phytochemicals have been isolated and characterized from fruits such as grapes and apples, vegetables such as broccoli and onion, spices such as turmeric, beverages such as green tea and red wine, as well as many other sources. Effect of extracted plant phytochemicals depends on

1. The nature of the plant material
2. Its origin
3. Degree of processing
4. Moisture content
5. Particle size

There are lots of medicinal plants which contain a number of phytochemicals and those phytochemicals are used medicine purpose to treat various kinds of diseases. In the following table a list is shown of phytochemicals having medicinal values.

Table a: Structural features and activities of various phytochemicals from plants **[4]**

Phytochemicals	**Structural features**	**Example(s)**	**Activities**
Phenols and Polyphenols	C3 side chain, - OH groups, phenol ring	Catechol, Epicatechin, Cinnamic acid	Antimicrobial, Anthelmintic, Antidiarrhoeal
Quinones	Aromatic rings, two ketone substitutions	Hypericin	Antimicrobial
Flavones Flavonoids Flavonols	Phenolic structure, one carbonyl group Hydroxylated phenols, C6-C3 unit linked to an aromatic ring Flavones + 3-hydroxyl group	Abyssinone Chrysin, Quercetin, Rutin Totarol	Antimicrobial Antidiarrhoeal
Tannins	Polymeric phenols (Mol. Wt. 500-3000)	Ellagitannin	Antimicrobial, Anthelmintic, Antidiarrhoeal
Coumarins	Phenols made of fused benzene and α-pyrone rings	Warfarin	Antimicrobial
Terpenoids and essential oils	Acetate units + fatty acids, extensive branching and cyclized	Capsaicin	Antimicrobial Antidiarrhoeal
Alkaloids	Heterocyclic nitrogen compounds	Berberine, Piperine, Palmatine, Tetrahydropalmatine	Antimicrobial, Anthelmintic, Antidiarrhoeal
Lectins and	Proteins	Mannose-specific	Antimicrobial

Polypeptides		agglutinin, Fabatin	
Glycosides	Sugar + non carbohydrate moiety	Amygdalin	Antidiarrhoeal
Saponins	Amphipathic glycosides	Vina-ginsenosides-R5 and -R6	Antidiarrhoeal

Mechanism of action of phytochemicals

Different mechanisms of action of phytochemicals have been suggested. They may inhibit microorganisms, interfere with some metabolic processes or may modulate gene expression and signal transduction pathways. Plant extracts and essential oils may exhibit different modes of action against bacterial strains, such as interference with the phospholipids bilayer of the cell membrane which has as a consequence a permeability increase and loss of cellular constituents, damage of the enzymes involved in the production of cellular energy and synthesis of structural components, and destruction or inactivation of genetic material. In general, the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents.

Table b: Mechanism of action of some phytochemicals **[4]**

Phytochemicals	**Activity**	**Mechanism of action**

Quinones	Antimicrobial	Binds to adhesins, complex with cell wall, inactivates enzymes
Flavonoids	Antimicrobial Antidiarrhoeal	Complex with cell wall, binds to adhesins Inhibits release of autocoids and prostaglandins, Inhibits contractions caused by spasmogens, Stimulates normalization of the deranged water transport across the mucosal cells, Inhibits GI release of acetylcholine
Polyphenols and Tannins	Antimicrobial Antidiarrhoeal Anthelmintic	Binds to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption, metal ion complexation Makes intestinal mucosa more resistant and reduces secretion, stimulates normalization of deranged water transport across the mucosal cells and reduction of the intestinal transit, blocks the binding of B subunit of heat-labile enterotoxin to GM1, resulting in the suppression of heat-labile enterotoxin-induced diarrhea, astringent action. Increases supply of digestible proteins by animals by forming protein complexes in rumen, interferes with energy generation by uncoupling oxidative phosphorylation, causes a decrease in G.I. metabolism
Phytochemicals	**Activity**	**Mechanism of action**
Coumarins	Antiviral	Interaction with eucaryotic DNA
Terpenoids and	Antimicrobial	Membrane disruption Inhibits release of autocoids and prostaglandins

essential oils	Antidiarrhoeal	
Alkaloids	Antimicrobial Antidiarrhoeal Anthelmintic	Intercalates into cell wall and DNA of parasites Inhibits release of autocoids and prostaglandins Possess anti-oxidating effects, thus reduces nitrate generation which is useful for protein synthesis, suppresses transfer of sucrose from stomach to small intestine, diminishing the support of glucose to the helminthes, acts on CNS causing paralysis
Lectins and Polypeptides	Antiviral	Blocks viral fusion or adsorption, forms disulfide bridges
Glycosides	Antidiarrhoeal	Inhibits release of autocoids and prostaglandins
Saponins	Antidiarrhoeal Anticancer Anthelmintic	Inhibits histamine release in vitro Possesses membrane permeabilizing properties Leads to vacuolization and disintegration of teguments

Chapter- 1

INTRODUCTION

1.1 Overview of *Corchorus capsularis*

Jute is known as golden fibre of Bangladesh. It is the main cash crop for the country, since Bangladesh supplies more than 95 percent of the world's requirement of this important fibre. The word jute is probably coined from the word *jhuta* or *jota*, an Orrisan word. Among the all species, white Jute (*C. capsularis*) is commercially important available and the present study was conducted on the subjected one. The centre of origin of this species is said to be Indo-Burma including South China. Jute grows under wide variation of climatic conditions and stress of tropic and subtropics. It is grown in India, Myanmar, Nepal, China, Taiwan, Thailand, Vietnam, Cambodia, Brazil and some other countries.

It was observed in different literatures that the green, leafy vegetable of *C. capsularis* is rich in beta-carotene for good eyesight, iron for healthy RBCs, calcium for strong bone and teeth, and vitamin C for smooth, clear skin, strong immune cells and fast wound-healing. Vitamins A, C and E present in the jute leaf sponge up free radicals, scooping them up before they can commit cellular sabotage. Antioxidants from jute leaves have been associated with protection from chronic diseases such as heart diseases, neoplasm, diabetes and hypertension. Ayurvedics use the leaves for ascites, algesia, piles and tumors. Elsewhere the leaves are used for cystitis, dysuria, fever and gonorrhea. The cold infusion is said to restore the appetite and strength .

Figure 1.1: Corchorus capsularis

1.2 Description of the crop jute:

Annual, much-branched herb 90-120 cm tall; stems glabrous. Leaves 6-10 cm long, 3.5-5 cm broad, elliptic-lanceolate, apically acute or acuminate, glabrous, serrate, the lower serratures on each side prolonged into a filiform appendage over 6 mm long, rounded at the base, 3-5 nerved; petioles 2-2.5 cm long, slightly pubescent, especially towards the apex; atipules subulate, 6-10 mm long. Flowers pale yellow; bracts lanceolate; peduncle shorter than the petiole; pedicles 1-3, very short. Sepals ca 3 mm long, oblong, apiculate. Petals 5 mm long, oblong spathulate. Style short; stigma microscopically papillose. Capsules 3-6.5 cm long, linear, cylindric erect, beaked, glabrous, 10-ribbed, 5-valved; valves with transverse partitions between the seeds. Seeds trigonous, black .

1.3 Taxonomy:

The genus *Corchorus* is classified under the subfamily Grewioideae of the family Malvaceae. It contains around 40 to 100 species.

The genus Oceanopapaver, previously of uncertain placement, has recently been synonymized under Corchorus. The name was established by Guillaumin in 1932 for the single species Oceanopapaver neocaledonicum Guillaumin from New Caledonia. The genus has been classified in a number of different families including Capparaceae, Cistaceae, Papaveraceae, and Tiliaceae. The putative family name "Oceanopapaveraceae"

has occasionally appeared in print and on the web but is a nomen nudum and has never been validly published nor recognised by any system of plant taxonomy.

The genus Corchorus was first described by Linnaeus in his great work Species Plantarum (1753). It is derived from the Ancient Greek word (korkhoros or korkoros) which referred to a wild plant of uncertain identity, possibly jute or wild asparagus **[5].**

1.4Properties

-Considered carminative, cardiac, laxative, febrifuge, and tonic.
-Leaves considered stimulant, laxative, demulcent, appetizer and stomachic.
-The corchorin considered toxic and poisonous; some studies suggest a digitalis-effect on the heart.
-Fiber, one of nature's stronger vegetable fibers, is referred to as the "golden fiber." It is long, soft, shiny, 1 to 4 meters in length, with a diameter of 17 to 20 microns, with high insulating and anti-static properties, with moderate moisture regain and low thermal conductivity.

Figure1.2: Corchorus capsularis

1.5 Health Benefits and use:

• It is rich in calcium, phosphorus, iron and potassium.

• It has also been determined that 100 grams of saluyot contains an ample amount of Vitamin A, thiamine, riboflavin, ascorbic acid, and is also rich in fiber.

• It is also gives diet.

• The Ilocanos use saluyot in their preparation of dinengdeng and bulangbulang.

• The content is good for eyesight, as the vegetable contains beta-carotene.

• It is used to reduce wrinkles, it is also contains anti-oxidant substances.

• It is used traditionally to address concerns related to inflammation and pain.

• It is also been connected with curing the chronic inflammation of the urinary bladder.

• Ayurvedics use the leaves for ascites, pain, piles, and tumors.

•Elsewhere the leaves are used for cystitis, dysuria, fever, and gonorrhea .

1.6Traditional use:

Leaf: demulcent, bitter tonic, stomachic, laxative, carminative anthelmintic, astringent and intestinal antiseptic; Infusion of dried leaf- bitter and commonly used as a stomachic tonic like chiretta, also used in fever, bilious trouble dysentery, liver disorders, intestinal colic, gastric catarrh, skin diseases, atonic dyspepsia. Mild jaundice and disorder of digestive system, for the treatment of dysentery, dried leaves are eaten with rice; Decoction of dried wot and unripe capsule ': good for diarrhoea; Capsule : used as a paste over swellings and abscesses, purgative and contains vitamin C **[6].**

1.7 Taxonomic Hierarchy:

Table 1.1: Taxonomic Hierarchy of *Corchorus capsularis*

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridaeplantae – green plants
Infrakingdom	Streptophyta – land plants
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants, phanérogames
Infradivision	Angiospermae – flowering plants, angiosperms, plantas com flor, angiosperma, plantes à fleurs, angiospermes, plantes à fruits
Class	Magnoliopsida
Superorder	Rosanae
Order	Malvales
Family	Malvaceae – mallows, mauves
Genus	Corchorus L.
Species	Corchorus capsularis L. – jute

1.8 Chemical constituents of *Corchorus capsularis:*

Leaves:

The phytochemical screening of the leaves of *Corchorus* capsularis showed the presence of fiavonoid, saponins, tannins, steroids, and triterpenes.It is also rich in vitamin, carotenoid, calcium, k, and dietary fibres. Leaves of Corchorus capsularis contains two functional compounds: Phytol and Monogalactosyl diacyl glycerol. *Corchorus* capsularis also contains capsin, a glycoside which is responsible for the major bitter taste of the leaves.

