Extraction, Identification and Estimation of Caffeine & Catechin from *Corchorus capsularis* leaves extract

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
Tasnim Tabassum
2009-3-70-014
East West University

DEDICATED

TO MY

Beloved PARENTS….

Certificate

This is to certify that the thesis of Extraction, Identification and Estimation of Caffeine & Catechin from *Corchorus capsularis* leaves extract 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 Tasnim Tabassum (ID#2009-3-70-014) 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.

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Chairperson & Associate Professor
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Dr. Repon Kumer Saha

Supervisor

Associate professor, Department of Pharmacy

East West University

Declaration by Research candidate

I, Tasnim Tabassum, hereby declare that the dissertation entitled "Extraction, Identification and Estimation of Caffeine & Catechin from *Corchorus capsularis* leaves extract" 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, associate 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 Signature of Candidate

Date: 10. 01 .2014 (Tasnim Tabassum)

Table of Contents

Corchorus capsularis

LIST OF TABLES

LIST OF FIGURE

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.

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Extraction of Caffeine & Catechin from *Corchorus capsularis*

Abstract:

Medicinal plants are the most important source of life saving drugs for the majority of world's population. People of all countries in the world have used hundreds to thousands of indigenous plants. Jute (*Corchorus capsularis*) is the most widely cultivated species for its nutritious leaves, bark, root, & Stems etc. The objective of this study was to isolate, identify and quantify caffeine & catechin from (*Corchorus capsularis*) leaves extract.

Caffeine was isolated from *Corchorus capsularis* leaves extract and was identified and quantified by phytochemical analysis of TLC (Thin layer chromatography technique), by determined caffeine's spot, UV detection in spectrophotometer with ƛ max- 260 n.m, measured caffeine's amount in 1mg jute leaf (0.234 mg), total phenolic content method detected phenol's amount in isolated caffeine (50.26 mg/g). The isolated caffeine was tested for antimicrobial and antioxidant activities, & results showed no antimicrobial activity and good antioxidant activity with IC 50 values of (285 µg/ml), and Brine shrimp assays exert moderate cytotoxic effect. Isolated caffeine's every experiment was compared with pure caffeine. Isolated caffein showed more cytotoxicity & better antioxidant activity then pure caffeine but similarity showed in antibacterial test, both pure & Isolated caffeine have no antibacterial effect. R.F (Retardation factor) of pure caffeine was 0.66 c.m & isolated caffeine was 0.98 c.m.

Catechine was isolated from *Corchorus capsularis* leaves extract and was identified and quantified by phytochemical analysis of (TLC) Thin layer chromatography technique. Catechin were tested for antimicrobial and antioxidant activities, brine shrimp lethality assays etc. Result of isolated catechin shows individual peak in U.V spectrometer with ƛ max- 264 & 320 n.m, well antioxidant effect with IC 50 values of (432 µg/ml), no antibacterial but moderate cytotoxicity observed by brine shrimps lethality tests (Compared with acetone because catechin was found jute leaves lower layer acetone fraction). Total phenol content method, detected phenol's amount in isolated catechin (44.25 mg/g). R.F (Retardation factor) of isolated catechin was 0.94.c.m

Rationale and objective of the work:

Success in phytochemical research is conditioned by a careful plant selection, based on various criteria such as observation of chemotaxonomic data used, for getting the highest activity of the plant material. The main reason for choosing the topic "Extraction, Identification and Estimation of Caffeine & Catechin" from *(Corchorus capsularis)* leaves extract" is that although many pharmacological, phytochemical and nutritious compounds have been isolated from (*Corchorus capsularis*) leaves, especially from its methanolic extract, no attempt has been made to isolate caffeine & catechin from this plant. Moreover research has proven various health benefits of caffeine & catechin, both are useful component in tea, coffee & having a wide range of medicinal applications. The work described in this dissertation is an attempt to isolate caffeine & catechin from *(Corchorus capsularis)* leaves extract and to evaluate their possible pharmacological, phytochemical and microbiological profiles.

Caffeine is a stimulant, improves central nervous system, it increases our heart rate and breathing rate, help to prepare body for work performance. Caffeine also has a direct effect on muscles. Calcium must be released within a muscle fiber in order for that fiber to contract, and caffeine may block the adenosine receptors attached to muscle fibers, triggering electrical activity of calcium, result is a stronger muscle contraction.

Catechins are a type of flavonoid found in certain foods; several forms exist, but all are potent antioxidants that may help to protect us from potentially damaging chemicals called free radicals.

The objective of this study is to establish *(Corchorus capsularis)* leaves extract as another new natural source of caffeine & catechin other than tea and coffee. Therefore, the objective of this work is to explore the possibility of developing new drug candidates from caffeine & catechin of this plant for the treatment of various diseases.

Medicinal Plants

Plants that possess therapeutic properties or beneficial pharmacological effects on animal body are generally classified as Medicinal plants. We can declared a general plant as a medicinal, when one or more of its organ, contains necessary substance that can be used for therapeutic purpose or used as a precursor for synthesis of useful drugs. When a plant is designated as 'medicinal', it is implied that the said plant is useful as a drug, therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants can possess some special qualities or virtues that make them medicinally important than other plants. It has now been established that the plants which naturally synthesis and accumulate some primary and secondary metabolites, like alkaloids, glycosides, volatiles oils, tannins, contain vitamins, minerals & showed medicinal properties termed as medicinal plants. **[1]**

Medicinal plants are the most important source of life saving drugs for our country & the majority of world's population. It has been estimated that in developed countries such as United States, plant drugs constitute 25% of total drugs which in fast developing countries such as China and India, the contribution is as much as 80%. Bangladesh is also a major country where people use a high percentage of medicinal plants for therapeutic activities against various kinds of disease. Volatile and penetrating plant extracts were used in therapeutic applications for physical well being from ancient times**. [2]**

Medicinal Plants as a source of modern drugs

In many cases, the sources of modern drugs from plants have been used by indigenous people. It has been shown that the average success rate of obtaining new medicines from plant sources is 1 in 125, while the success rate of obtaining efficacious medicines from synthetic chemicals is about 1 in 10,000. Since the advancement of modern allopathic medicine, the tendency toward overlooking medicinal plants had been generally increased by the researchers.In recent years, the emphasis is shifting back to medicines prepared by plants origin ingredients. The reasons behind this lie in emergence of complex problems caused by excess dependence to the modern medicine. Problems like drug-resistant to the microorganisms and serious side-effects caused by

numbers of modern drugs are typical examples of these. The modern western Medicine do not always offer sufficiently effective cures to diseases like diabetes and arthritis, which are affecting millions of people throughout the world. So to research deeper and thoroughly in medicinal plants stands as an essential task**. [3,4]**

Figure- Medicinal Plant

Necessity of studying Medicinal Plants

1. Many of the modern medicines are produced indirectly from medicinal plants, eg: Aspirin
2. Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine & Bangladeshi medicine also.
3. Many food crops have medicinal effects, for example garlic.
4. Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species.
5. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons.
6. Cultivation and preservation of medicinal plants protect biological diversity, for example metabolic engineering of plants.
7. Studying medicinal plants helps us to identify alternative and complementary medicine & also helps to find the lead compound diversification to treat various diseases. **[5]**

Corchorus capsularis

Chapter – 1

Introduction

1.1 Overview of *Tiliaceae* Family

Tiliaceae is a botanical name for a family of flowering plants, & listed by nomenclatural databases such as IPNI. In the northern regions the *Tiliaceae* family is known as Tilia. **[6]**

The family *Tiliaceae* has a very lively inventory history. The family *Tiliaceae* is trees, shrubs, or rarely herbs comprising about 50 genera and 450 species that are further characterized by the presence of branched or stellate hairs. The leaves are simple and always alternate, stipules are present. The flowers are actinomorphic and always bisexual. The perianth consists of a valvate calyx with 5 distinct parts or basally connate sepals and a corolla of an equal number of petals or sometimes the corolla is sepaloid or absent. The gynoecium is a single compound pistil of 2-10 carpels, and a 2-10-loculed superior ovary with 1-several axile ovules in each locule & an equal number of stigmas. The fruit of this family is variable. The androecium consists of usually many stamens that are distinct or basally connate or in fascicles. **[7]**

1.1.1 Genus: *Corchorus*

Corchorus is a genus of about 40-100 species of flowering plants in the family Malvaceae, native to tropical and subtropical regions throughout the world**.**

1.1.2 Description

The jute plants are tall, usually annual herbs, generally a height of 2-4 m, unbranched or with only a few side branches. The flowers are small (2-3 cm diameter) and yellow colour, with five petals; the fruit is a many-seeded capsule. The leaves are lanceolate, alternate, simple, 5-15 cm long, with an acuminate tip and a finely serrated or lobed margin. It thrives almost anywhere, and can be grown all the year-round. **[8]**

1.1.3 Taxonomy

The genus *Corchorus* has been classified in different families as Capparaceae, Cistaceae, Papaveraceae, and Tiliaceae. The putative family name "Oceanopapaveraceae" has occasionally appeared in print and on the web but has never been validly published by any system of plant taxonomy. The genus Corchorus was first described by Linnaeus in his Plantarum (1753). Genus *Corchorus* is derived from the Greek word (korkhoros) which referred to a wild plant of uncertain identity, possibly jute or wild asparagus. The genus *Corchorus* is classified under the subfamily Grewioideae of the family Malvaceae. The genus Oceanopapaver, has recently been synonymized under Corchorus. The name was established by Guillaumin in 1932 for the single species Oceanopapaver, neocaledonicum, Guillaumin from New Caledonia. **[8]**

a) Corchorus aestuans

b) Corchorus olitorious

Figure: 1.1 Some Plants of Tiliaceae Family

1.2 Common Name of *Corchorus capsularis*

There are a number of common names of *Corchorus capsularis* in the language of Bengali, Hindi, Marathi, and English. Some of them are listed below.

Table1.1: Some Common Name of Corchorus Capsularis **[9]**

Common name	Area
Mara shaak	Assamese
Koshta	West Bengal, Bangladesh
Malukhiyah	North Africa, Middle East
White Jute	English
Rama	Nigeria
Krain Krain	Seria Leone
Mrenda, Murere	Kenya
Khudra	North Sudan
Saluyot	Phillipines
Baipo	Thai
Patta Shaak	Hindi
Chonche	Marathi
Senabu	Kannada

1.3 Broad or Taxonomical Classification [10]

Kingdom	Plantae: Planta, vegetal plants
Sub Kingdom	Viridaeplantae: green plants
Infra Kingdom	Streptophyta: land plants
Division	Tracheophyta: vascular plants, tracheophytes
Subdivision	Sparmatophytina: spermatophytes, seed plants, phanero games
Infra division	Angiospermae: flowering plants, angiosperms
Class	Magnoliopsida
Superorder	Rosanae
Order	Malvales
Family	Tiliaceae
Genus	Corchorus L
Species	*Corchorus capsularis L*

1.4 Jute (The Golden Fibre)

Jute is the dicotyledonous fibrous plant of the genus *Corchorus,* family Tiliaceae. Jute is known as the golden fibre of Bangladesh. It is the most important cash crop for our country. Jute fibre is produced mainly from two important species: White Jute (*Corchours capsularis*), and Tossa Jute (*Corchorus olitorius*). The origin of white jute is said to be Indo-Burma & South China and Tossa Jute is grown in Africa. Jute is grown in Bangladesh, India, Myanmar, Nepal, China, Vietnam, Cambodia, Taiwan, Thailand Brazil and some other countries. Although jute is grown in almost all the districts of Bangladesh but Dhaka, Faridpur, Tangail, Jessore, Sirajganj, Bogura, and Jamalpur are considered as the better growing areas in our country**.**

1.4.1 Distribution & Habitat

Jute is grown in the month of March, April & the temperature required 18-33□C. Jute is cultivated in the rainy season. In Bangladesh, sowing starts at the end of February and continues up to the end of May. Cultivation largely depends upon moisture conditions. *C. capsularis* is generally grown in lower lands, water logging conditions. *C. olitorious* is more susceptible to water logging, cultivated in medium to lower medium lands. Jute can be grown in a number of soil types, with optimum fertility and soil pH ranging need from 5.0-8.6. It needs long day light for growth. After sowing, four to five months are needed for harvesting of crops. The fibre is obtained from the bast or phloem layer of the stem**. [11]**

Figure 1.2: Corchorus capsularis (Jute Leaf)

1.4.2 Botanical Description of the plant parts-

The Jute plants are tall, usually annual herbs. They are reaching a height of 2–4 m, generally un-branched or with only a few side branches. The leaves are alternate, simple, lanceolate, light green color & not more than 5–15 cm long and a finely serrated or lobed margin. The flowers are small (2–3 cm diameter) and yellow color, with five petals; their fruit is a many-seeded capsule. It thrives almost anywhere, and can be grown year-round. **[12]**

1.4.3.1 Uses of Jute Leaves

1. Jute is better known as a fiber crop, it is also a medicinal “vegetable", eaten from Tanganyika to Egypt as a favourite food. Jute used as vegetables in Africa, Southeast Asia, and Middle East.

2. Japan has been importing dry jute leaf from Africa and they are using it as the substitute of coffee and tea.

3. In Europe, jute leaves are used as soup, known as "Molukhyia."

4. In Bengal, jute leaves are used as a condiment & added to the daily diet with rice.

5**.** Jute leaves are rich in vitamins, calcium, potassium, iron, carotinoids and dietary fibers.

1.4.3.2 Medicinal Use of jute Leaves

1. Besides food Jute is also used as herbal medicine to prevent dysentery, worm and constipation.

2. Jute leaf contains antitumor promoters; Phytol and Monogalactosyl-diacylglycerol.

3. Jute Leaves reduce risk of cancer but investigations & research are ongoing in this matter.

4. The mutant of Jute CM-18 contains higher protein and carotene contents than parent variety. Fiber, Alkaloid and Vitamin C contents are more or less similar with CVL-1 (parent compound)

5. Injections of olitoriside improve cardiac insufficiencies and have no cumulative attributes; hence, it can serve as a substitute for strophanthin (Dried material-Nalita in India).

6. Tussah jute is a folk remedy for aches and pains, dysentery, enteritis, fever, dysentery, pectoral pains, and tumors. It is reported to be demulcent, de obstruent, diuretic, lactagogue, purgative, and tonic, also.

7. 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.

8. Jute leaf as a vegetable contain an abundance of antioxidants with a significant α-tocopherol equivalent to Vitamin E , have been associated with protection from chronic diseases such as heart disease, cancer, diabetes, and hypertension as well as other medical conditions. **[13, 14]**

1.4.3.3 Other Uses of Jute

In Bengal, sacks and saris made of jute were commonly started to use in the Middle Age continuing now a day's also. Its use was popularized primarily in Western Europe, particularly at Dundee. Jute is used as packaging materials like gunny bag, twill, carpet backing, wool pack, twine, hessian, mats, canvas, rug, handicrafts, wall cover, and furnishing fabrics of different types and natures. Now a day, it has been used as a popular raw material for packaging. Finely carded and highly absorptive fiber made from jute & used for surgical dressings. Before being used as a commercial commodity it was used in different parts of the world to make household and farm implements such as ropes, handmade clothes, wall hangings, etc. Paper also made from Jute. Dundi (UK) purchases high class jute of all grades, especially white and tossa. USA, South America Belgium, Italy, is the buyers of fine quality jute. Jute was cultivated in ancient times in Bengal. At that time it was a popular plant and its leaves were used as a vegetable and for medicinal purposes. **[13, 14]**

1.4.4.1 Chemical Constituents of Jute Leaves

1.4.4.2 Leaves

Per 100 g, the leaves are contain 43-58 g calories, 4.5-5.6 g protein, 0.3 g fat, 7.6-12.4 g carbohydrate, 80.4-84.1 g H2O, 1.7-2.0 g fiber, 2.4 g ash, 266-366 mg Ca, 97-122 mg P, 7.2-7.7 mg Fe, 12 mg Na, 444 mg K, 6,410-7,850 µg 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 oxydase and chlorogenic acid. The folic acid content is substantially higher than other folacin-rich vegetables.

Table1.2: Nutritional comparisons between jute leaf and spinach leaf (per 100 gm each)

Ingredients	Jute Leaf	Spinach Leaf
Calories (kilo cal)	73	25
Protein (g)	3.6	3.3
Carotene (mg)	6400	5200
Calcium (mg)	298	55
Vitamin C (mg)	64	65
Iron (mg)	11	3.9
Lipid (g)	0.6	0.2

Jute, green leafy vegetable is rich in beta-carotene for good eyesight, calcium for strong bones and teeth, and vitamin C for smooth skin, iron for healthy red blood cells, strong immune cells, and fast wound-healing. Vitamins A, C and E present in Saluyot, (jute as a food in Phillipines) "sponge-up" free radicals, scooping them up before they can commit cellular sabotage.

One-half cup cooked Saluyot leaves contains: 1.3g protein, 0.3g fat, 3.1g carbohydrates, 0.4g fiber, 87.3mg calcium, 22.5mg phosphorous, 1.0mg iron, 0.02mg thiamin, 0.04mg riboflavin, 0.3mg niacin, and 10mg Ascorbic Acid or vitamin C. Salyut has an antioxidant activity of 77% or α-tocopherol (48.9**)** equivalent (vitamin E). **[11, 13]**

1.4.4.3 Seeds

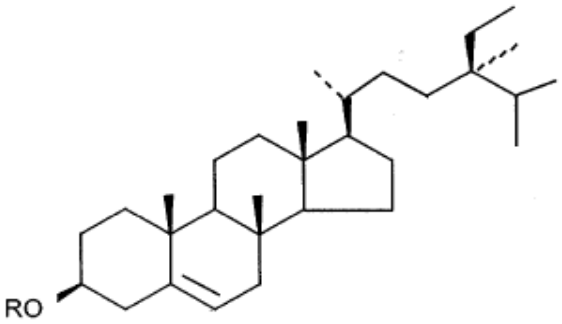
Active principle of the jute seed is corchorin. Corchortoxin, another cardiac agent found from the seeds. Seed contains 2.25% raffinose.11.3-14.8% Oil(estrogenic), which contains 16.9% palmitic acid, 3.7% stearic acid, 62.5% linoleic acid, 0.9% linolenic acids,1.8% behenic acid, 1.1% lignoceiic acid, 9.1% oleic acid and large portions of B, Mn, Mo, and Zn.

Table1.3: Some Compound Isolated from Corchorus capsularis Plant Parts as:

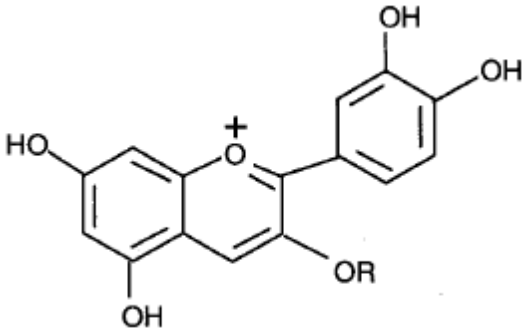
Compounds	Plant Parts
1.Cardiac Glycoside	Seeds
2.Strophanthidin Glycoside	Seeds
3.Capsulasone Corchorol	Leaves
4.Capsularol	Leaves
5.Phenolics	Leaves, Bark
6.Triterpines	Leaves, Root
7.Sterols	Seeds, Leaves, Root

1.4.4.4 Bark & stem

The bark and the stem of unretted jute plant (*Corchorus capsularis*) were containing various free, glycoside and ester-linked phenol acids. From an 80% aqueous ethanol extract of (*Corchorus capsularis*) contained p-coumaric acid, ferulic, caffeic, vanillic and p-hydroxybenzoic acids were identified and quantified. P-Coumaric acid was the major component, and β-sitosterol was isolated also. **[15]**

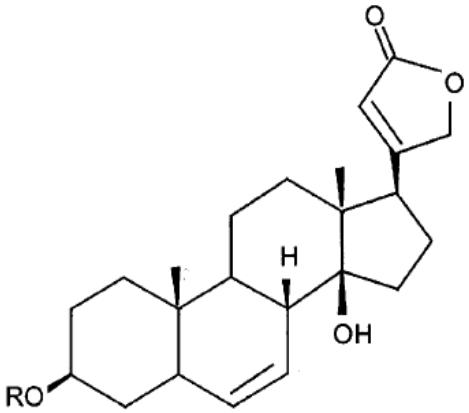


β -Sitosterol (R=H)

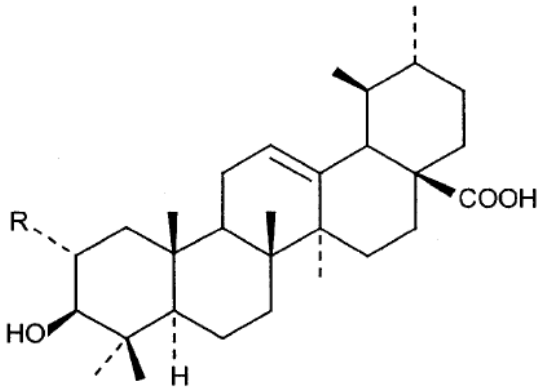


Cyanidin (R=H)

Cyanidin glucoside (R= Glucose)



Corchoroside –B (R=α- LRamnopyranose)



Ursolic acid(R=H),corosolic acid(R=OH)

Figure 1.3: Some Structures of Chemical Components of Corchorus capsularis

Chapter – 2

Literature Review

Of Jute Leaf

2. Pharmacological Investigational Reviews of Jute Leaves

2.1 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. Corchoroside A&B isolated from *Corchorus olitorius* seeds give digitalis like action. Esterification of –OH group of (3'-4'position of Corchoroside A & 19 position of Corchorosol A by acetic acid led to a new acetylated cardenolides. Corchorosol derivatives from Corchorus capsularis are more effective on heart diseases than corchorosides. Showed decrease parenteral & increase acetylating degrees with Corchorus derivatives**.** All cardenolides, oligoglycosides (Corchorusosides A-E) are isolated from *Corchorus olitorius* seeds. It was tested with lagendroff perfuse rabbit hearts, showed that low concentration of seed extract increase left ventricular pressure & coronary blood flow & at a high concentration significantly decreased them with a sharp increase of heart rates with ventricular fibrillation**.**

2.2 Anticancer Activities

Alcoholic extract of entire C.aestuans showed anticancer against human epidermal carcinoma of nasopharynx in tissue cultures.

2.3 Anticonvulsant Activities

Methanolic extract of *Corchorus olitorious* showed significant anticonvulsive activities by altering the levels of catecholamine and brain amino acid in mice.

2.4 Antihistaminic Activities

Corchokoinoside A 40, B 41 & Roseoside (6S-9R) -43 from *Corchorus olitorius* inhibit histamine release from rat peritoneal exudates from cell induced by antigen antibody reaction & shows it's antihistaminic action.