Per 100 g, the leaves of *C. capsularis* are reported to contain 43-58 calories, 80.4-84.1 g water, 4.5-5.6 g protein, 0.3 g fat, 7.6-12.4 g total carbohydrate, 1.7-2.0 g fibre, 2.4 g ash, 266-366 mg Ca, 97-122 mg P, 7.2-7.7 mg Fe, 12 mg Na, 444 mg K, 6.41-7.85 mg beta-carotene equivalent, 0.13-0.15 mg thiamine, 0.26-0.53 mg riboflavin, 1.1-1.2 mg niacin, and 53-80 mg ascorbic acid. Leaves contain oxidase and chlorogenic acid. The folic acid content is substantially higher than other folacin-rich vegetables, ca 800 µg per 100 g (ca 75% moisture) or ca 3200 µg on a zero moisture basis.

Seed:

Active principle of the jute seed is corchorin, a glucoside ten times bitterer than quinine sulfate. Study yielded corchortoxin, another cardiac agent from the seeds. Study yielded another bitter, corchsularin from the seeds. Seed contains 2.25 percent of raffinose. Oil contains the glycerides of oleic acid, 39.18%; glycerides of linolic acid, 44.63 %; a small quantity of "crude archidic acid," 0.169%; and palmitic and stearic acid.

Bark & stem:

The polysaccharides and lignin are the major constituents in bark, stem and fibre of *Corchorus* capsularis . Glucose, fructose, sucrose, six low molecular weight sugar alcohols and two inositols are identified and quantified in bark. Bark and stem of *Corchorus* capsularis contain various free glycosidic and ester- linked phenolic acid. The ferulic and p-coumarin acids are the major components of phenolic acid in fresh bark **[7].**

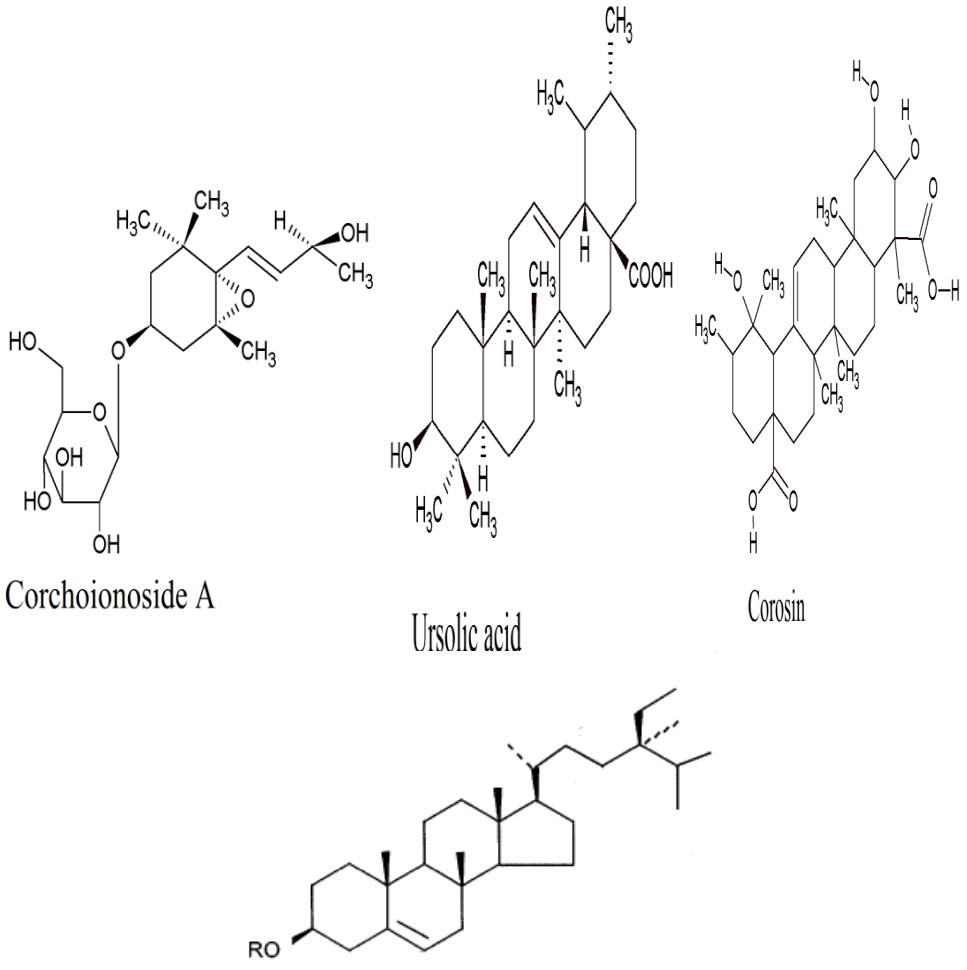


Figure1.3: Structures of some chemical constituents of *Corchorus capsularis*

Chapter- 2

LITERATURE REVIEW

2.1 Reviews on Phytochemical Investigation

The phytochemical screening of the leaves of *Corchorus* capsularis showed the presence of fiavonoid, saponins, tannins, steroids, and triterpenes.It is also rich in vitamin, carotenoid, calcium, k, and dietary fibres.

C. capsularis leaves also contain bitter tasting glycoside capsin, isocapsin & some more polar saponin component. The leaves of C. capsularis have yielded a novel dammarane triterpene glycoside which has been characterized on the basis of spectral data, as the 25, 30-0-β-diglucopyranoside of 20, 24-epoxy-3β, 12 β, 25, 30- tetrahydroxydammarane (capsugenin).

From the leaves of Corchorus capsularis a new dammarane triterpene glycoside, capsin, has been isolated. Capsin was identified as the 3-glucoside of 20, 24-epoxy-3β, 12β, 25, 30-tetrahydroxydammarane from spectral data. Capsin was tentatively assigned the (20S, 24S)-configuration by comparison with data available for similar compounds. One of the oxidation products of the aglycone appears to be a friedo-type derivative, formed by concerted methyl migration on decarboxylation of a C-30 carboxylic acid intermediate .Active principle of the jute seed is corchorin, a glucoside ten times bitterer than quinine sulfate. Corchortoxin, another cardiac agent found from the seeds.The polysaccharides and lignin are the major constituents in bark, stem and fibre of *Corchorus* capsularis . Glucose, fructose, sucrose, six low molecular weight sugar alcohols and two inositols are identified and quantified in bark **[8].**

2.2 Reviews on Biological Investigations

2.2.1 Anti-oxidant activity

The present study was carried out to determine the free radical scavenging properties of some plants found in Malaysia such as, *Muntingia calabura*; *Bauhinia purpurea*; *Dicranopteris linearis*; *Melastoma malabathricum*; *Corchorus capsularis*. The air-dried leaves of each plant (20 g) were soaked in distilled water (1:20; w/v) for 72 h at room temperature. The collected supernatants were tested for the free radical scavenging activity against the DPPH and superoxide anion radical scavenging assays. All extracts were found to show remarkable antioxidant activity in both assays with the percentage of inhibition (%) yielded 94–99% and 83–100%, respectively. Phytochemicals screening of all plants demonstrated the presence of flavonoids, saponins, triterpenes and steroids, but not alkaloids. Tannins were detected only in the leaves of *M. calabura*, *D. linearis*, *M. malabathricum*. The ability to scavenge free radicals indicates these plants could be used as a new source of antioxidant agents, and the activity seen could be attributed to the synergistic effect of various bioactive compounds present in these extracts, particularly of the flavon-oids type. Further study has been designed in our laboratory to isolate and to identify the bioactive com-pounds responsible for the observed antioxidant activity **[9].**

2.2.2 Cardiovascular Activities

Corchortoxin (strophanthidin) is a cardiac aglycone isolated from Corchorus capsularis seeds, showed a cardiac activity. These activities are similar to digitalis genius but jute seed's extract showed better activities than it is. Corchorosol derivatives from Corchorus capsularis are more effective on heart diseases than corchorosides. Showed decrease parenteral & increase acetylating degrees with Corchorus derivatives.

2.2.3 Antinociceptive and Anti-inflammatory Properties

The antinociceptive and anti-inflammatory properties of *Corchorus capsularis* leaves chloroform extract were investigated in experimental animal models. The antinociceptive activity was measured using the writhing, hot plate and formalin tests, while the anti-inflammatory activity was measured using the carrageenan-induced paw edema test. The extract, obtained after 72 h soaking of the air-dried leaves in chloroform followed by *in*

vacuo evaporation to dryness, was weighed and prepared by serial dilution in DMSO in the doses of 20, 100 and 200 mg/kg. The extract was administered 30 min prior to subjection to the respective assays. The extract was found to exhibit significant (p<0.05) antinociceptive and anti-inflammatory activities. As a conclusion, the present study confirmed the traditional claims of using *C. capsularis* to treat various ailments related to inflammation and pain **[10].**

2.2.4 Hepatobiliary Activities

Corhcorus capsularis green leaves powders are cholesterol free, lowered hepatic cholesterol condition and increased neutral fecal bile acid condition & neutral sterol excretion in rats.

2.2.5 Activity against gastrointestinal nematodes

Effects of neem, betel leaf, devil‟ s tree, jute and turmeric against natural gastrointestinal nematodes in sheep and on some hematological parameters (TEC, Hb and PCV) and body weight were studied. Thirty (30) naturally parasitized sheep were randomly divided into six groups(A, B, C, D, E and F), each consisting of five sheep. Ten percent water extract of leaves of neem, betel leaf, devil‟ s tree and jute were administered orally to the sheep of group A, B, C and D, respectively. Sheep of group E was treated orally with 10% water extract of rhizome of turmeric. Sheep of group F was kept as infected control group. Fecal samples, hematological parameters and body weight were examined before treatment and on 7th, 14th, 21st and 28th day. A significant (p<0.01) reduction of EPG count was found following administration of neem (37.60-47.03%), betel leaf (6.43-14.00%), devil‟ s tree (3.04-11.04%), jute (0.50-5.26%) and turmeric (0.46-8.30) in sheep. The EPG count of the control group (F) were significantly (p<0.01) increased up to the last day of experimental period. After treatment with neem, betel leaf, devil‟ s tree, jute and turmeric total erythrocyte count (TEC), hemoglobin (Hb) content and packed cell volume (PCV) were increased significantly (p<0.01 and p<0.05) in sheep. The body weight was increased significantly (p<0.01 and p<0.05) in neem, betel leaf, devil‟ s tree, jute and turmeric treated sheep. On the other hand, body weight was decreased in

untreated control group. The present study reveal that 10% water extract of neem was moderately effective and betel leaf, devil‟ s tree, jute and turmeric were relatively less effective against gastrointestinal nematodes in sheep .