2.5 Antimalarial Activities

Aqueous extract of *Corchorus olitorious* showed a strong growth of inhibition (>96%) of the malaria parasite plasmodium falciparum. **[15]**

2.6 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. **[15]**

2.7 Renal & Hematological changes

Low doses of the plant extract genus Corchorus did not exhibit any change of serum creatinine protein levels but the high dose level significantly increases creatinine levels. **[15]**

2.8 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 immuno blotting 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. **[16]**

2.9 Antinociceptive and Anti-inflammatory Properties

The antinociceptive and anti-inflammatory properties of *Corchorus capsularis* leaves chloroform extract were investigated in animal models. The antinociceptive activity was measured using the writhing, hot plate and formalin tests, while the anti-inflammatory activity was measured using the carrageen an-induced paw edema test. The extract, obtained after 72 h soaking of the air-dried leaves in chloroform after 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. This study confirmed the traditional claims of using *C. capsularis* to treat various ailments related to inflammation and pain. **[17]**

2.10 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*. 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*. The extracts exhibited the high antimicrobial activity. This could be adduced to the presence of phytochemical constituents and can be of prophylactic importance. **[18]**

2.11 Anti-oxidant activity

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, and 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. **[19]**

2.12 Antiestrogenic Activities

Methanolic extract of *Corchorus olitorius* seeds arrested normal oestrus cycle of adulated female mice & significantly decrease the weight of ovaries and uterus**. [15]**

2.13 Inhibitory effect on Nitric Oxide

Corchori fatty acids (A 65, B 66, C 67) (an element of *Corchorus capsularis*) showed an inhibitory effect on Lipopolysaccharides induced NO production in cultured mouse peritoneal macrophages**. [15]**

2.14CytoToxicity

Contain HCN& several cardiac glycosides. The LD50 of tissue extracts to mice report that the The "lethal dose" of Corchoroside A to cats is 0.053-0.0768 mg/kg and Corchoroside B 0.059-0.1413.

2.2 Reviews on Phytochemical investigation

2.2.1Phytochemical constituents of Jute Leaf-

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. Later on one more new triterpinic glucoside Capsugenin 30-O-glucopyranoside was isolated from mature leaves of Corchorus capsularis**.**

2.2.2 Polysaccharides & other sugars

Fructose & Galactose were identified in the bark of *Corchorus capsularis.* Free sugars Sucrose, Raffinose, Arabinose, Fructose, Glucose, Galactose have been reported in the leaves & seeds of *Corchorus capsularis*.

2.2.3 Phenolic Compounds

Isolation of *Corchorus capsularis* gives Characterizaton of cyanidin glucoside.

2.2.4 Sterols

β -isosterols isolated from *Corchorus capsularis* root leaves & seeds.

2.2.5 Triterpinoids

A Triterpinoid corosin was isolated from *Corchorus capsularis* refluxing with HCL gave corosic acid {C30H44O6 [α] D 26 = +127 (0.9% in Methanol)}. **[15]**

Chapter- 3

Crude Isolation

From

Corchorus capsularis

3. Plant Selection

Plant selection is greatly effected in success of research work there are thousands of medicinal plants in Bangladesh. Among these plants it was not easy to select any plants for the research purpose. Specific plant & their parts contained specific medicinal values. Thus, unless it is already known which plants with their parts (as Bark, Root, Leaves & Seeds etc) contain the highest level of the active compounds it is important to collect multiple plant parts, or the whole plant to ensure the extracts prepared is the representative of the large range of primary & secondary metabolites.

From the literature review it was seen that there is little work on the plant *Corchorus capsularis* (leaves) with its Chloroform fraction (Caffeine isolation), Acetone fraction (Catechin isolaton) . So I got a chance to select the leaves of *Corchorus capsularis* as a sample for my research work to see whether the isolated caffeine & catechin have any Antioxidant, Cytotoxic or Antimicrobial activities or not.

3.1 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 Chadpur District under Comilla division, on 1st July 2013.

3.2 Plant Identification

To aid taxonomic experts in confirming the field identification and to get a permanent scientific record (accession number) a voucher specimen was prepared on 29th September, 2013. In the voucher specimen the dried leaves of sample plant were attached and some information like local name, medicinal use, location of the sample plant were also written on that voucher specimen. Finally, from BNH (Bangladesh National Herbarium) I got the identification or accession number of collected sample on 29th September, 2013, and the accession number got 37901 with *Corchorus capsularis* and *Tiliaceae* scientific name and family name of the plant respectively.

3.3 Drying of Plant Sample

After the collection of sample it needs to be dried to make the sample extract. 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 research procedure. In general the plant material should be dried at temperature below 30□C to avoid the decomposition of thermo labile compounds. Degradation of active compound in case of drying can give a wrong result. Also shed drying of the sample was completed. The Jute plant leaves were dried for 10 days.

3.4 Grinding of Dried Sample

Grinding of sample makes easy extraction procedure. Small amount of plant material can be grind by 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 the amount of sample. It also decreases the amount of solvent required for the extraction. The dried samples were ground to coarse powder with a mechanical grinder and powdered samples were kept in clean closed glass containers before extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination of previously ground material or other foreign material deposited on it. The total amount of grinded sample was 2 kg.

3.5 Maceration of Powdered Sample

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 to the solvent, with 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. if the concentrations of active principle in the solid material and the solvent are the same. Then, there will be no mass transfer of the active principle from plant material to the solvent. Mass transfer of the active principle also depends on its solubility in the solvent; heating of the solvent can enhances the mass transfer. After getting the dried sample extract was prepared with it. **[20]**

Figure: 3.1 Grinding of Jute Leaves

3.6 Crude Drug Isolation from Jute Leaf

3.6.1 Equipment & Materials

1000 ml Beaker	Measuring Cylinder	Funnel
Clay triangle	Chloroform	Hot pads
Distilled Water	Separator funnel stand	Hot Plate
Analytical balance	Reverse-phase filter paper	Pipette
Stirring rod	Conical flask	Jute Leaf
Hot water bath	Regular filter paper	Watch glass
500ml separator funnel		

3.6.2 Procedure:

100 gm jute leaf powder was measured and added with 1000ml of distilled water in a beaker.

The beaker was then heated by using a hot plate (The solution was boiled in 95□c.).

↓

The content of the beaker was then cooled and filtered by using cloth and filter paper for getting clear extract solution. 500 ml filtrate was collected.

↓

The filtrate was then placed into separating funnel and allowed to settle for some time.

↓

20 ml of $CHCl_3$ was added and the funnel was inverted back and forth ten times, stopping every three times to allow gas to escape. The organic layer in the funnel was released into a 2^{nd} beaker.

↓

The 20 ml $CHCl_3$ mixing with jute extract was repeated with a total 200 ml $CHCl_3$, each time after separation, releasing the organic layer into the 2^{nd} beaker.

↓

Separated two different layer of Chloroform (175 ml) & jute leaf extract (525 ml).

↓

By using a rotary evaporator evaporated the chloroform layer & gets crude drug from Jute leaf & recondensed chloroform. ($CHCl_3$ was evaporated in 62□c).

In this way crude isolation was completed from *Corchorus capsularis***.**

3.6.3 Thin Layer Chromatography:

Chromatography is the separation of two or more compounds or ions by the distribution between two phases, one is moving and the other is stationary phase. These two phases can be solid-liquid, liquid-liquid or gas-liquid.

Thin-layer chromatography (TLC) is a solid-liquid form of chromatography where the stationary phase is normally a polar absorbent and the mobile phase can be a single solvent or combination of solvents. TLC is a quick, inexpensive micro scale technique that can be used to:

• Determine the number of components in a mixture

• Verify a substance's identity

• Monitor the progress of a reaction

• Determine appropriate conditions for column chromatography

• Analyze the fractions obtained from column chromatography. **[21]**

3.6.4 Materials and Chemicals Needed:

- TLC plates
- 4 capillary tubes
- TLC chamber with lid
- Ruler and pencil
- Caffeine standard, Chloroform drug sample (from Jute leaf)
- TLC solvent: (solvent system-2: intermediate polar)

  - Chloroform: Ethyl acetate: Formic acid (5:4:1)

3.6.5 Procedure:

1 TLC plate was obtained. The plates were marked as spot 1, 2, 3 & 4. A pencil was used to lightly mark a straight line about 0.5 cm from both end of the plates.

We gave total four spots on the TLC plate. One spot was from the jute leaf extract, one was Chloroform (Crude drug layer), & other two was pure caffeine & chloroform's spots.

With a capillary tube a small spot of the Caffeine Standard was spotted on the plates. With another capillary a spot with the Extracted Crude drug from *C. capsularis* leaf was spotted carefully. To avoid confusion, the spots were lightly labeled below the pencil line.

- The TLC plates were developed by placing them in the TLC chamber that has been filled with the developing solvent. The solvent was allowed to migrate up the TLC plates until it reached the marked top end line.
- The plates were then removed immediately and the solvent was allowed to evaporate. After that it was visualized under UV light and all the spots were marked.

3.6.6 Charring of T.L.C Plate:

- For charring purpose the plate was exposed to 10% sulphuric acid solution, dried & then heated in a hot plate for 5-10 minutes.
- **Result**-We again shows two individual peaks with Chloroform layer & pure caffeine more well then before with brown color after charring.

3.6.7 Staining with DPPH (2, 2-diphenyl-1-picrylhydrazyl):

0.4% DPPH solution was prepared with methanol as solvent and labeled as stock solution. From the stock solution 10% DPPH solution was taken in a petri dish and the TLC plate was dipped in it. The plate was visually observed for a color change after sometime. All the processes were carried out in dark place. The antioxidant active regions became yellow in color.

3.7.1 TLC of Crude drug Isolation from *corchorus capsularis*

Fig 3.2: TLC plate 1 under open eye

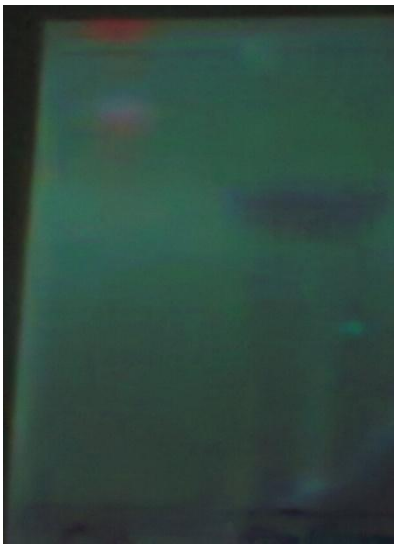


Fig 3.3: TLC plate 1 under UV light

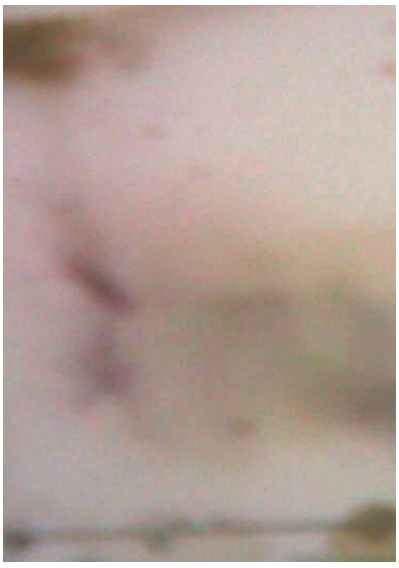


Fig 3.4: After charring with 10% H_2SO_4 solution

Fig 3.5: after dipped in DPPH solution

3.7.2 R.F (Retardation Factor) Value Calculation:

R.F value: Distance traveled by solute (Extract spot phase)

Distance traveled by solvent (Mobile phase)

R.F value for Caffeine = 5**.0** c.m = 0.77 c.m

6.0c.m

R.F value for crude extract = 6.0c.m = 0.92 c.m

6.5c.m

- **Open Eye Observation-** Shows individual peak of Chloroform layer (light green color)
- **Observation under U.V light-**
- 1. Under U.V. light we show two individual peaks.
- 2 One was the peak of pure Caffeine (Deep Brown Color)
- 3. One was the peak of Chloroform (Lower Layer of Jute Extract) (Red Color)
- **Charring of T.L.C Plate-**

Result-We again shows two individual peaks with Chloroform layer & pure caffeine more well then before with brown color after charring.

Staining with DPPH (2, 2-diphenyl-1-picrylhydrazyl):

0.4% DPPH solution was prepared with methanol as solvent and labeled as stock solution. From the stock solution 10% DPPH solution was taken in a Petri dish and the TLC plate was dipped in it. The plate was visually observed for a color change after sometime. All the processes were carried out in dark place. The antioxidant active regions became yellow in color indicates the presence of flavonoid compound.

Chapter – 4

Part: 1

Caffeine Isolation

From Jute Leaf

4. History of Caffeine

Coffee, (*Coffea Arabica)* is the preeminent species of caffeine. Cofee was believed to be the first species, contain caffeine as main compound. The pharmacologically active principle of coffee is "caffeine", discovered in the 17th Century by Dr. Sylvestre Dufour. The popular "Kaldi Legend" about the discovery of caffeine was as: "Coffee was first discovered when Kaldi, a goat-herd in Ethiopia, observed his goats dancing & unusually frisky after eating berries from a bush. Curious about this, he ate the berries, & found they gave him a renewed energy. The news of this energy laden fruit quickly spread throughout the region. Hearing about this amazing fruit, monks dried the berries in water, & drank the liquid to provide energy & stimulation. This famous "Kaldi story" is known as a history of caffeine identification from coffee. **[22]**

4.1 Caffeine: Chemical properties

Caffeine an alkaloid of the methylxanthine family is a naturally occurring substance found in the leaves, seeds or fruits of over 63 plants species worldwide. In its pure state, it is a white powder. Its chemical formula is $C_8H_{10}N_4O_2$.Its systematic name is 1, 3, 5-trimethylxanthine.The most commonly known sources of caffeine are coffee, cocoa beans, cola nuts and tea leaves**.**

4.2 Caffeine Health Benefits

Caffeine ease depression by increasing dopamine in the brain, replenishes muscle glycogen concentrations increases stamina after exercise. Caffeine cleanses the colon; reduce fatty liver disease (with non-alcohol related fatty liver). Caffeine removes sleep & tiredness. Caffeine relieves muscles pain. Caffeine helps to prevent Alzheimer's disease. Caffeine protects cataracts. Caffeine prevented skin cancer in hairless mice. **[23]**

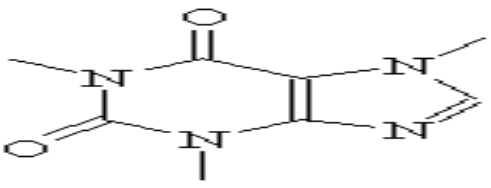


Fig 4.1: Structure of Caffeine

4.3 Caffeine Extraction from Jute Leaf

4.3.1 Introduction:

Extractions from solids can be performed by utilizing the different chemical properties of various solvents. The primary solvent used in the extraction of caffeine is water. Caffeine is sparingly soluble in water at ambient temperatures & highly soluble in water at 100^{o}C. The boiling of coffee beans, jute leaves and other materials dissolves to produce caffeine. We can take advantage of the solubility properties of caffeine in water to create an aqueous solution of caffeine. First, the caffeine was dissolved from jute leaves by boiling them in water. The solution was allowed to cool at room temperature. Although the solubility of caffeine is low at room temperature; the caffeine will remain in solution and need to be extracted with another solvent. (Here NaoH were used).

The solubility of caffeine in chloroform is quite high at room temperature. When chloroform is added to the aqueous caffeine solution, the caffeine is transferred to the chloroform. Chloroform is much denser than water and insoluble in it. The chloroform will form a layer under the water and can be separated from it. **[24]**

Figure 4.2: Caffeine Extraction from Jute leaf

4.3.2 Equipment and Materials:

1000 ml Beaker	100ml Measuring Cylinder	Funnel
Clay triangle	Chloroform	Separator funnel
Distilled Water	Separator funnel stand	Hot Plate
Analytical balance	Reverse-phase filter paper	Pipette
Stirring rod	125ml Erlenmeyer flask	Jute Leaf
Hot water bath	Regular filter paper	Watch glass

4.3.3 Procedure:

Part 1: Dissolution of Caffeine in Water

1. Weighed 100 g of jute leaf and placed them in the beaker. Recorded actual weight of it, added 1000ml of distilled water to it.
2. Boiled the water containing the jute leaf on a boiling water bath for 45-50 minutes while stirring occasionally. In the boiling period the temperature was 95-96□C.
3. After the boiling period is over, removed the beaker from the heat and allowed to cool it for 45 minutes.
4. After the solution has cooled, squeezed the jute leaves by a cloth to remove all the liquid from it. Disposed of the jute leaves.
5. Filtered the solution through regular filter paper to remove all the solid particles.

Part 2: Transfer of Caffeine from Water to Chloroform

1. Added 200ml of chloroform into the 600 ml Jute leaf solution. (With continuous stirring for 20 minutes)

2. Allowed the chloroform to settle to the bottom.

3. With the help of a 250 ml separator funnel we separated two different layer of Chloroform (175 ml) & jute leaf extract (625 ml).

Part: 3 Final Isolation of Caffeine with NaoH

1. Then this 175 ml Chloroform solution was mixed with 40 ml NaOH solution.(with continuous stirring for 20 minutes) then kept the solution for 30 minutes.

2. Get two different layer of Chloroform (165 ml) & NaOH (50 ml) (after separated it with a Separator funnel).

3. Then kept the Chloroform (Isolated Caffeine) layer, open air drying for five days. **[24]**

After five days we get dried isolated caffeine**.**

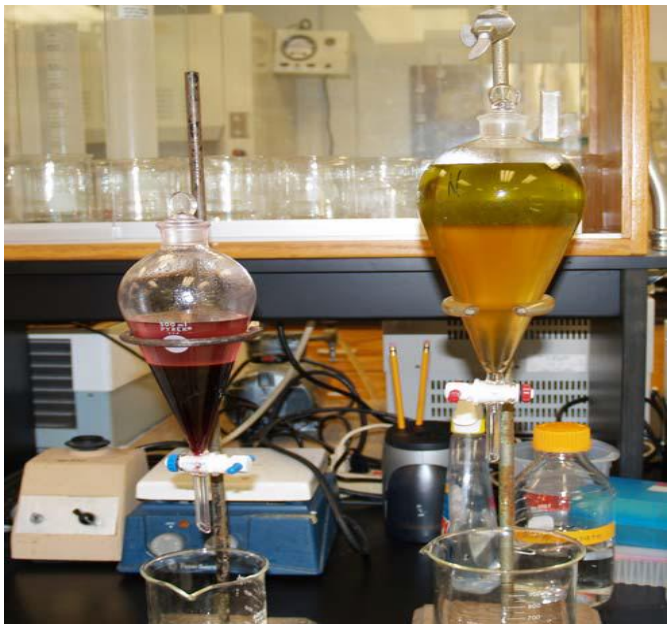


Figure: 4.3 Separation of Caffeine from Jute Leaf

Part: 2

Isolated Caffeine Methods Of Experiment

4.4 Solvents, Reagents, Equipments for Experiment

4.4.1 Chemicals and Other Reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl), Sulfuric acid, Folin reagent, Ciocalteu reagent, Acetone, Phosphate buffer, L-Ascorbic acid, potassium ferricyanide, Sodium carbonate, distilled water ,Sodium nitrite, Aluminium chloride, Sodium hydroxide, Hydrogen peroxide, Normal saline, Hydrochloric acid, Acetic anhydride, Alcoholic ferric chloride, 5-aqua copper sulphate, Sodium potassium tartrate, Sodium Chloride, Dichloromethane, Pure Caffeine etc.

4.4.2 Equipments and Other Tools

In case of the extraction procedure and for various phytochemical tests many equipments and materials were used. Some of them are analytical balance, beaker (in various size),hot water bath, fume hood pipette, micro-pipette, rotary evaporator, hot air oven, dryer, storage cabinet, spatula, test tube, volumetric flask, conical flask, test tube holder, test tube rack, aluminum foil paper, scotch tape, refrigerator, water bath, electronic shaker, ultra violate lamp, mask, gloves, lab coat, sprayer, reagent bottle, TLC plate, TLC tank, scale, pencil, TLC plate cutter, capillary tube, mortar and pestle, laminar air flow cabinet, loop, burner, micropipette tip, Petri dishes, glass rod, cotton, filter paper, funnel, hot plate, centrifugal machine, autoclave machine, glassware washers, stirrer, UV spectroscopy, ephedrine tube, Whatman‟ s filter paper, paper disc, incubator, vortex machine, PH meter.

4.4.3 Solvent System

Dimethylsulfoxide (DMSO), Acetone, Chloroform, Distilled water,, Diethyl ether, Acetic acid, Formic acid, Dichloromethane, Ethyl acetate, Dichloromethane, Benzene, Sodium hydroxide etc.

4.5 Thin layer chromatography

4.5.1 Thin layer chromatography (TLC) is a chromatography technique is coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide, or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (mobile phase) is drawn up the plate via capillary action. Different analytes ascend the TLC plate at different rates & separation is achieved. **[21]**

4.5.2 Thin Layer Chromatography(T.L.C)Analysis:

Reagent Media- 1.Chloroform -5 ml
2. Ethyl Acetate-4 ml
3. Formic Acid-1 m
4. Pure Chloroform.
5. Pure Caffeine, Jute Caffeine

Apparatus- 1.TLC tank
2.Capillary Tube
3.Watch Glass
4. TLC plate
5.Pipette Pumper

Procedure-

1. Gave total five spots on the TLC plate. One spot was from the jute leaf extract, one was with Chloroform, one for NaoH & other two was pure caffeine & chloroform's spots.

2. Then placed the TLC plate in TLC Tank. .After ten minutes when run of TLC solvent was completed then we placed the TLC Plate under U.V. light.

Antioxidant Screening-

For detection of Flavanoids the plate was dipped into 0.04% DPPH Solution & allowed it to be dried (30 minutes) while keeping in a dark place.

Charring of T.L.C Plate-

For charring purpose the plate was exposed to 10% sulphuric acid solution, dried & then heated in a hot plate for 5-10 minutes.

4.6 Anti-Oxidant Tests (DPPH Test) (1, 1-diphenyl-2-picrylhydrazyl radical)

4.6.1 Principle

The DPPH reagent has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. 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. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this 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. **[25]**

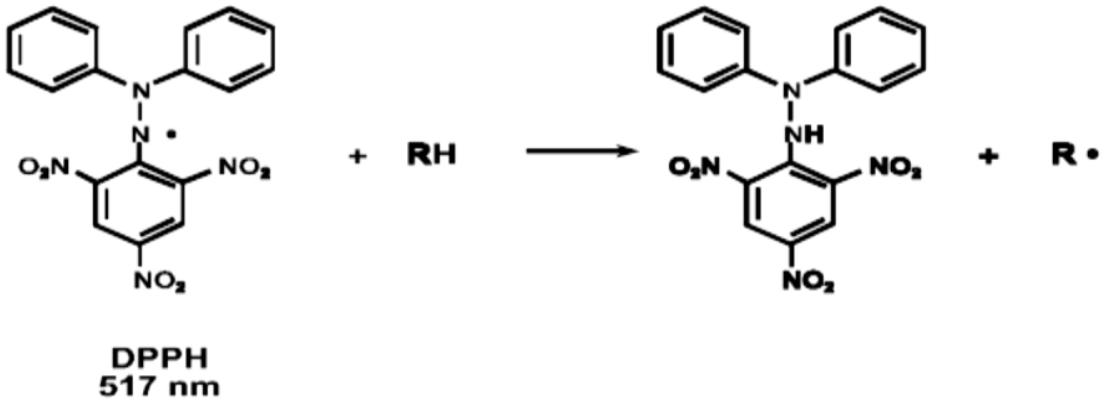


Figure 4.4: Antioxidant Test with Free Radical Scavenging

4.6.2 Apparatus

Test Tube	U.V Spectrophotometer
Jute Caffeine extracts	Water
Beaker	Analytical Balance
DPPH	L-Ascorbic Acid

4.6.3 Preparation of DPPH Reagent

0.008 gm DPPH was weighed & dissolved in 20 ml distilled water. (400 µg/ ml). The Reagent was kept in dark place or in amber color bottle.

4.6.4 Preparation of Extract Solution

0.005 gm of Isolated Caffeine Extract was weighted in volumetric flask & the volume was made up to 50 ml with water. (The concentration was 100 µg/ ml.)

4.6.5 Preparation of standard solution

0.005 gm pure caffeine was weighted in volumetric flask & the volume was made up to 50 ml with water. The concentration was 100 µg/ ml.