2.2.6 Antitumor Promoters

Two antitumor promoters against tumor promoter-induced Epstein-Barr virus activation were isolated from the leaves of jute (*Corchorus capsularis* L.). The antitumor-promoting activity was examined by an immunoblotting analysis. Their active components were identified as phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) and monogalactosyldiacylglycerol (1,2-di-*O*-α-linolenoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol) by spectroscopic data and chemical and enzymatic reactions. The content of the latter in four cultivars of *C. capsularis* L. and *C. olitorius* L. was found to vary with the cultivar. Amount of each active component increased by treatment of the leaves with hot water **[11].**

2.2.7 Antibacterial activity

The phytochemistry and antimicrobial potential of *Corchorus olitorius* leaf extracts on four bacterial isolates was investigated using both agar diffusion and tube dilution methods. Aqueous and methanolic extracts were tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Staphylococcus aureus*. Extracts concentration of 500, 125, 125 and 62.5 mg /ml were used while tetracycline was used as the standard drug. The phytochemical investigation revealed the presence of hydrocyanin, cardiac glycosides (+++) and tannins (+), flavonoids (++), anthraquinones (++) and saponins (++). Agar and tube dilution tests of both aqueous and methanolic extracts indicated that the extracts had antimicrobial activities against the four bacterial isolates, though the methanolic extracts had wider diameter of inhibition and activity indices than the aqueous extracts. Susceptibility increased with concentrations and highest susceptibility was observed against *E. coli* and other isolates at 500 mg/ml-1. The extracts exhibited the high antimicrobial activity (Activity Index, A.I with respect to the standard drug used). This could be adduced to the presence of phytochemical constituents

and can be of prophylactic importance. Its highest potency against *E. coli* justifies its therapeutic use by traditional healers in South-Western Nigeria for gastroenteritis with good results .

2.2.8 Renal & Hematological changes

Low doses of the plant extract of Corchorus genus did not exhibit any significant change of creatinine serum protein levels but the high dose level significantly increases creatinine levels.

Multiple weekly dose of methanolic extract of Corchorus capsularis (15, 20, 25 kg. i.p) on liver & kidney functions & hematological parameters of mice were studied, but no significant alterations of R.B.C count & Hemoglobin content were shows **[12].**

Chapter- 3

PREPARATION OF PLANT EXTRACT FOR EXPERIMENT

3.1 Plant selection:

From the literature review it is seen that there is little of work on the plant *Corchorus capsularis* about the pharmacological activity of the plant. But there is a least of work has been found about chemical investigation of this plant, especially about the leaves of this plant. So I got a chance to select the leaves of *Corchorus capsularis* for my research work to see whether the leaves have antioxidant and antimicrobial activity or not.

3.2 Plant collection:

After selection of plant it is must to collect the plant parts for the research purpose. Throughout Bangladesh the plant *Corchorus capsularis* is available. The plant sample was collected from Dhaka on 27th June, 2013.

3.3 Plant Identification:

It is advisable to attempt field identification of sample collected. Finally, from BNH (Bangladesh National Herbarium) the identification or accession number of collected sample was got and the accession number is 37901 with *Corchorus capsularis* and *Tiliaceae* scientific name and family name of the plant respectively.

3.4 Drying of plant sample:

After the collection of sample it needs to be dried to make the sample extract. In general the plant material should be dried at temperature below 30 0C to avoid the decomposition of thermolabile compounds. So sun drying can be very effective but drawback is sometimes water molecules are absorbed by the sample and hence microbial growth can

affect the phytochemical study. The leaves were dried in the sun light thus chemical decomposition could not take place.

3.5 Grinding of dried sample:

Small amount of plant material can be milled using grinder or blender. But if the sample is in high amount then it is easier to get powdered sample by grinding from a spice mill. Grinding improves the efficiency of extraction by increasing surface area. It also decreases the amount of solvent required for the extraction. The dried samples were ground to coarse powder with a mechanical grinder (Blender) and powdered samples were kept in clean closed glass containers pending extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other foreign matter deposited on the grinder.

3.6 Maceration of dried powdered sample

3.6.1 Principle:

The choice of extraction procedure depends on the nature of the plant material and the components to be isolated. The principle of solid-liquid extraction is that when a solid material comes in contact with a solvent, the soluble components in the solid material move to the solvent**.** Thus, solvent extraction of plant material results in the mass transfer of soluble active principle (medicinal ingredient) to the solvent, and this takes place in a concentration gradient. The rate of mass transfer decreases as the concentration of active principle in the solvent increases, until equilibrium is reached, i.e. the concentrations of active principle in the solid material and the solvent are the same. Thereafter, there will no longer be a mass transfer of the active principle from plant material to the solvent. Since mass transfer of the active principle also depends on its solubility in the solvent, heating the solvent can enhances the mass transfer. Moreover, if the solvent in equilibrium with the plant material is replaced with fresh solvent, the concentration gradient is changed **[13].**

3.6.2 Procedure:

After getting the sample as dried powdered, the sample (500 gram) was then soaked in 1100mL of Methanol for two days, the process is known as maceration technique. A plastic jar was taken and washed thoroughly with ethanol and dried. Then the dried powder sample was taken in the jar. After that Methanol (80 mL) was poured into the jar up to 1-inch height above the sample surface as it can sufficiently cover the sample surface. The plastic cover with aluminium foil was closed properly to resist the entrance of air into the jar. This process was performed for two days. The jar was shaking in several times during the process to get better extraction. After the extraction process the plant extracts was filtered with sterilized cotton filter. The cotton was rinsed with ethanol and fitted in a funnel. The filtrate was collected in a beaker. Again sample (500 gram) was then soaked in 1000mL of Methanol with previous filtrate (300 ml) for two days and after the extraction process the plant extracts was filtered with sterilized cotton filter. The cotton was rinsed with ethanol and fitted in a funnel. The filtrate was collected in a beaker. Again sample (500 gram) was then soaked in 800mL of Methanol with previous filtrate (500 ml) for two days and after the extraction process the plant extracts was filtered with sterilized cotton filter. The cotton was rinsed with ethanol and fitted in a funnel. The filtrate was collected in a beaker.

3.7 Rotary:

After maceration process, sample gone through rotary process by which we got concentrated solution of sample.

3.8 Vacuum Liquid Chromatography

3.8.1 Principle:

Chromatographic purification is an integrated part of organic synthesis. The Dry Column Vacuum Chromatography presented here, has excellent resolving power, is easily applied to large scale chromatography (up to 100 g) and is fast. Furthermore, the technique is

economical and environmentally friendly due to significant reductions in solvent and the amount of silica used. Therefore, it is an excellent alternative to the commonly used Flash Column Chromatography for purification in organic synthesis.

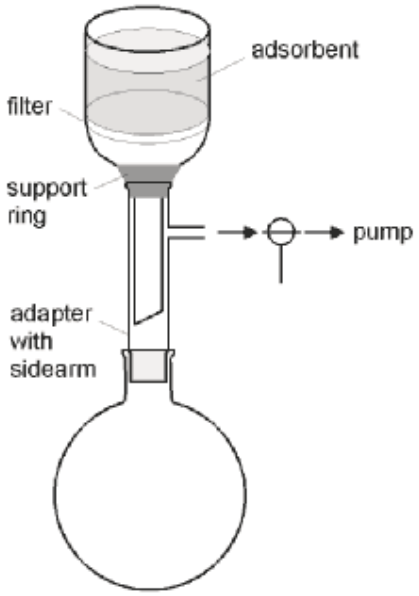


Figure 3.1: Vacuum Liquid Chromatography

3.8.2 Solvent selection:

1. N-hexane
2. Ethyl acetate
3. Methanol
4. Diethyl ether
5. Chloroform
6. Dichloromethane
7. Butanol

3.8.3 Procedure

The experimental procedure can be summarized as follows:

1. At first sample from rotary was mixed with 130 grams silica and filled in a sintered glass funnel of the appropriate size.
2. Vacuum is applied and the surface is pressed firmly to give a completely level, well compacted bed approximately 4.5–5.5 cm high.
3. Then solvents were poured into sintered glass funnel according to their polarity, non polar solvents were poured first then polar solvent poured.

4. The mixture to be separated according to the polarity of solvents.
5. Fractions were collected separately and fractions are monitored by TLC.

Chapter- 4

METHODS AND MATERIALS

4.1 THIN LAYER CHROMATOGRAPHY (TLC)

4.1.1 Principle:

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on aluminium foil, a sheet of glass, or plastic, which coated with a thin layer of adsorbent material, usually silica gel. Thin layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. The polarity of the solvent systems was increased to get a clear graph of all the possible compounds present in the extract **[14].**

Table 4.1: Compositions of various solvent systems for TLC

Nonpolar Basic solvent	**Intermediate polar Basic Solvent**	**Polar Basic solvent**
Benzene 9mL **Ethanol 1mL** **Ammonium hydroxide 0.1mL**	Chloroform 5Ml Ethyl acetate 4mL Formic acid 1mL	Ethyl acetate 8mL Ethanol 1.2mL Water 0.8mL

4.1.2 Apparatus:

1. TLC tank
2. TLC plate
3. Pencil
4. Scale
5. Spray bottle
6. Capillary tube

4.1.3 Reagents:

1. Chloroform (EMSURE Chloroform, Germany)
2. Ethyl acetate (Ethyl acetate, Germany)

3. Formic acid (UNI-CHEM Formic acid, Germany)
4. DPPH solution
5. H2SO4 solution
6. water

4.1.4 Procedure:

1. Intermediate polar solvent system was prepared.
2. In the next step, TLC plate was prepared and in TLC plate eight spots were spotted which represent each fraction
3. After spotting TLC plate was exposed to the solvent system
4. After completing of TLC, plate was exposed to 10% sulphuric acid.
5. After charring plate was dried and heated for charring which will assist in the fixation of spot and spot will be prominently visible.
6. Plate was also dipped into DPPH solution in dark place and dried.
7. Again, in the TLC plate three spots were spotted which were representative of n hexane, butanol and chloroform fractions and followed the same procedure above.

4.2 TOTAL POLYPHENOLIC ASSAY

4.2.1 Principle:

Total phenolic content of each extract was determined by Folin–Ciocalteu procedures. A sample's reducing capacity actually measured by Folin–Ciocalteu reagent made in Germany. Chemical nature of the FC reagent of extract is not known but it is believed that it should contain heteropolyphosphotunstates - molybdates. Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly (PMoW11O40)4-. It is believed that electron-transfer reaction occurs between reductants and Mo normally and the molybdenum is easier to be reduced in the complex **[15].**

4.2.2 Apparatus:

1. analytical balance
2. Test tube
3. Spatula
4. Pipette
5. Pipette pumper
6. Uv- spectrophotometer

4.2.3 Reagents:

1. Folin-Ciocalteu (Folin-Ciocalteu, Germany)
2. Salicylic acid
3. Sodium bicarbonate
4. Distill water
5. Methanol (METHANOL G.R.GRADE, Bangladesh)

4.2.4 Procedure:

1. 1 mg/100 µl or 100 µl of plant extract was taken in a test tube

2. 5 ml of Folin – ciocalteu (Diluted 10 fold) reagent solution was added into the test tube

3. 4 ml of Sodium carbonate solution was added into the test tube.

4. The test tube was incubated for 30 minutes at 200C to complete the reaction **[15].**

5. Then the absorbance of the solution was measured at 765 nm using a spectrophotometer against blank.

6. Methanol was taken as typical blank solution.

7. The Total content of phenolic compounds in plant methanol extracts in salicyclic acid equivalents was calculated by the following formula equation

$C = (c \times V)/m$

Where:

C = total content of phenolic compounds, mg/g plant extract,

c = the concentration of salicylic acid established from the calibration curve, mg/ml;

V = the volume of extract, ml;

m = the weight of pure plant methanolic extract, g.