4.6.6 Preparation of positive Control

0.005 gm Ascorbic acid was weighted & the volume was made up to 50 ml with water. The concentration was 100 µg/ ml.

4.6.7 Blank Preparation:

The blank was prepared with 5ml water & DPPH solution with 100µl (control).

4.6.8 Procedure (Extract Preparation)-

Five test tubes was marked with the following concentration as 01(40µg/ml), 02(80µg/ml),

03(120µg/ml) , 04(160µg/ml), 05(200µg/ml).With this concentration plant (jute leaf) extract solution 1ml, 2ml, 2ml, 4ml and 5ml were taken in 5 different test tubes and the volume adjusted to 5 ml water . Then 100 µl DPPH solutions were adjusted to each test tube.

The same procedure was repeated with Standard Caffeine

Incubation period was 30 minutes, then the absorbance values measured at 517 nm on a spectrophotometer and converted into the percentage antioxidant activity using the equation,
DPPH antiradical scavenging capacity (%) = [(Ab.of blank – Ab.of sample)/Ab.of blank] ×100

The IC50 values were calculated by the linear regression model using plots, where the abscissa represented the concentration of tested plant extracts and showed the average percent of scavenging capacity. IC50 values denote the concentration of the sample required to scavenge 50% of DPPH radicals. **[26]**

4.7 Brine Shrimps Cyto toxicity Test (Isolated Caffeine)

4.7.1 *Artemia* (Brine Shrimp)

Artemia is an important live food for aquarium fish, and the larval stages of marine fish and commercial crustaceans. *Artemia* are brachiopod crustaceans found in salt lakes. Great Salt Lake in North America is one of the main sources of brine shrimp. *Artemia* can live in water up to about 300 ppt. The best salinity for culture is seawater or 30-35 ppt. They survive at temperatures of 6-35□C but usually cultured at 25-30□C.

4.7.2 Procedure for hatching brine shrimp

1. At first, set up the tanks as a water bath, the outer tank contains an aquarium heater to maintain a temperature of 25-30oC in the hatching container. Freshwater is used in the water bath & seawater was added to the inner container. (38 g salts per liter).
2. Added the airline from the aerator into the inner container.
3. Once the seawater has reached the correct temperature, added 0.2g of dry *Artemia* cysts and mixed with the water.
4. Position a light over the culture helped synchronize hatching.
5. After 24-30 hours the brine shrimp hatched.
6. To separate hatched brine shrimp from empty cysts turned off the air and removed the light over the container. In this way the empty cysts will float and the brine shrimp concentrated in the water column.
7. Use pipette to remove the brine shrimps.
8. The brine shrimp then used in the cytotoxicity experiment. **[27]**

4.7.3 Brine Shrimps Cytotoxicity & Lethality Test (Isolated caffeine)

Prepared Brine Shrimps (sample) with 1 liter= 38 g (Nacl Solution)

After Brine Preparation PH need 8.5(Adjusted with NaoH solution)

4.7.3.1 Hatching- 1.Light

2. Aeration

3. O2 Circulation

4. Nauplii /Egg (Brine Shrimp)

4.7.3.2 Observation Period- 48 hours

4.7.3.3 Shrimp Collection-

1 .Six test tubes were need.

2. 10 Nauplii were collected in every test tube.

3. Nauplii collected from brine shrimp samples.

4.7.3.4 Sample Preparation-

20 mg Pure & Standard Caffeine sample were measured & dissolved it in 2 ml hot water.

4.7.3.5 Sample Dilution (Pure Caffeine)

1. Stock solutions concentrations was 10 mg/ml
2. Then the stock solution was diluted with the following concentration- (Serial Dilution) 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, 0.3125 mg/ml.(1 ml sample was taken from every test tubes.)

3 .Rest of the volume of every test tube (except the first one) was adjusted with 1ml Hot water. & the total volume of every test tube was 2 ml.

One blank was prepared without given any caffeine, only brine shrimp sample (Control group)

For Cytotoxicity measurement, compared it with the drug sample.

Same Procedure & Concentration of Isolated Caffeine (from Jute Leaf) sample dilution was followed in here.

4.7.3.6 Brine Shrimps Addition with Caffeine's sample-

Caffeine's sample (100 µl) was taken from every test tube. (After dilution)

Then the sample of pure Caffeine was placed in brine shrimps test tubes with accurate labeling of the dilution serial of sample.(was labeled with 1-6 & 6 No. was blank.)

Then the volume of every test tube was adjusted with Brine.

Same procedure of Shrimp addition with drug sample was followed for Isolated (Jute) Caffeine.

4.7.3.7 Observation-

Then the test tube of Brine Shrimps with drug Sample (pure & Isolated Caffeine) was kept in 24 hours (under light) for observation & after 24 hours, cytotoxicity data of brine shrimp was collected. From this data made brine shrimps % alive & LD-50 concentrations.

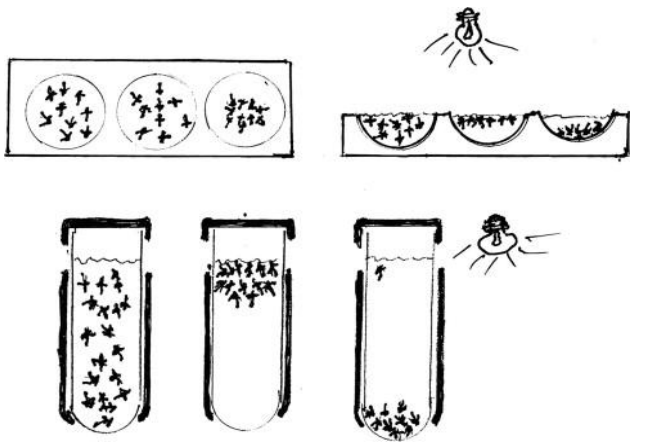


Figure 4.5: Cytotoxicity test of Brine Shrimps

Figure 4.6: Brine Shrimps

4.8 Total Phenolic Assay

4.8.1 Principle

The total phenolic content in Jute Leaf (Caffeine) extracts was determined by Folin–Ciocalteu Reagent (FCR). The FCR measures a sample's reducing capacity by measures the total amount of polyphenols presence within it.

4.8.2 Reduction Reaction

The Folin Ciocalteu reagent contains hetero poly phosphotun states, molybdates ion. Reversible one or two-electron reduction reactions lead to blue species, possibly (PMoW11O40)4-. Molybdenum ion is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Molibdenum (VI): **[28]**

4.8.3 Procedure-

4.8.3.1 Salicylic Acid Preparation-

1gm salicylic acid was mixed with 100 ml distilled water.

4.8.3.2 Na2Co3 Preparation-

10.6 gm Na2Co3 was mixed with 100 ml distilled water.

4.8.3.3 Folin-Ciocalteu reagent preparation

10 ml Folin-Ciocalteu reagent was mixed with 90 ml distilled water. (Total 100 ml was prepared in an amber color bottle).

4.8.3.4 Blank Preparation-

5ml chloroform was used here as a blank sample.

4.8.5 Assay Procedure-

Salicylic acid was taken in 5 test different concentrations as 5ml, 2.5 ml, 1.25 ml, 0.625 ml & 0.3125 ml. Rest of the volume of every test tubes was adjusted with water up to 5ml.(Without first one).Then 100 µl concentration was taken with 100-1000 µl micropipette. Then was taken it

another 5 test tubes.4 ml Na2Co3 was taken in every test tube. Then 5 ml Folin-Ciocalteu reagent was added in every test tube. Then keep the test tubes in dark place for 30 minutes. (For incubation)

Assay for Pure & Isolated Caffeine-1mg dried Jute leaf sample was taken in a test tube & 1ml chloroform was added with it. Then 100 µl concentrations were taken. Pure Caffeine was used here as a standard & prepared the same concentration & procedure as isolated Caffeine from Jute leaf sample. After 30 minutes the absorbance of salicylic acid, Isolated & Standard Caffeine sample was taken in U.V Spectrophotometer. (ƛ max- 765 n.m.)

The Total content of phenolic compounds in Jute leaf Caffeine extracts in salicylic acid equivalents (SAE) was calculated by the following formula equation -
T = (C x V)/M; here-
T = total content of phenolic compounds, mg/g plant extract, in SAE;
C = the concentration of salicylic acid established from the calibration curve.
V = the volume of extract.
M= the weight of pure plant Jute Caffeine extract.

4.9 Chemical analysis by UV spectroscopy

4.9.1 Principle:

Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-mirrored device. The sample beam (colored magenta), passes through a small transparent container (cuvette) containing a solution of the compound as a transparent solvent. The other beam, the reference (colored blue), passes through an identical cuvette containing only the solvent. The intensities of these light beams are then measured by electronic detectors. The intensity of the reference beam, which should have little or no light absorption, is defined as $\mathbf{I_0}$. The intensity of the sample beam is defined as **I**. Over a short period of time, the spectrometer automatically scans all the component wavelengths in the manner described. The ultraviolet (UV) region is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm. **[29]**

Ultraviolet-visible spectroscopy is a method of detection using visible light and its adjacent light wavelengths. A UV-visible spectrometer is an instrument that uses a light source to pass through a sample chamber and detects metal ions and organic compounds. The UV-visible spectrometer is a powerful tool used in many laboratories**.** A light source shutter controls the amount of light from a specialized lamp that passes through the sample. The shutter is the only moving component of a UV-visible spectrometer. The advantage of this system lies in the simplistic design of the instrument. This rapid analysis is achieved only through proper calibration. The UV-visible technique is non-destructive to the sample and has a high sensitivity for detecting organic compound. **[29]**

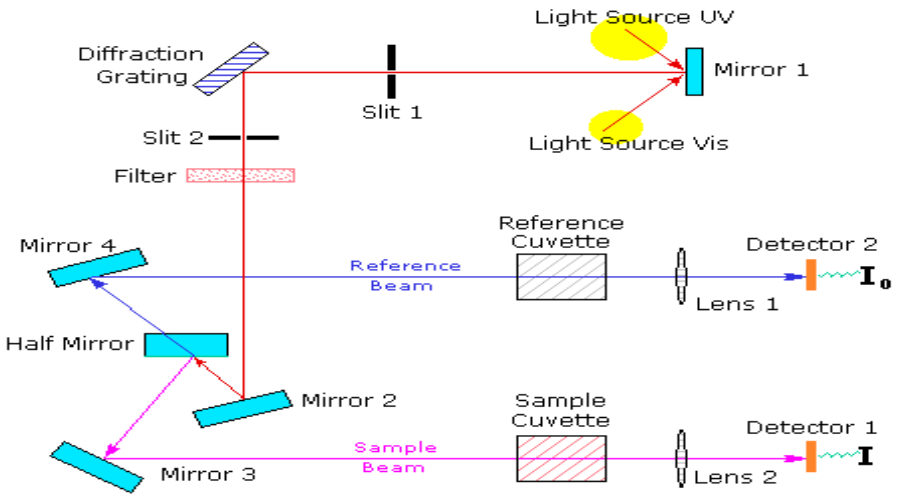


Figure 4.7: Instrumentation of a UV-Visible spectrometer

4.9.3 Determination of Caffeine Concentration in Isolated Caffeine from Jute Leaf in U.V. spectrophotometer

Pure & Isolated Caffeine Sample was measured with same concentration & dissolved in Hot water. After dilution, measured the amount of Caffeine in isolated caffeine sample (compared with pure caffeine) with the help of Spectrophotometer (ƛ max.-260 n.m).

4.9.3.1 Preparation of sample of Pure Caffeine-

5 mg pure Caffeine was dissolved in 50 ml distilled water. Then the Concentration of pure Caffeine was 100 µg/ml (Dissolved in hot water bath for 15 minutes, 80□c temperature).

4.9.3.2 Sample Dilution from stock solution-

At first 5 test tubes were taken & then serial dilution was performed as following concentration- 0.5, 1, 1.5, 2 & 2.5 ml Caffeine was taken from stock solution & 9.5, 9, 8.5, 8, 7.5 ml water was serially mixed with it.