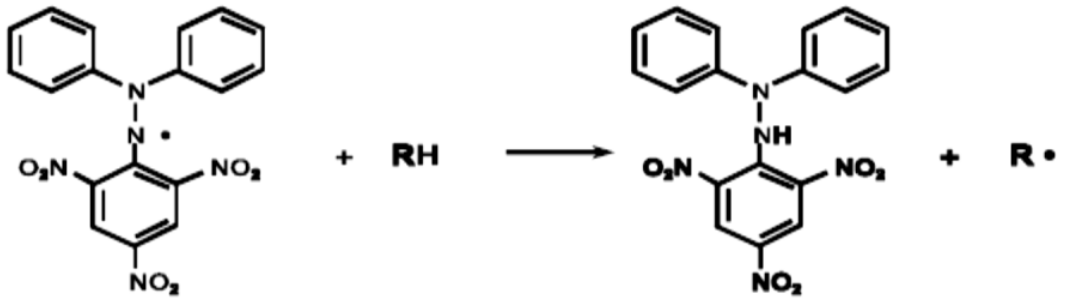
7. By the equation, $y = 0.011x$, $R^2=0.998$ equivalent amount are measured.

4.3 ANTI-OXIDANT TESTS

DPPH Test (1, 1-diphenyl-2-picrylhydrazyl radical)

4.3.1 Principle:

The most commonly used antioxidant test is DPPH Test (1, 1-diphenyl-2-picrylhydrazyl radical)**.** In DPPH test, at ambient temperature antioxidant efficiency is measured so it eliminate the risk of thermal degradation of molecules tested. The hydrogen or electron donation abilities of extracts are measured from the bleaching of the purple colored methanol solution of 1, 1-diphenyl-2-picrylhydrazyl. In the radical form this DPPH molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule. With this method it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 517 nm **[16].**



DPPH
517 nm

4.3.2 Apparatus:

1. Analytical balance
2. Test tube
3. Beaker
4. Spatula
5. Pipette
6. Pipette pumper
7. Uv- spectrophotometer

4.3.3 Reagent:

1. DPPH
2. Ascorbic acid
3. Methanol (METHANOL G.R.GRADE, Bangladesh)
4. Water

4.3.4 Procedure:

1. The DPPH radical scavenging method was used for the determination of the antioxidant capacity of the extracts. Different concentrations of the plant extract (10, 20, 30, 40,and 50µl,) were added at an equal volume (5ml) to methanol solution of DPPH

.

2. Different concentrations of Ascorbic acid (10 mg/ml) were used as the standard antioxidant

3. After 30 min at room temperature, the absorbance values were measured at 517 nm on a spectrophotometer and converted into the percentage antioxidant activity using the equation, DPPH antiradical scavenging capacity (%) = [1 – (Abof sample – Abof blank)/Abof control] × 100.

4. DPPH solution plus methanol was used as a control.

4.3.5 Standard Preparation:

1. 250 mg Ceevit Tablet (Square) was crushed in mortar and pestle.

2. 10 ml distilled water was added and the solution was filtered.

3. The solution was taken in 5 test tubes to prepare 5 different concentrations.

4. (100, 200, 400, 60, and 800µl,) solutions were taken in 5 different test tubes and the volume adjusted to 5 ml with water in all the test tubes.

4.3.6 Sample Preparation:

1. 10, 20, 30, 40, and 50µl concentrations of plant extract were taken in 5 different test tubes and the volume adjusted to 5 ml with methanol in all the test tubes.

4.3.7 Control Preparation:

DPPH solution plus methanol was used as a control.
In all the test tubes 100 µL DPPH solution was added in dark and left for 30 minutes. After that UV absorbance was measured in UV machine at 517 nm.

4.4 ANTI-MICROBIAL ASSAY

4.4.1 Principle:

Performance of antimicrobial susceptibility testing is an important task of the clinical microbiology laboratory. The goals of testing are to detect plant extract resistance in common pathogens .Disc diffusion method is the most widely used testing method. Disc diffusion method provides flexibility and possible cost savings .Each method has strengths and weaknesses that is organisms may be accurately tested by the method. Some methods provide quantitative results (eg, minimum inhibitory concentration), and

all provide qualitative assessments using the categories susceptible, intermediate, or resistant.

4.4.2 Principle of Disc Diffusion Method:

The disk diffusion susceptibility method is simple and well-standardized. Bacterial inoculums are applied to the surface of a large agar plate. Antibiotic discs and disc of test materials are placed on the inoculated agar surface. Plates are incubated for 16–24 h at 35°C prior to determination of results. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition.The zones of growth inhibition are measured to the nearest millimeter around each of the antibiotic disks. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium.The results of the disk diffusion test is qualitative **[17].**

4.4.3 Apparatus and Reagents:

1. Nutrient Agar Medium

2. Petri dishes

3. Filter paper

4. Laminar air flow hood (Esco Laminar Flow Cabinet, USA)

5. Autoclave (HIRAYAMA, Japan)

6. Sterile cotton

8. Refrigerator

9. Micropipette

10. Incubator

11. Ethanol

12. Sterile forceps

4.4.4 Test Materials of *Corchorus capsularis :*

Methanolic extract of *Corchorus capsularis* leaves were taken as test sample.

4.4.5 Test Organisms

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

Table 4.2: List of micro-organisms used

Gram positive Bacteria	**Gram negative Bacteria**	**Yeast**	**Fungus**
Bacillus subtilis	*Shigella boydii*	*Candida albicans*	Bacillus megaterium
Staphylococcus aureus	Salmonella typhi	*Saccharomyces cerevisiae*	
Beta hemolytic streptococcus	E.coli		
Bacillus cereus	Kleb siella		
Streptococcus pyrpgen	Vibrio mimicus		

4.4.6 Preparation of the Medium

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

4.4.7 Sterilization Procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood (name) and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 1210C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

4.4.8 Preparation of the Test Plate

The test organisms were transferred from the subculture to Petri dish containing about 10 ml of melted and sterilized agar medium. Then a sterilized cotton bud was taken and dipped into the bacterial/ fungal suspension. Then the bacterial/fungal sample is applied to the Petri dish with the help of this cotton bud.

4.4.9 Preparation of Discs

4.4.9.1 Standard Discs

These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, Vancomycin (30µg/disc) standard disc was used as the reference.

4.4.9.2 Preparation of Sample Discs with Test Sample

Measured amount of each test sample was dissolved in specific volume of solvent to obtain the desired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank Petri dish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

4.4.10 Diffusion and Incubation

The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi. The plates were then kept in a refrigerator at 40C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 370C for 24 hours.

4.4.11 Determination of Antimicrobial Activity by Measuring the Zone of Inhibition

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

4.5 BRINE SHRIMP LETHALITY BIOASSAY

4.5.1 Principle:

Brine shrimp lethality bioassay is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of pharmacological activities e.g. anticancer, antiviral, and pharmacological activities of natural products etc.Bioactive compounds are almost always toxic in high doses. Pharmacology is simply toxicology at a lower dose or toxicology is simply pharmacology at a higher dose. Thus (in-vivo) lethality, a simple zoological organism (Brine shrimp napulii- *Artemia salina*) can be used as a convenient monitoring for

screening and fractionation in the discover of new bioactive natural products **[18].** Natural product extracts, fractions or pure compounds can be tested for there bioactivity by this method. This bioassay is indicative of cytotoxicity and a wide range of pharmacological activity of natural products. Brine shrimp is the English name of the genus *Artemia* of aquatic crustaceans. *Artemia*, the only genus in the family Artemiidae,

4.5.2 Hatching of shrimp:

Artificial sea water was prepared by dissolving 38 g of NaCl in one liter of distilled water and was filtered to get a clear solution. A rectangular tank was divided in to two unequal compartments by a porous separator. The larger compartment was darkened while the smaller one was kept illuminated. The eggs of *Artemia salina* were hatched at room temperature (25-30 ºC) for 18-24 h. The larvae (nauplii) were attracted by the light and moved to the smaller compartment through the holes. They were then collected by a Pasteur pipette.

4.5.3 Apparatus and Reagents:

1. Test tube

2. Vial

3. Magnifying glass

4. Pasteur pipette

5. N-hexane
6. Butanol
7. Methanol
8. Ethyl acetate

4.5.4 Procedure:

1.Solid sample was dissolved in respective solvent and liquid samples were transferred to vials to get concentrations of 10, 5, 2.5, 1.25 and 0.625 in 4 ml artificial sea water with ten nauplii in each vial.

2. Blank contain 4 ml artificial sea water with ten nauplii .

3. After 24 hr incubation at room temperature (25-30 ºC), the number of viable naupliis were counted using a magnifying glass.

4.6 HAEMAGGLUTINATION ASSAY (HA)

4.5.5 Principle

Many viruses attach to molecules present on the surface of RBCs. A consequence of this is that at certain concentrations, a viral suspension may bind together (agglutinate) the RBCs, thus preventing them from settling out of suspension. Since agglutination is rarely linked to infectivity, attenuated viruses can therefore be used in assays. By serially diluting a virus suspension into an assay tray (a series of wells of uniform volume) and adding a standard amount of blood cells, an estimation of the number of virus particles can be made. While less accurate than a plaque assay, it is cheaper and quicker (taking just 30 minutes). This assay may be modified to include the addition of an antiserum. By using a standard amount of virus, a standard amount of blood cells, and serially diluting the antiserum, one can identify the concentration of the antiserum (the greatest dilution which inhibits hemagglutination). The linking together of the red blood cells by the viral particles results in clumping. This clumping is known as haemagglutination [19].

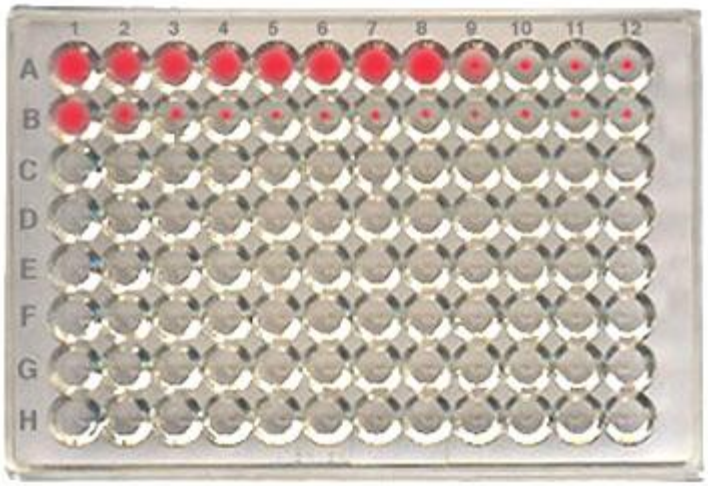


Figure 5.1: Haemagglutination Experiment

4.6.2 Apparatus

1. Centrifuge machine
2. Ephindorf tube
3. Micro pipette
4. Cotton
5. Refrigerator
6. Test tube
7. Eppendorf tube box

4.6.3 Reagents

1. Isotonic phosphate buffer
2. Blood

4.6.4 Procedure

1. Stock solution of the test sample was prepared at concentration of 1 mg/100µl and each solution was serially diluted.

2. Fresh blood from healthy person was collected only for the test of Haemagglutination Assay (HA).

3. Then the all bloods were centrifuged and the erythrocytes were separated

4. 1% erythrocyte suspension was prepared in phosphate buffer (pH 7.4).

5. 100µl ml of the test sample dilution was taken with 100µl ml of 1% erythrocyte and incubated at 25°C.

6. After incubation, the results were noted. Smooth button formation in bottom indicated negative activity, while a rough granular deposition at bottom showed positive activity.

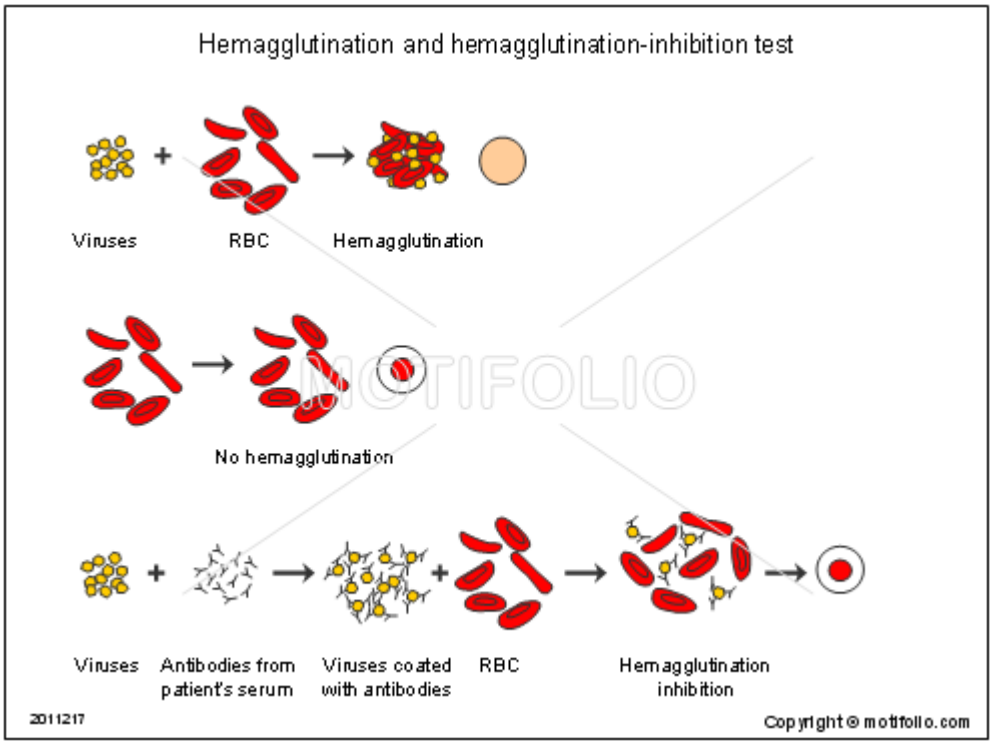


Figure 5.2 : Hemagglutination and hemagglutination –inhibition test

Chapter- 5

RESULT AND DISCUSSION

5.1 Thin Layer Chromatography (TLC)

The results obtained after TLC of the methanolic extract of the *Corchorus capsularis* leaf in solvent system 2 is given below-

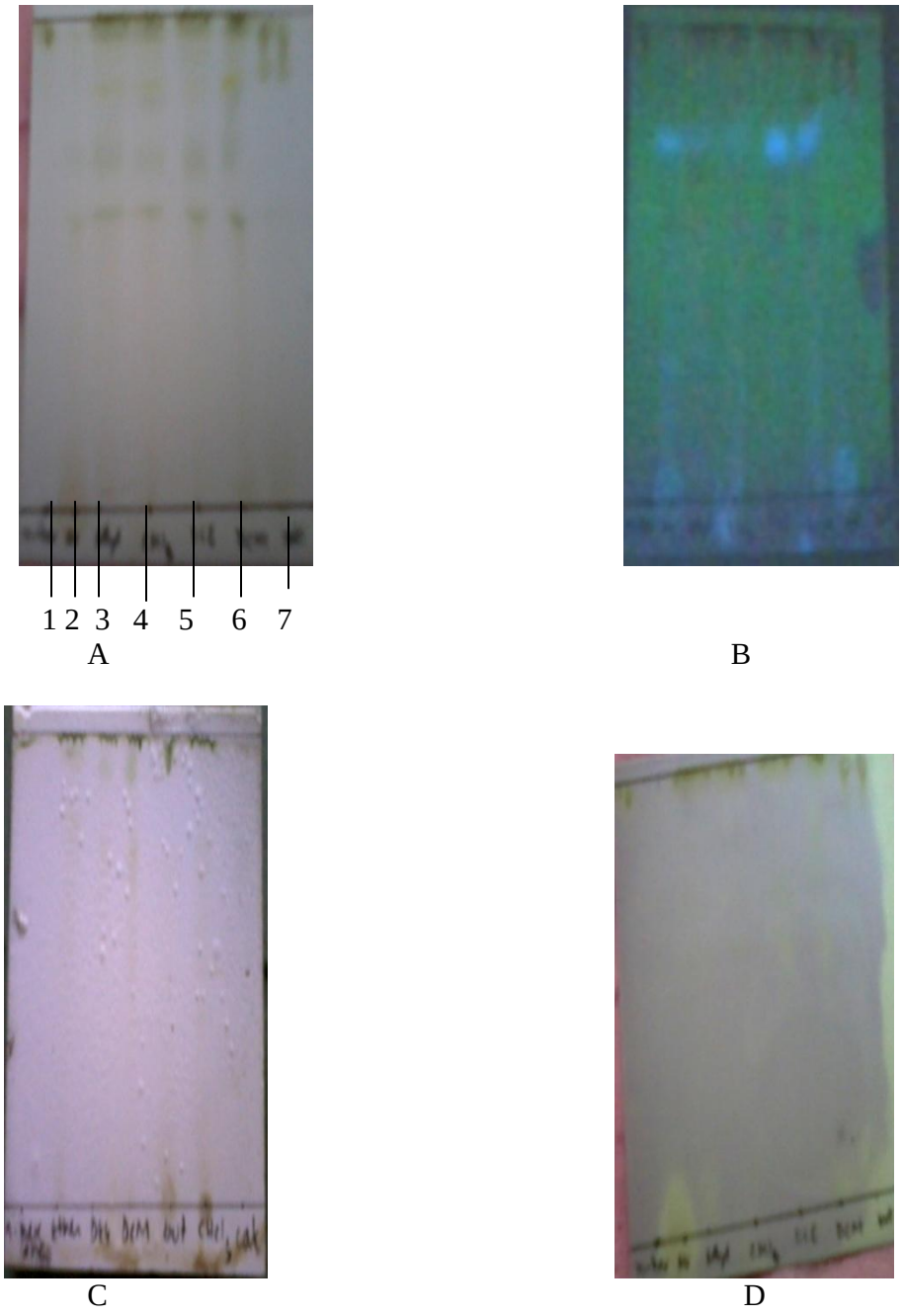


Figure 5.1 : Results for TLC in intermediate polar solvent. Here 1= N-hexane, 2= Methanol, 3=Ethyl acetate, 4= Chloroform, 5= Diethyl ether, 6= Dichloromethane and 7= Butanol. It is applicable for above 4 plates. (A= naked eye view; B = UV light view; C= after charring and D= after application of DPPH).

The naked eye view of the TLC was mentioned in the plate “a” which showed some clear spot. Then the plate was observed under UV which is shown in the plate “b”. It showed some spots which indicate the presence of different compounds in that sample. After charing of the TLC plate with sulfuric acid has showed (plate c) spots at the bottom as well as at the top of the TLC plate.

Spraying of DPPH solution on the TLC plate have shown significant formation of pale yellow color (plate 4) in the place of the spots which indicates significant free radical scavenging property of that fraction

Again results obtained after TLC of the butanol, n hexane and chloroform fraction of the methanolic extract of the *Corchorus capsularis* leaf in solvent system 2 is given below

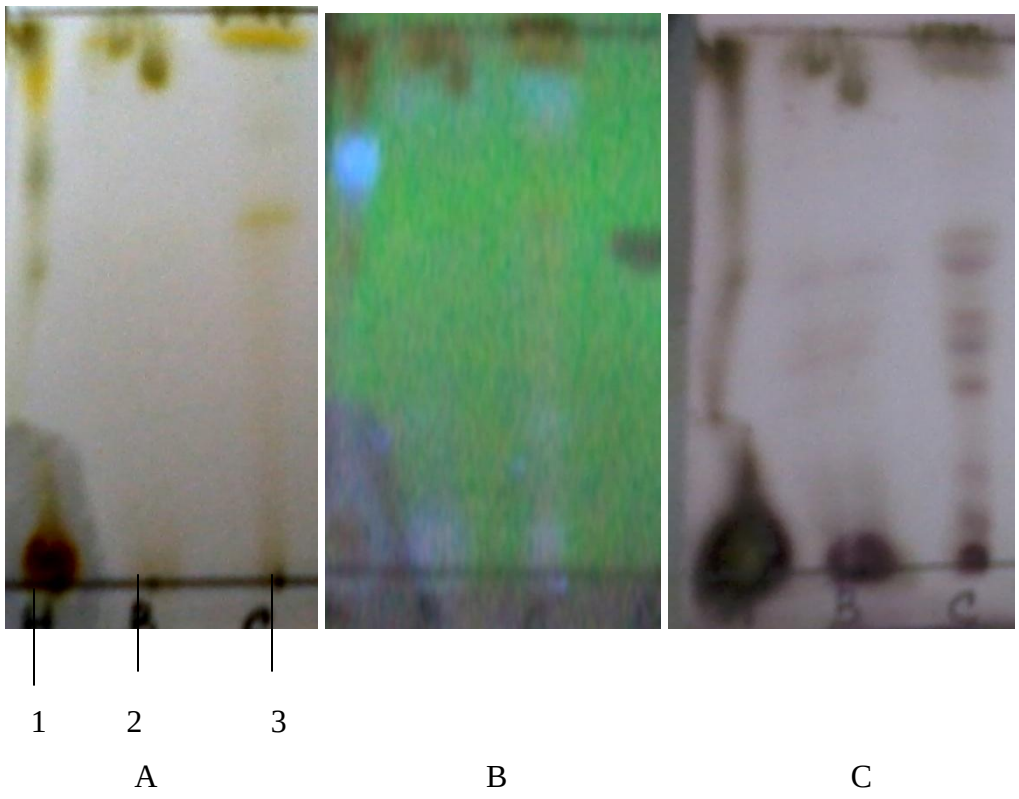


Figure 5.2: Results for TLC of the butanol, n hexane and chloroform fraction in intermediate polar solvent. Here 1= N-hexane, 2= Butanol and 3= Chloroform. (A= naked eye view; B = UV light view; C= after charring)

5.2 Anti-oxidant Tests

Anti-oxidant tests were classified by various sections like DPPH Test, Reducing power activity test, Total phenolic assay, Total flavanoid assay, Hydrogen peroxide radical scavenging assay. Samples were subjected as methanolic extract to various standard methods to determine various scavenging capacity and amount that is equivalent to various standards like ascorbic acids, salicylic acids.