4.9.3.3 Preparation of sample of Isolated (Jute Caffeine)

1mg dried sample of Isolated Caffeine was dissolved in 10 ml distilled water. (Dissolved hot water bath for 15 minutes, temperature-80□C). Then the Concentration of pure Caffeine was 100µg/ml. Then 1 ml from stock solution was taken & added with 9ml distilled water. Then the concentration of Isolated Caffeine was 10µg/ml.

Absorbance of Pure & Standard Caffeine was taken in U.V. spectrophotometer (ƛ max.-260 n.m) & compared the amount of Caffeine in both standard & Isolated Caffeine sample.

4.10 Antimicrobial assay:

The antimicrobial screening is the first stage of antimicrobial drug research. It is performed to ascertain the susceptibility of various fungi and bacteria to any extract samples, drug or any agent. This test measures the ability of each test sample to inhibit the in vitro fungal and bacterial growth. This ability can estimate by disc diffusion method.

4.10.1Disc diffusion method:

When a filter paper disc impregnated with a chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a **"**zone of inhibition**" [30].** Isolated Caffeine, from jute leaf extract was tested for antimicrobial activity of disc diffusion method.

4.10.2 Preparation of the Medium

1**.** 5.6 gm Nutrient agar powder was mixed with 200 ml distilled water**.**

2. Then the agar media was sterilized by autoclave machine

4.10.3 Sterilization Procedure

1**.** UV light was switched on, one hour before working in the Laminar Hood

2. Whatman's filter paper was punched, and 6 mm disks were collected in a beaker. Beaker Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121□C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized by autoclave machine & then kept in laminar hood.

3. Antimicrobial screening was done in Laminar Hood.

4.10.4 Preparation of the Test Plate

The test organisms were transferred from the subculture to petridish containing about 10 ml of sterilized agar medium. A sterilized cotton bud was taken and placed into the bacterial/ fungal subculture suspension. Then the bacterial/fungal sample was applied to the petridish with the help of sterilized cotton bud. All procedure was completed under laminar air flow**.**

4.10.5 Preparation of Standard Discs

Standard Discs were used as positive control to ensure the activity of standard antibiotic against the test organisms. Vancromycin (brand name Gentamycin) (30 µg/disc) used here as standard disc.

4.10.6 Preparation of Sample Discs with Test Sample

10 µl of isolated caffeine (600µg/µl) and pure caffeine (1000µg/µl) were loaded per disc.

4.10.7 Test Organisms

Different bacterial strains of gram positive, gram negative bacteria and fungi were used to carry out this assay. Table: 4.1 List of microorganisms

Gram positive Bacteria	Gram negative Bacteria	Fungi
1.*Streptococcus pyrogen*	*1.Pseudomonas aureus*	1.*Candida albicans*
2.*Staphylococcus aureus*	2.*Kleb siella*	2.*Saccharomyces cerevisiae*
3.β *hemolytic streptococcus*	3.*E.coli*	3.*Aspergillus niger*
	4.*Salmonella P.typhi*	

4.10.8 Procedure:

1. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts were placed on nutrient agar medium, contained the test microorganisms.

2. Standard antibiotic discs were used as positive control.

3. The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the micro organisms.

4. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter.

Part: 3

Isolated Caffeine

Result & Discussion

4.11.1 Result: TLC of Isolated Caffeine from Jute leaf

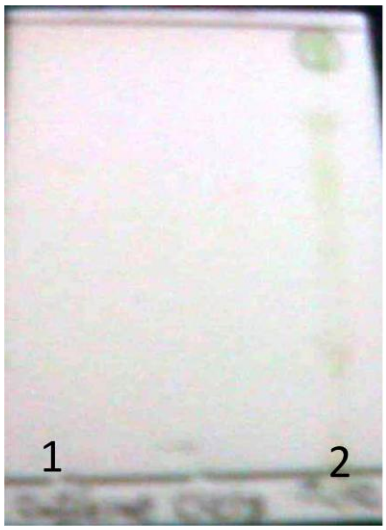


Fig 4.8: TLC plate under open eye

Fig 4.9: TLC plate under UV light

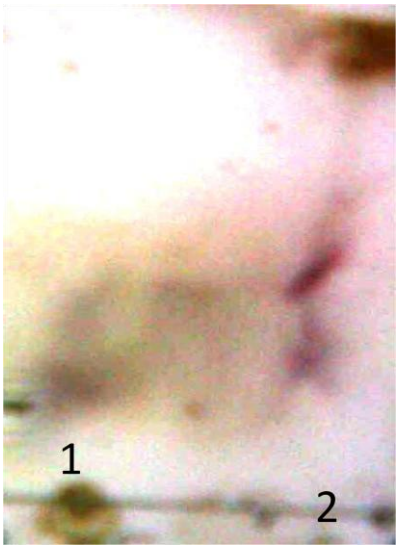


Fig 4.10 After charring with 10% H_2SO_4 solution

Fig 4.11:After dipped in DPPH solution

TLC plate 1 = Pure Caffeine, 2 = Isolated Caffeine

4.11.2 R.F (Retardation Factor) Value Calculation:

R.F value: Distance traveled by solute (Extract spot phase)

Distance traveled by solvent (Mobile phase)

R.F value for Pure Caffeine = 4.0 c.m = 0.66 c.m

6.0c.m

R.F value for Isolated caffeine = 5.9 c.m = 0.98 c.m

6.0c.m

4.11.3 Result:

Open Eye Observation- Showed individual peak of isolated caffeine, Green color, (in spot 2).

Observation under U.V light-

1. Under U.V. light we showed two individual peaks.

2 One was the peak of pure Caffeine, Deep Brown Color, (in spot 1).

3. One was the peak of Chloroform, Lower Layer of Jute Extract, Red Color, (in spot 2).

Charring of T.L.C Plate

After charring of the TLC plate with sulfuric acid, visualized two spots, one was for pure caffeine in spot 1 & other was isolated caffeine in spot 2.

Antioxidant Screening

Spraying of the DPPH solution in the TLC plate shows moderate yellow color isolated caffeine (in spot 2), which indicates the presence of antioxidant (flavonoid) compounds in the sample.

4.12 Chemical analysis of U.V spectroscopy (Isolated Caffeine)

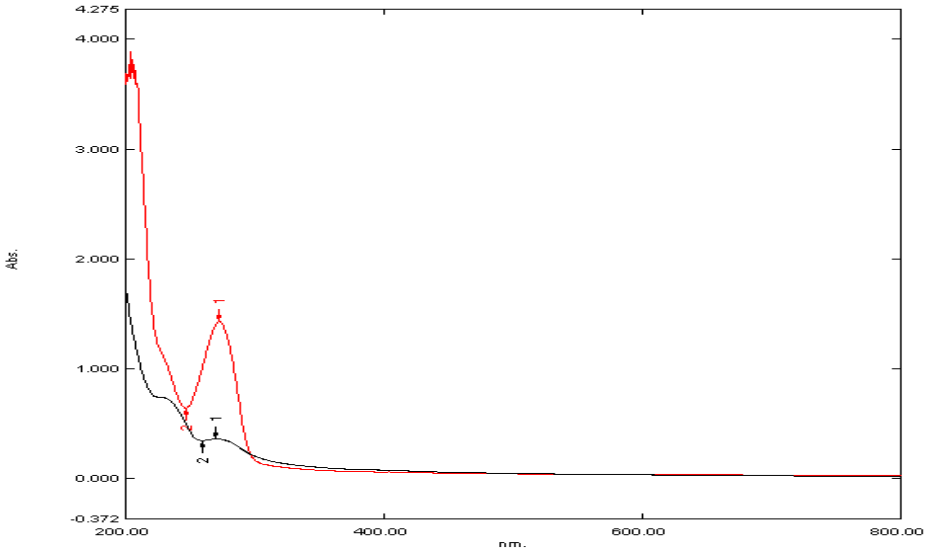


Figure 4.12: Chemical analysis of U.V spectroscopy

Result:

U.V spectrophotometer give peak on ƛmax=272 n.m for pure caffeine (black line) & isolated caffeine (red line) give peak on ƛmax=272 n.m & the concentration of both pure & isolated caffeine sample was 0.1 mg/ml (Dissolved in hot water). This value indicates the presence of caffeine in jute leaf corchorus capsularis.

Explanation:

From the scanning curve we find that the λ max for caffeine for jute was 272nm. After analysis of isolated caffeine's structural compound, (compared also with pure caffeine's structure) and the responses of its chromophores to UV radiation we can give the U.V analysis explanation. For our experiment the caffeine was dissolved in hot water. As water is a polar solvent and can form hydrogen bonds with the presence of O and N atoms, caffeine undergoes keto-enol tautomerism. The enol form is more favored in water, the λ max can be calculated theoretically using Woodward‟ s rules for enones and the structure of the enol form helps us to understand H-Based form on the structure of the enol form of caffeine,. Here in jute leaf, the original enone ring helps to show the λ max of caffeine in 272nm.

4.13 Antioxidant Test

Table: 4.2 Determination of Free Radical Scavenging Capacity of Ascorbic Acid, Pure & Isolated Caffeine

Name	Concentration µg/ml	Absorbance	% Scavenging
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	52.38
Ascorbic Acid (S5)	200	0.098	53.33
Blank	0	0.210	0
Name	**Concentration µg/ml**	**Absorbance**	**% Scavenging**
Pure Caffeine(P1)	40	0.168	20
Pure Caffeine (P2)	80	0.163	22.38
Pure Caffeine (P3)	120	0.154	26.66
Pure Caffeine (P4)	160	0.143	31.90
Pure Caffeine (P5)	200	0.135	35.71
Blank	0	0.210	0
Name	**Concentration µg/ml**	**Absorbance**	**% Scavenging**
Isolated Caffeine(C1)	40	0.148	29.52
Isolated Caffeine (C2)	80	0.141	32.85
Isolated Caffeine (C3)	120	0.134	36.19
Isolated Caffeine (C4)	160	0.128	39.04
Isolated Caffeine (C5)	200	0.119	43.33

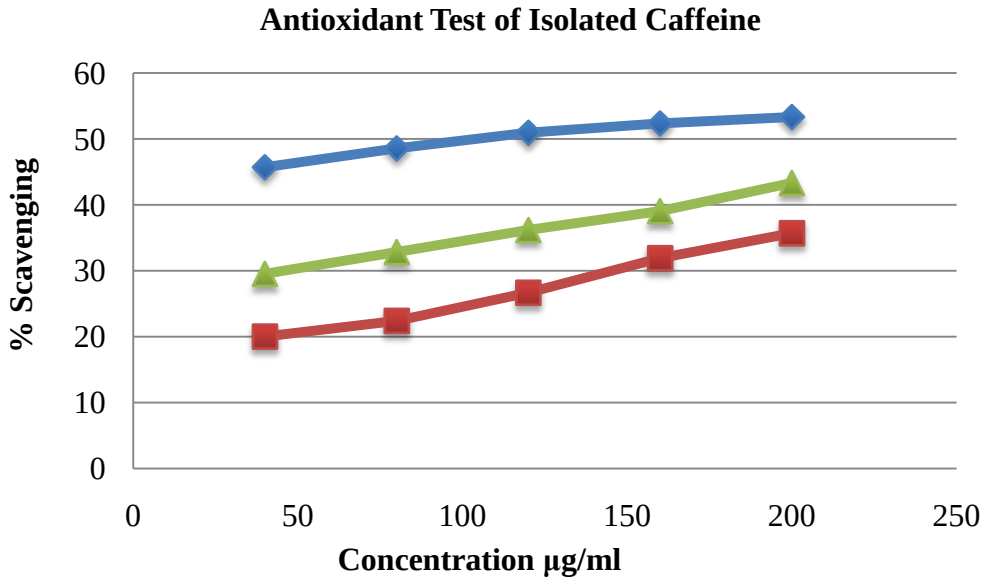


Fig 4.13 DPPH Scavenging potential of Corchorus capsularis: Isolated Caffeine (Green line), Pure Caffeine (Red Line), & Ascorbic acid (Blue Line)

Result:

Corchorus capsularis (Jute leaves) Isolated Caffeine extract was subjected to free radical scavenging (antioxidant) activity, & it showed an IC 50 value of 0.285 mg/ml.(285.23 µg/ml),by the equation of $y=0.084x+26.04, R^2=0.996$.