5.2.1 DPPH Test

The crude methanolic extract of *Corchorus capsularis* (leaves) was subjected to free radical scavenging activity and it showed significant free radical scavenging activity.

The percentage inhibition of free radical DPPH of crude methanolic extract of *Corchorus capsularis* (leaves) are given in table 5.1 and figure 5.3

Table 5.1: Determination of free radical scavenging capacity for the standard (ascorbic acid)

Name	Concentration (µg/µl)	Absorbance	% of inhibition
Ascorbic Acid (S1)	40	0.114	45.71
Ascorbic Acid (S2)	80	0.108	48.57
Ascorbic Acid (S3)	120	0.103	50.95
Ascorbic Acid (S4)	160	0.100	52.39
Ascorbic Acid (S5)	200	0.098	53.33

.

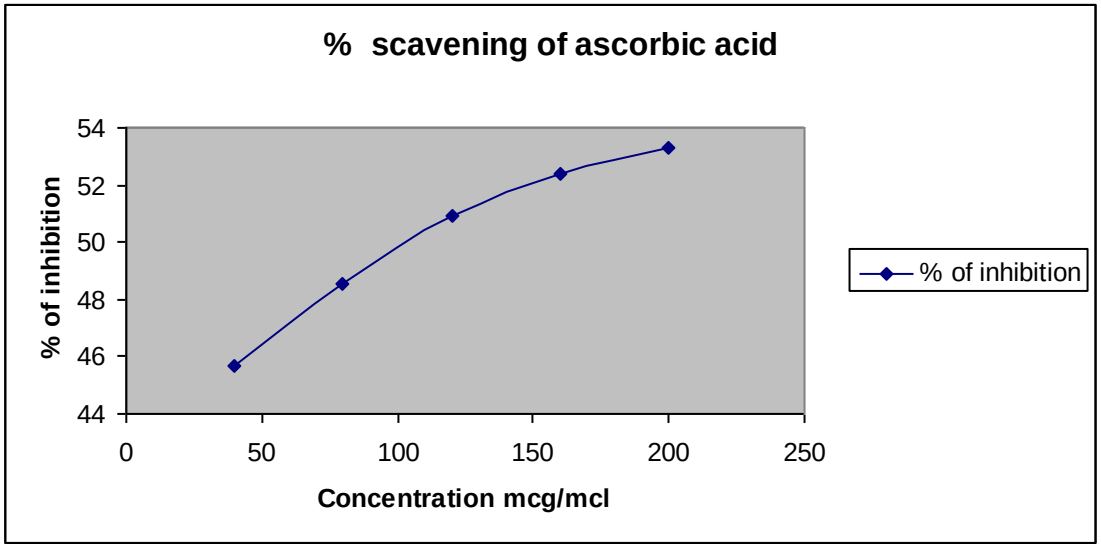


Figure 5.3: % scavenging of ascorbic acid

Table 5.2: Determination of free radical scavenging capacity for *Corchorus capsularis* crude methanolic extract

Name	Concentration (µg/µl)	Absorbance	% of inhibition
n-hexane 10	5	0.007	97.43
n-hexane 20	10	0.006	97.79
n-hexane 30	15	0.041	84.93
n-hexane 40	20	0.082	69.85
n-hexane 50	25	0.094	65.44
Butanol 10	5	0.255	27.21
Butanol 20	10	0.131	51.84
Butanol 30	15	0.120	55.88
Butanol 40	20	0.105	61.39
Butanol 50	25	0.106	61.03
Methanol 10	5	0.019	93.01
Methanol 20	10	0.038	86.03
Methanol 30	15	0.040	85.29

Methanol 40	20	0.074	72.79
Methanol 50	25	0.065	76.10
Ethyl acetate 10	5	0.239	12.13
Ethyl acetate 20	10	0.225	17.28
Ethyl acetate 30	15	0.174	36.03
Ethyl acetate 40	20	0.162	40.44
Ethyl acetate 50	25	0.173	36.39
Name	Concentration (µg/µl)	Absorbance	% of inhibition
Dichloromethane 10	5	0.409	-50.37
Dichloromethane 20	10	0.392	-44.18
Dichloromethane 30	15	0.332	-22.06
Dichloromethane 40	20	0.358	-31.62
Dichloromethane 50	25	0.347	-27.57
Chloroform 10	5	0.346	-27.21
Chloroform 20	10	0.365	-30.51
Chloroform 30	15	0.388	-42.65
Chloroform 40	20	0.454	-66.91
Chloroform 50	25	0.739	-171.69

Diethyl ether 10	**5**	**0.299**	**-9.93**
Diethyl ether 20	10	0.346	-27.21
Diethyl ether 30	15	0.363	-33.46

Diethyl ether 40	20	0.342	-25.74
Diethyl ether 50	25	0.284	-4.41

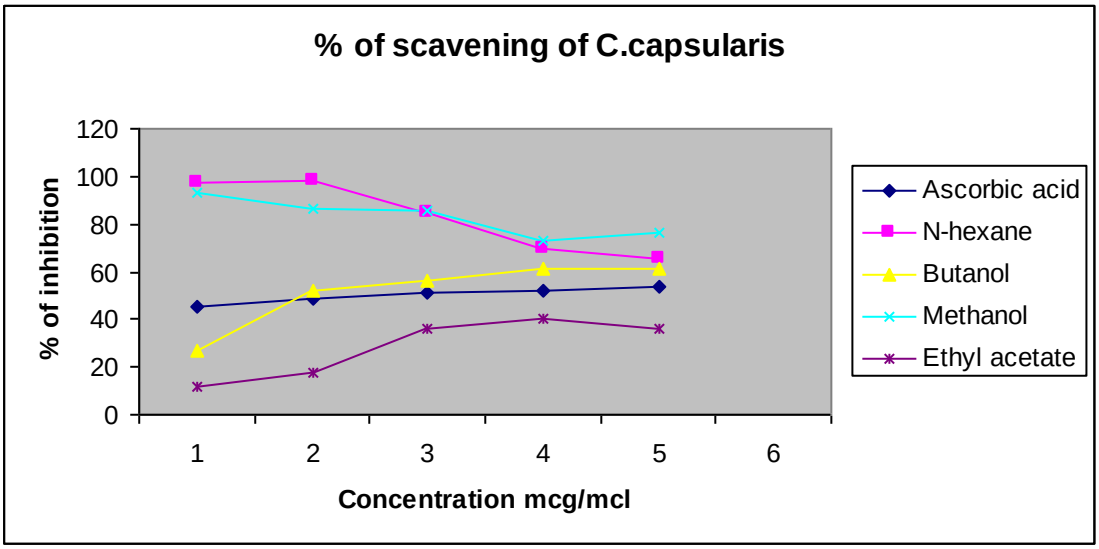


Figure 5.4: DPPH scavenging potential of C. capsularis crude methanolic extract with Ascorbic acid.

Values from table 5.2 show that Corchorus capsularis has antioxidant activity. Extract was tested for the free radical scavenging activity against the DPPH and superoxide anion radical scavenging assays. Extracts were found to show remarkable antioxidant activity in both assays with the percentage of inhibition (%) yielded 94–99% and 83–100%, respectively.

5.2.2 Total Polyphenolic Assay

The crude methanolic extract of *Corchorus capsularis* (leaves) was subjected to total phenolic content determination. Based on the absorbance values of the extract solution, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of Salicylic acid equivalents, result of the colorimetric analysis of the total phenolics are given in table.

Table 5.3: Standard curve preparation by using Salicylic acid

Salicylic Acid Concentration (Mg/ml)	Absorbance	Regression line	R2
5	0.162		
2.5	0.135	Y= 0.011x+0.105	R2= 0.998
1.25	0.119		
0.625	0.112		
0.3125	0.110		

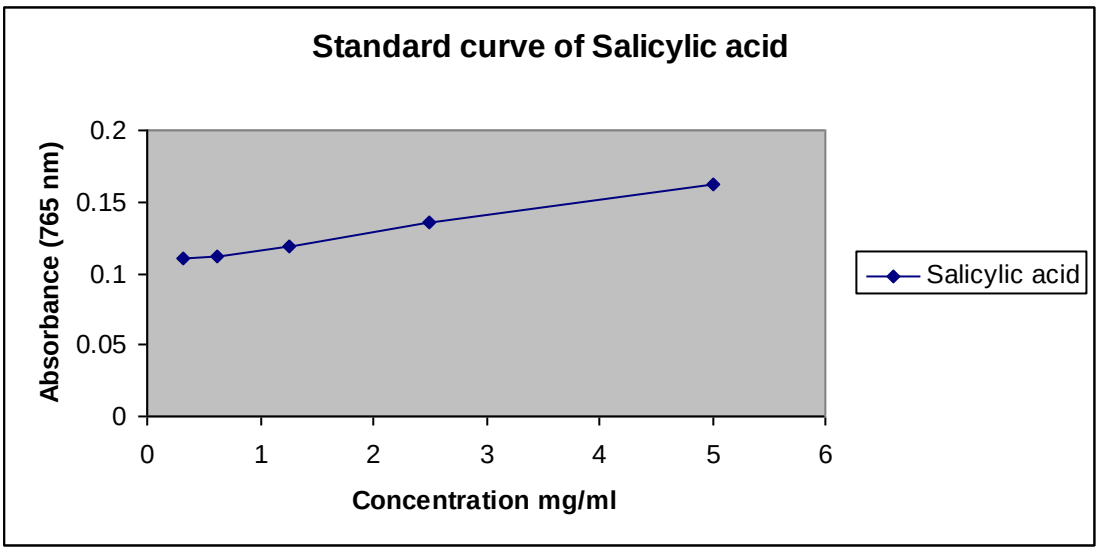


Figure 5.5: Standard curve of Salicylic acid (Y= 0.011x+0.105 and R2= 0.998)

Table 5.4: Determination of total phenolic content

Sample	Concentration (µg/µl)	Absorbance	Total Phenolic content (mg/ml)
n-hexane	100 µl	0.228	0.0111
Butanol	100 µl	1.281	0.10691
Methanol	100 µl	0.466	0.03282
Ethylacetate	100 µl	1.066	0.08736
Chloroform	100 µl	2.362	0.20518
Diethylether	100 µl	0.159	0.004
Dichloromethane	100 µl	0.256	0.01373

5.3 Anti-microbial Assay

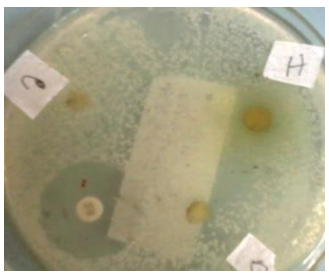
The methanolic crude extract of *Corchorus capsularis* (leaves) was subjected to the various bacterial cultures and from that zones of inhibition were measured. With the values obtained from the table are plotted in the figure which shows the zones of inhibition against specific microorganisms.