Here Pure Caffeine, showed free radical scavenging activity with an IC 50 value of 0.342 mg/ml.(342.74 µg/ml),by the equation of $y=0.102x+15.04, R^2=0.987$.

Ascorbic acid, showed free radical scavenging activity with an IC 50 value of 0.117mg/ml.(117.65 µg/ml),by the equation of $y=0.047x+44.47, R^2=0.956$.

4.14 Brine shrimp lethality test (Isolated Caffeine)

Table 4.3: Percentage of Nauplii alive

Sample	Concentration (mg/ml)	No. of nauplii alive	% alive
Pure caffeine	5	0	0
	2.5	3	33.3
	1.25	4	44.4
	0.625	6	66.6
	0.3125	7	77.7
	Control	10	100
Isolated caffeine	5	0	0
	2.5	2	22.2
	1.25	4	44.4
	0.625	5	55.5
	0.3125	8	88.8
	Control	10	100

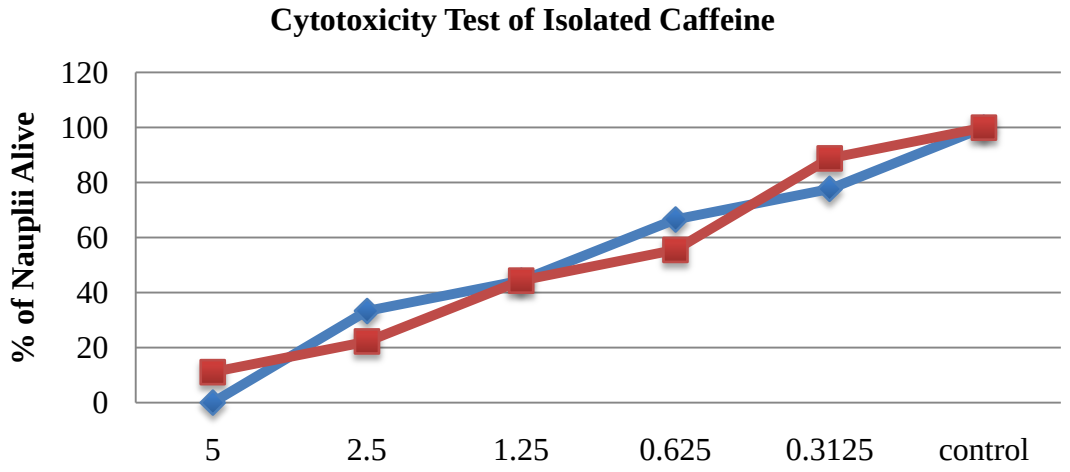


Fig 4.14: % of nauplii alive curve, Isolated Caffeine (Red Line), Pure Caffeine (Blue Line)

Table 4.4: Percentage of lethality Test

Sample	Concentration (mg/ml)	No. of nauplii dead	% of lethality	LD_{50} (mg/ml)
Pure caffeine	5	10	100	**0.9375**
	2.5	7	70	
	1.25	6	60	
	0.625	4	40	
	0.3125	3	30	
	Control	1	10	
Isolated caffeine	5	10	100	**0.625**
	2.5	8	80	
	1.25	6	60	
	0.625	5	50	
	0.3125	2	20	
	Control	1	10	

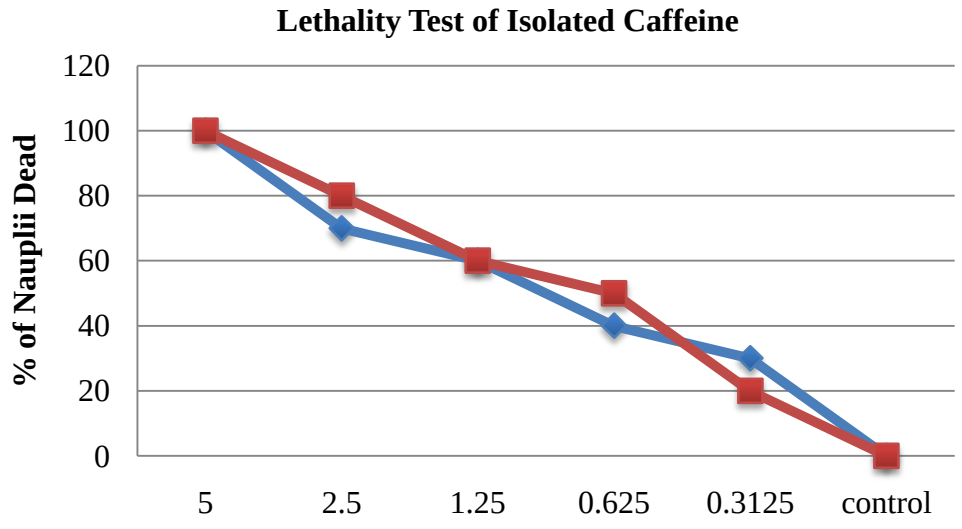


Fig 4.15: % of lethality Test, Isolated Caffeine (Red Line), Pure Caffeine (Blue Line)

4.15 Determination of Total phenolic content (Isolated Caffeine)

Table 4.5: Assay values of standard solution of salicylic acid

Serial no.	Concentration (mg/ml)	Absorbance (at 765 nm)
01	5	0.162
02	2.5	0.135
03	1.25	0.119
04	0.625	0.112
05	0.3125	0.11

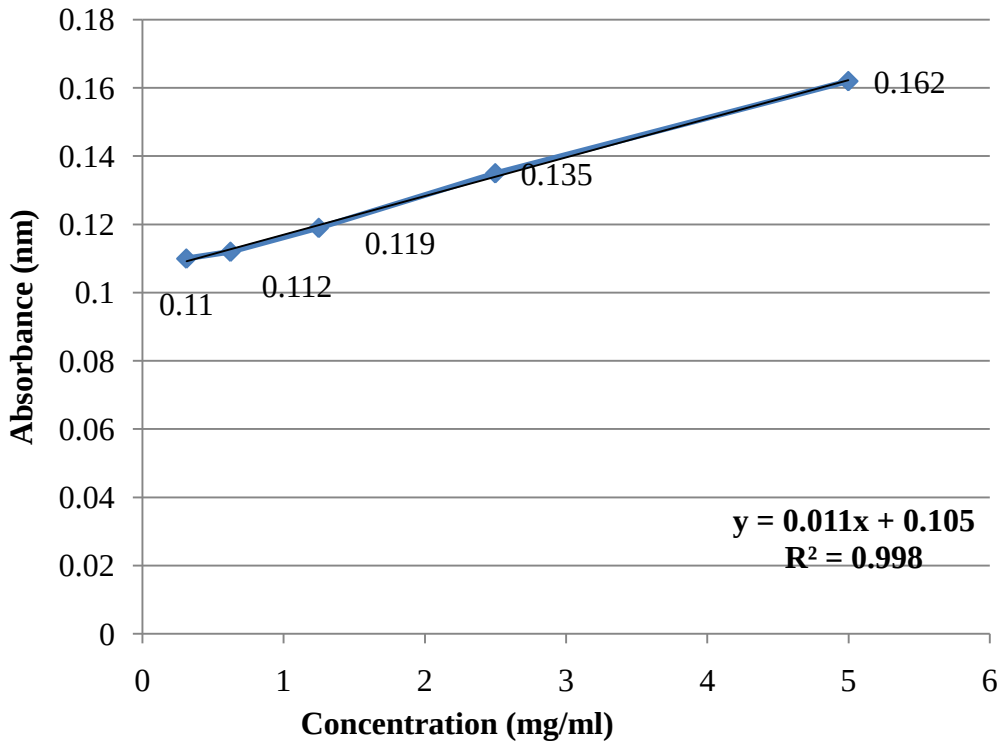


Fig 4.16: Standard curve of salicylic acid

Table 4.6: Total Phenolic Content of Pure Caffeine & Isolated Caffeine

Sample name	Absorbance at 765 nm	Concentration (mg/ml) [x= y-0.105/0.011]	TPC (mg/g) [T= CxV/M)	Average with SD
Pure Caffeine	0.151	4.2	42	**38.2±3.41**
	0.144	3.54	35.4	
	0.146	3.72	37.2	
Isolated Caffeine	0.157	4.72	47.2	**50.26±2.75**
	0.161	5.09	50.9	
	0.163	5.27	52.7	

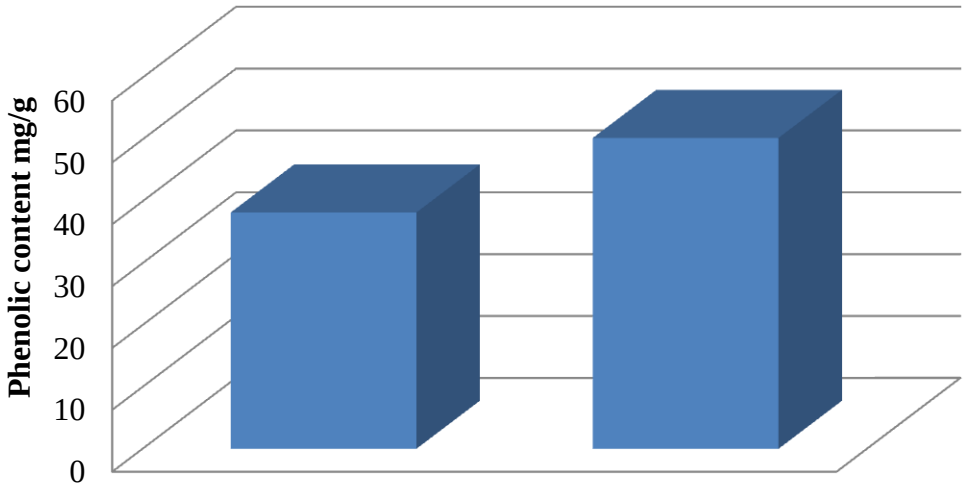


Fig 4.17: Graph of TPC of pure caffeine (38.2) & isolated caffeine (50.26)

Result: From the standard curve, the total phenolic compounds as Salicylic acid equivalent (SAE) of the pure caffeine and isolated caffeine was 38.2 mg/g and 50.26 mg/g respectively.