Table 5.5: Anti microbial screening was done on 6 sub types

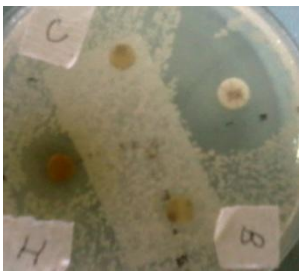
Nature of bacteria	Name of the microorganisms	Solvent	Zone of inhibition
Gram (-ve)	*E. coli*	Methanol Butanol Ethyi acetate n- hexane DCM Chloroform	0.8cm 1.0cm 0.8 cm 0.9cm 0.6 cm 0.7 cm
Fungi	*Candida albicans*	Chloroform n-hexane butanol	1.0cm 1.0cm 1.0cm
Fungi	*Saccharomyces cerevisiae*	Butanol Methanol n-hexane	1.5cm 1.3cm 0.8cm
Gram (+ve)	*Staphylococcus aureus*	n-hexane DCM Chloroform Ethyl acetate Butanol methanol	1.1cm 0.8cm 0.9cm 1.0cm 1.1cm 0.7cm
Fungi	*B. megaterium*	Butanol Ethyl acetate Chloroform n-hexane methanol DCM	1.1cm 0.7cm 1.0cm 0.9cm 0.7cm 1.0cm
Gram (+ve)	*B. hemolytic streptococcus*	Chloroform Butanol n-hexane	0.7cm 0.9cm 1.0cm

Table 5.6: Anti microbial screening was done on 12 sub types with positive control

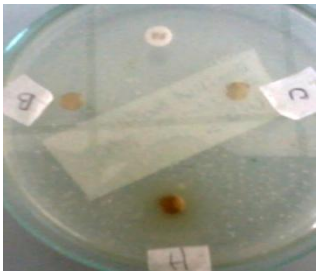
Nature of bacteria	Name of the microorganisms	Butanol	N-hexane	Chloroform	Standard (Vancomycin)
Gram (+ve)	*Beta hemolytic streptococcus*	10 mm	18 mm	0 mm	28 mm
Gram (+ve)	*Bacillus subtilis*	0 mm	15 mm	0 mm	29 mm
Gram (+ve)	*Bacillus cereus*	0 mm	17 mm	0 mm	25 mm
Gram (+ve)	*Staphylococcus aureus*	0 mm	20 mm	15 mm	30 mm
Gram (+ve)	*Streptococcus pyrogen*	0 mm	13 mm	0 mm	30 mm
Gram (-ve)	*Salmonella typhi*	0 mm	18 mm	0 mm	28 mm
Gram (-ve)	*Shigella boydii*	0 mm	13 mm	0 mm	23 mm
Gram (-ve)	*Kleb siella*	0 mm	9 mm	0 mm	30 mm
Gram (-ve)	*Vibrio mimicus*	0 mm	12 mm	0 mm	24mm
Fungi	*Candida albicans*	18 mm	15 mm	10 mm	25 mm
Fungi	*Saccharomyces cerevisiae*	O mm	15 mm	0 mm	26 mm
Fungi	*Bacillus megaterium*	0 mm	15 mm	0 mm	25 mm



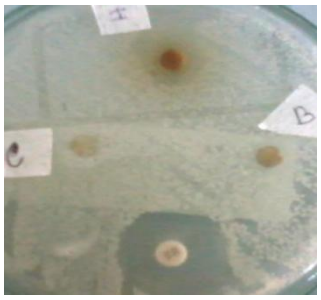
Bacillus cereus



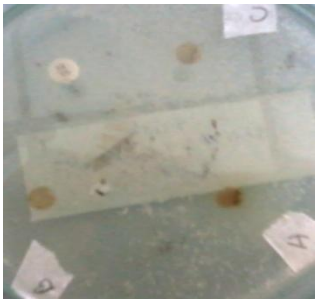
Bacillus megoterium



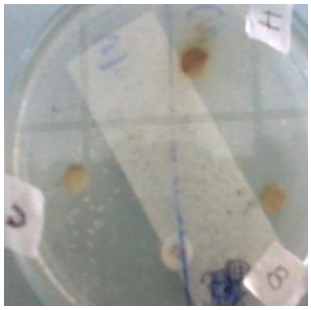
Bacillus subtilis



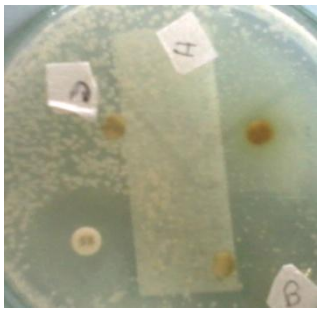
Beta hemolytic streptococcus



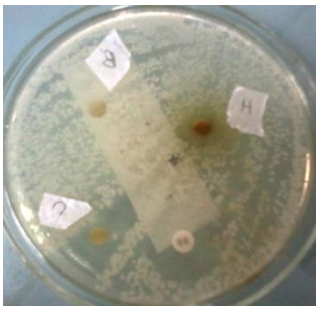
Kieb siella



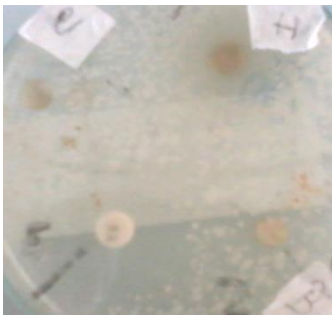
Candida albicans



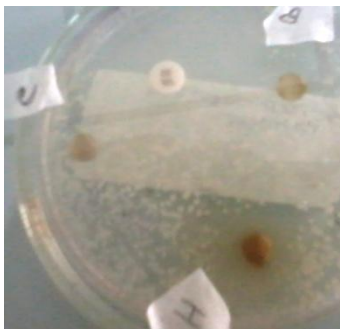
Saccharomyces cerevisiae



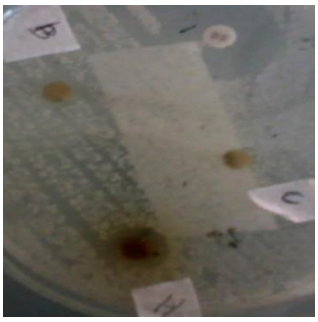
Salmonella typhi



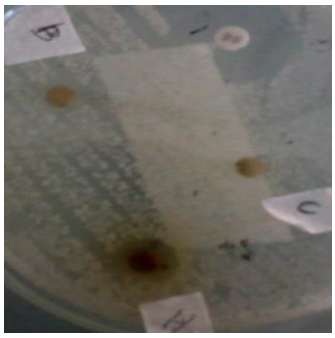
Shigella boyd



Staphylococcus aureus



Streptococcus pyrogen



Vibrio mimicus

Figure 5.6: Antimicrobial assay, Here, H=N-hexane, B=Butanol, C=Chloroform and White disc= Vancomycin.

Values from table 5.6 show that *Corchorus capsularis* has antimicrobial activity. N-hexane fraction of methanolic extract of leaves of *Corchorus capsularis* showed good acivities against gram positive bacteria, gram negative bacteria and fungi. Jute leaf (dried) (*Corchorus capsularis*) extract showed antibacterial activity against soft rot bacteria *Ecc* P-138 *in vitro* and effectively reduced the bacterial soft rot disease of different potato varieties in storage conditions. Since jute leaves are readily available without any cost in Bangladesh, the application of dry jute leaf extracts is a viable alternative to toxic chemical pesticides to control the soft rot diseases in Bangladeshi potatoes **[20].**

Values from table 5.6 indicate that Vancomycin is more sensitive to gram positive bacteria and little resistant to gram negative bacteria. . Gram positive bacteria showed 100% sensitivity to vancomycin in gram staining test by conventional method. Of the gram negative bacteria, 99.42% were resistant to vancomycin . Vancomycin test is simple, inexpensive and can be used in addition to Gram staining for rapid identification of bacterial cultures.

5.4 Brine Shrimp Lethality Test

Brine Shrimp lethality test was done which indicates cytotoxicity as well as a wide range of pharmacological activities.

Table 5.7: Determination of cytotoxicity of *Corchorus capsularis*

Sample	Concentration (mg/ml)	No. of nauplii alive	% of nauplii alive
n-hexane	5	0	0
	2.5	0	0
	1.25	0	0
	0.625	2	28.57
	0.3125	2	28.57
	control	7	100
Methanol	10	0	0

	5	0	0
	2.5	1	14.28
	1.25	1	14.28
	0.625	1	14.28
	control	7	100
ethyl acetate	10	1	14.28
	5	1	14.28
	2.5	2	28.57
	1.25	2	28.57
	0.625	2	28.57
	control	7	100
butanol	10	1	14.28
	5	0	0
	2.5	4	57.14
	1.25	5	71.14
	0.625	4	57.14
	control	7	100

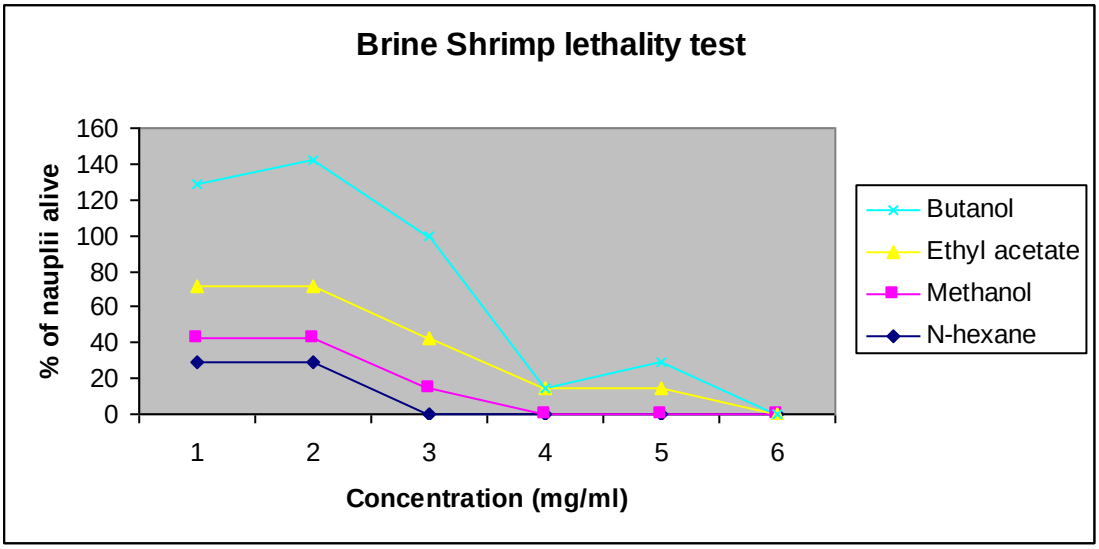


Figure 5.7: Cyto toxicity activity of methanolic extract of *Corchorus capsularis*

5.5 UV scanning

UV scanning was done on N-hexane fraction of methanolic extract of *Corchorus capsularis*.

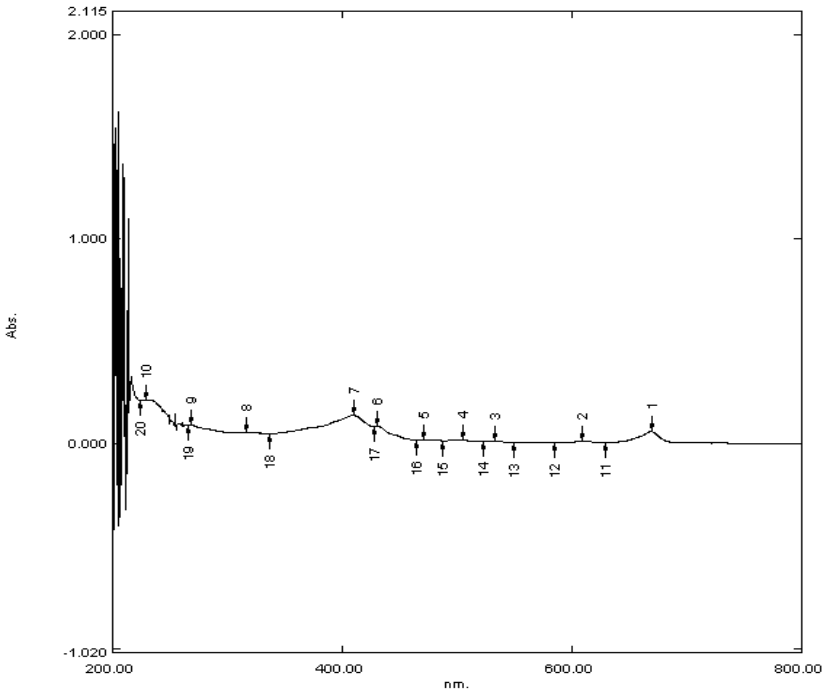


Figure 5.8: UV scanning of N-hexane fraction of methanolic extract of *Corchorus capsularis*.

Table 5.8: Wavelengths and descriptions of compounds found from UV scanning

No.	P/V	Wavelength nm. Abs.	Description
1		669	0.06
2		609	0.011
3		533	0.015
4		505	0.017
5		470.5	0.021
6		431	0.084
7		409.5	0.141
8		316.5	0.056
9		268.5	0.091
10		229.5	0.215
11		629.5	0.008
12		584.5	0.005
13		549	0.007
14		523	0.011
15		487.5	0.015
16		465	0.021
17		428	0.083
18		336.5	0.051

UV scanning was done on N-hexane fraction of methanolic extract of *Corchorus capsularis*. Almost eighteen different wavelength and description of compounds found from UV scanning and values from table 5.8, wavelength 409.5 nm is significant cause laboratory measurements of water vapor absorption using cavity ring-down spectroscopy revealed a broad absorption at 405 nm with a quadratic dependence on water monomer concentration, a similar absorption with a linear component at 532 nm, and only linear absorption at 570 nm in the vicinity of water monomer peaks. D_2O absorption is weaker and linear at 405 nm. Van't Hoff plots constructed at 405.26 nm suggest that for dimerization. This transition peaks at 409.5 nm, could be attributed to the 8th overtone of water dimer and the 532 nm absorption to the 6th overtone **[21].**

5.6 Haemagglutination Assay (HA)

Haemagglutination activity was found of 10µg/µl , 5µg/µl , 2.5µg/µl , 1.25µg/µl, 0.625µg/µl N-hexane fraction of methanolic extract of leaves of *Corchorus capsularis*.

Table 5.9: Haemagglutination test for methanolic extract of *Corchorus capsularis* (leaves)

Name	**con 10 µg/µl**	**con 5µg/µl**	**con 2.5 µg/µl**	**con 1.25 µg/µl**	**con 0.625µg/µl**	**con 0.3125µg/µl**	**Con 0.153µg/µl**	**con 0.0781µg/µl**	**con 0.0391µg/µl**	**Con 0.0195µg/µl**	**Con0.0098µg/µl**	**Con0.0049µg/µl**
n-hexane	+	+	+	+	+	-	-	-	-	-	-	-

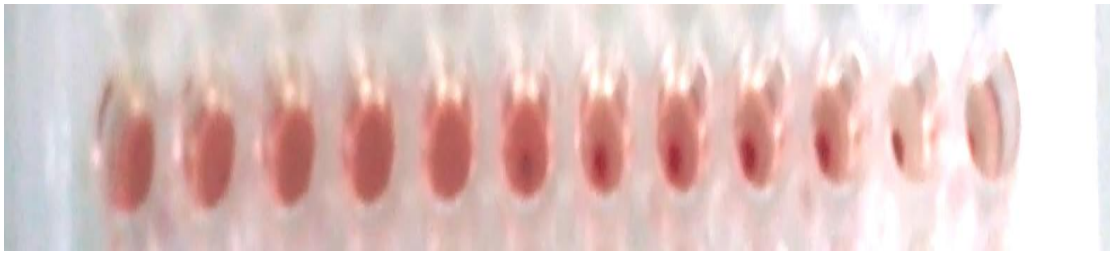


Figure 5.9: Haemagglutination test for methanolic extract of *Corchorus capsularis* (leaves) (n-hexane fraction)

From the table 5.9 and figure 5.9 it is seen that haemagglutination activities were found in 10µg/µl, 5µg/µl, 2.5µg/µl,1.25µg/µl and 0.625µg/µl N-hexane fraction of methanolic extract of leaves of *Corchorus capsularis*. From the table it is also seen that increase the activity with the increased concentrations.

Values from table 5.6 show that N-hexane fraction of methanolic extract of leaves of *Corchorus capsularis* showed good activities against gram positive bacteria, gram negative bacteria and fungi but its haemagglutination activities were found only in higher concentrations of the extract.

Bunium bulbocastanum was screened for its antibacterial, antifungal, phytotoxic and haemagglutination activities.The crude methanolic extract and n-hexane fraction showed significant (89%) and good activity (61%) against Staphylococcus aureus but crude methanolic extract and n-hexane fraction of B. bulbocastanum were unable to agglutinate RBCs of the human blood indicating that this species lack phytolectin so, there is no strong and significant relation between antimicrobial assay and haemagglutination assay **[22].**

Chapter- 6

CONCLUSION

CONCLUSION

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

Therefore, plant materials can be potential sources of chemically interesting and biologically important drug entrant. And for this purpose the plant can be further screened against various diseases in order to find out its unexplored efficacy with a gaze to the future with a great deal of expectancy.

Crude methanolic extract of *Corchorus capsularis* leaves of the family *Tiliaceae* is traditionally used in various disease conditions. Such as atonic dyspepsia, liver disorders and as febrifuge. Also used for chronic cystitis, gonorrhea, dysuria, worms in children, hepatic and intestinal colic, and for gastric catarrh. In my experiment it shows very positive result for Anti-oxidant activity, Antihaemolytic activity, Anti-inflammatory activity & good Haemaglutination activity. The plant also shows moderate antimicrobial activity. There are some established research reports regarding the phytochemical and pharmacological properties of this plant. Still there are plenty of scopes to establish a variety of properties which are significantly beneficial to mankind.

REFERENCES

1. Sarker, S.D., Nahar, L., Chemistry for Pharmacy Students General, Organic and Natural Product Chemistry ,*Journal of Natural product Chemistry*, **1**, 283-359 (2007).

2. Abo, K.A., Ogunleye, V.O., Ashidi, JS., Antimicrobial poteintial of Spondias mombin,
Croton zambesicus and Zygotritonia crocea, *Journal of Pharmacological Research,* **5**, 494-497 (1991).

3. Voravuthikunchai, S.P., Kitpipit, L., Activities of crude extracts of Thai medicinal plants on methicillin- resistant Staphylococcus aureus, *Journal of Clinical Microbiology and Infection*, **9**,236 (2003).

4. Tiwari, P., Kumar, B., Phytochemical screening and Extraction, *Journal of Internationale Pharmaceutica Sciencia*, **1**, 98-106 (2011).

5. Islam, T.M., A Comprehensive Review of *Corchorus Capsularis*: A Source of Nutrition, Essential Phytoconstituents and Biological Activities. *Journal of Biomedical and Pharmaceutical Research*, **2,**100 (2013)

6. Islam, M. M., Jute (Corchorus capsularis L. & C. olitorius L.) leaf, *Vegetable for nutrition and medicine for human health and beauty*.**1,**92 (2010)

7. Duke, J. A., Medicinal use of Jute ,*Handbook of Energy Crops*, **1**, 120-123 (1983)

8. Quader, M. A., Gray, A. I., Waterman, P. G., Lavaud, C., Massiot, G., Hasan, C. M. & Ahmed, M.D. Capsugenin-25, 30-O-β-diglucopyranoside: A New Glycoside from the Leaves of Corchorus capsularis, *Journal of Natural Products*, **53**, 527–530 (1990).

9. Zakaria, Z.A., Free Radical Scavenging Activity of Some Plants Available in Malaysia. *Iranian journal of Pharmacology & Therapeutics*, **6**, 87-91 (2006).

10. Zakaria, Z.A., Sulaiman, M.R., Gopalan, H.K., Ghani, Z.D.F.A., Mohd, R.N.S.R., Jais, A.M.J., Abdullah, F.C., Antinociceptive and Anti-inflammatory Properties of Chorchorus capsularis Leaves Chloroform Extract in Experimental Animal Models, *The Pharmaceutical Society of Japan,***127**, 359-365 (2007).

11. Furomoto, T., Wang, R., Okazaki, K., Feroj, A.F.M., Antitumor Promoters in Leaves of Jute (Corchorus capsularis and Corchorus olitorius). *Food Sci. Technol*. **8**, 239–243 (2002).

12. Adegoke, A. A., Adebayo-Tayo, B., Antibacterial activity, *Journal of Medicinal Plants Research*, **3**, 155-159 (2009).

13. Singh, J., Maceration technique, *Maceration, Percolation and Infusion Techniques of Extraction of Medicinal and Aromatic Plants*,**2,**120 (2012).

14. Beckett, A.H., Stenlake, J.B., Principle of Thin layer chromatography, *Practical pharmaceutical chemistry*, **4**, 115-128 (1962.)

15. Velioglu, Y. S., Mazza, G., Gao, L., Oomah, B. D., Antioxidant activity and total phenolics in selected fruits, vegetables and grain product,. *J Agric Food Chem*, **46**, 4113-4117 (1998).

16. Bondet, V., Brand-Williams, W., Berset, C., Kinetics and mechanism of antioxidant activity using the DPPH free radical method, *Lebensmitt Wissensch Technol* ,**30,** 609–615 (1997).

17. Barry, A.L., Principle & practice of Microbiology, *Lea & Fabager,* **3**, 21-25 (1976).

18. Anderson, J.E, Chan,g C.J., McLaughlin, J.L. Bioactive components of Allamanda schottii, *J Nat Prod*, **2**,307-8 (1988).

19. Hirst, G.K., The quantitative determination of influenza virus and antibodies by means of red cell agglutination, *Journal of Experimental Medicine*, **75**, 49–64 (1942).

20. Rahman, M.M., Khan, A.A., Ali, M.E., Mian, I.H., Akanda, A.M., Botanicals to Control Soft Rot Bacteria of Potato. *The Scientific World Journal*,**2012**,6 (2012).

21. Hargrove, J., Water dimer absorption of visible light, *Atmospheric Chemistry and Physics Discussions*, **7**, 11123-11140 (2007)

22. Khan, I., Ahmad, H., Ali, N., Ahmad, B., Tanoli, H., Screening of Bunium bulbocastanum for antibacterial, antifungal, phytotoxic and haemagglutination activities, *Centre of Biotechnology and Microbiology, University of Peshawar*, **4,**787-91 (2013)