4.16 Determination of caffeine in *Corchorus capsularis* by UV-VISIBLE spectroscopy

Table 4.7: Assay values of standard solution of pure caffeine

Serial no.	Concentration (µg/ml)	Absorbance (nm) at 260 nm
01	5	0.234
02	10	0.478
03	15	0.671
04	20	0.878
05	25	1.133

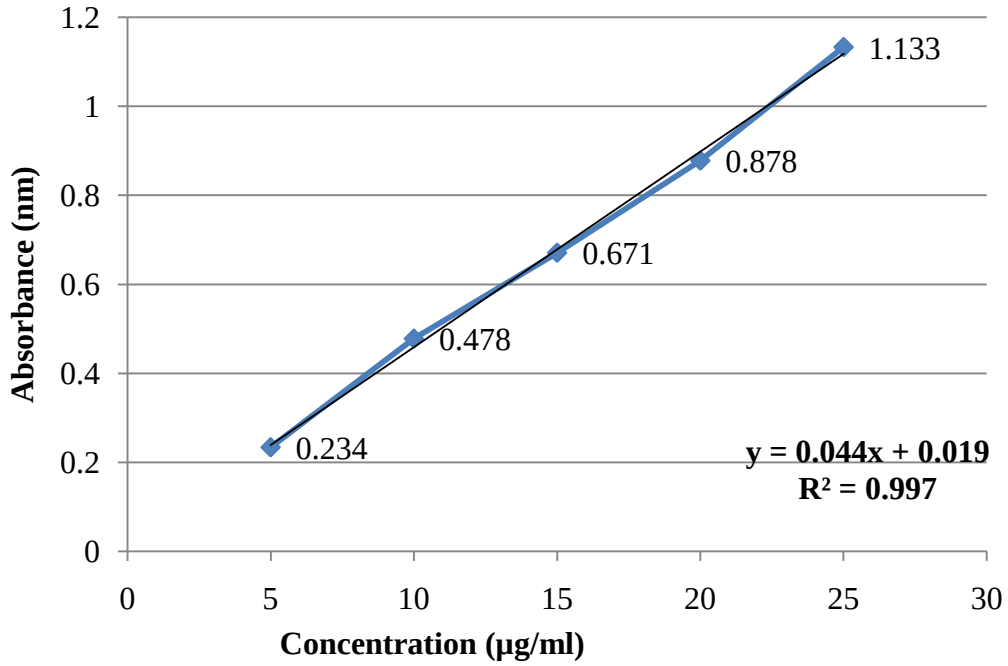


Fig 4.18: Standard curve of pure caffeine

Table 4.8: Assay of isolated caffeine by UV absorbance method

Serial no.	Absorbance at 260 nm	Concentration (µg/ml) [x= y-0.019/0.044]	Amount (mg)
1.	0.122	x= 2.34	**0.234**

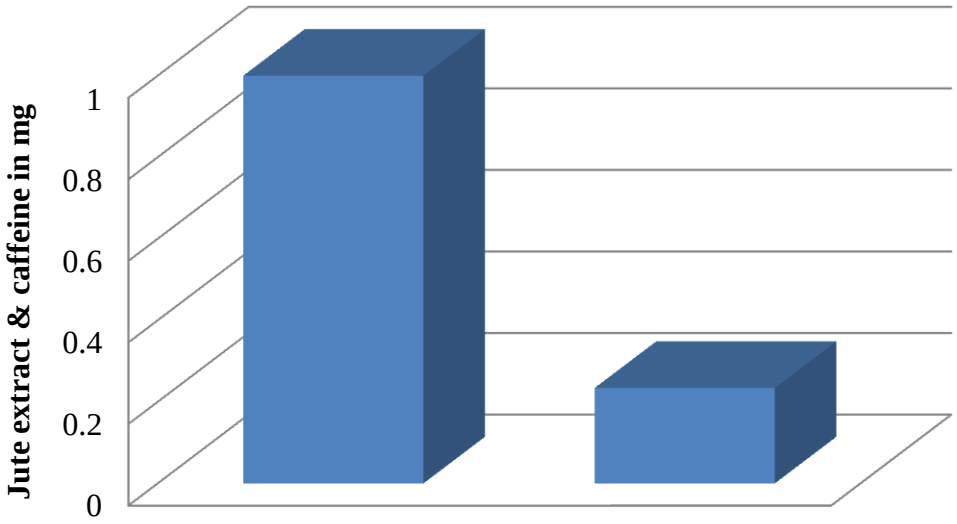
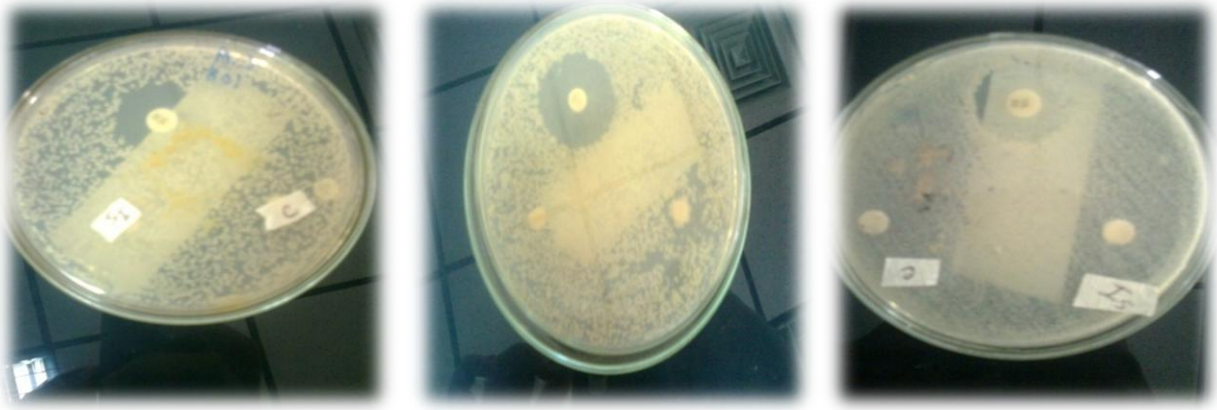


Fig 4.19: Presence of Caffeine (0.234 mg) in Jute Extract (1 mg)

Result: The amount of caffeine was measured from a standard curve of 1 mg *Corchorus capsularis* leaves extract contain 0.234 mg. caffeine.

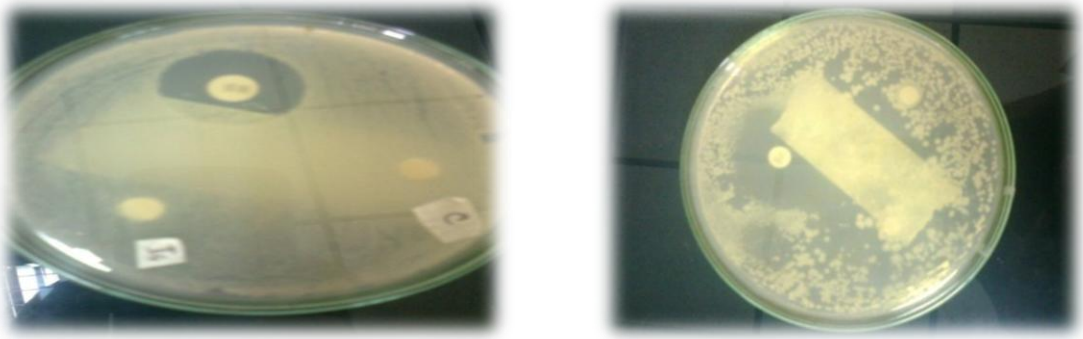
4.17 Antimicrobial assay (Isolated Caffeine)



Streptococcus pyrogen (gram +)

Candia albicans *(Fungi)*

E.coli *(gram-)*



Staphylococcus aureus *(gram +)*

Saccharomyces cerevisiae

Fig 4.20: Antimicrobial assay of Pure & Isolated Caffeine

Table 4.9: Antimicrobial screening of Corchorus capsularis (Jute Leaf) (Isolated Caffeine)

Type	Species	Zone of inhibition (mm)		
		Pure Caffeine (1000µg/µl)	**Isolated Caffeine (600µg/µl)**	**Vancomycin (30µg/ml)**
Gram (+)	***Streptococcus pyrogen***	-	-	25 mm.
	Staphylococcus aureus	-	-	20 mm.
	β-hemolytic streptococcus			18 mm
Gram (-)	***Salmonella typhi***	-	-	22 mm.
	E.coli	-	-	20 mm.
	Pseudomonas aureus			20 mm.
	Kleb siella			15 mm.
Fungi	***Candida albicans***	-	-	22 mm.
	Saccharomyces cerevisiae	-	-	20 mm.

Result: Both pure and isolated caffeine has no antimicrobial activity.

4.18 Discussion Isolated Caffeine:

Antioxidant Test -.

Phytochemicals screening of *Corchorus capsularis* leaf demonstrated the presence of flavonoids, saponins, triterpenes and steroids, but not alkaloids. 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 flavanoids type. **[31]**

From experiment DPPH Scavenging potential & IC 50 value of Pure Caffeine were 0.342 mg/ml & DPPH Scavenging potential & IC 50 value of Isolated Caffeine was 0.285 mg/ml. The results of caffeine antioxidant tests showed that caffeine effectively scavenges ·OH with a reaction rate is a constant of $5.9 \times 10^{9} m^{-1} sec^{-1}$ that is comparable with those of other efficient ·OH radical scavengers & give antioxidant property. **[32]**

Methanolic extract of *Corchorus capsularis* showed DPPH Scavenging potential & IC 50 value of 0.301 mg/ml.

Because of presence of flavonids in *Corchorus capsularis* leaf & because of antioxidant activity is present in pure caffeine, Isolated caffeine of *Corchorus capsularis* leaf extract showed antioxidant activities with an I.C.50 value of 0.285 mg/ml.

Cytotoxicity Test-

Caffeine's cytotoxicity is related with its carcinogenic and anticarcinogenic properties. The reaction of 1, 3, 7-trimethylxanthine (caffeine) with the hydroxyl radical (·OH), as investigated by electron spin resonance (ESR) spin trapping. The ·OH was generated by the Fenton reaction ($Fe^{2+} + H_2O_2$) as well as by the reaction of chromium (V) with H_2O_2. ESR measurements provide evidence that a caffeine-derived oxygen-centered radical is formed in the reaction of caffeine with ·OH and responsible for carcinogenic (oxidative D.N.A breakage by hydroxyl radicals, oxidative D.N.A degradation in the presence of copper or chromium ion) & anticarcinogenic properties of caffeine and related methylxanthine compounds**. [33]**

Phytochemicals screening of *Corchorus capsularis* leaf contains HCN, which may responsible for cytotoxicity. The LD50 of tissue extracts to mice report that the "lethal dose" of Corchoroside A to mice are 0.053-0.0768 mg/kg and Corchoroside B 0.059-0.1413**.[15]**

Caffeine is a mild stimulant in central nervous system. The LD50 of tissue extracts to rats report that the "lethal dose" of Caffeine for rats are 200 mg/kg body weight**. [15]**

Isolated caffeine sample from *Corchorus capsularis* leaf extract showed LD50 values of 0.625 mg/ml, which was compared with pure caffeine LD50 values of 0.9375 mg/ml.

Antibacterial Test-

The phytochemistry and antimicrobial potential of *Corchorus capsularis* leaf extracts 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*. 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 indicates than the aqueous extracts. Susceptibility increased with concentrations and highest susceptibility was observed against *E. coli*. The *Corchorus capsularis* methanolic extracts exhibited the high antimicrobial activity**. [18]**

But with comparison of pure caffeine, isolated caffeine sample of *Corchorus capsularis* showed no antibacterial activity.

Total Phenolic test

Isolation of *Corchorus capsularis* gives characterizaton of cyanidin glucoside, indicates the presence of phenolic compounds**. [15]**

The phenolic content found in the crude methanolic extract of *Corchorus capsularis* (leaves) was 0.226 mg equivalent of Tannic acid (TAE) per mg of dried extract

The phenolic content found in *Corchorus capsularis* (leaves) isolated caffeine extract was 50.26 mg with salicylic acid equivalent (SAE) per gram of dried extract.

The phenolic content found in pure caffeine was 38.20 mg with salicylic acid equivalent (SAE) per gram. From literature, get caffeine has total phenolic content of 35.3mg/g.

Because of presence of cyanidin glucoside in *Corchorus capsularis* (leaves) & phenolic compounds are present in caffeine, isolated caffeine showed total phenolic content of 50.26 mg/g which is higher than pure caffeine respectively.

Chapter – 5

Part: 1

Catechin Isolation

From Jute Leaf

5.1Catechin

Catechin is a flavan-3-ol (secondary metabolite) type of compound. It belongs to the chemical family of flavonoids. The name of the catechin derives from **catechu**; it is the boiled extract of *Mimosa catechu* (*Acacia catechu L.f*).

Catechins are mainly found in a variety of plant, but also present in foods and beverages. Catechins are classified as the following compounds: catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate etc. High concentrations of catechin are found in red wine, broad beans, black grapes, apricots and strawberries.

5.2.1 History of catechin-

L-Epicatechin can be found in cacao beans and was first called kakaool or cacao-ol. Maximilian Nierenstein at first identified the presence of catechin in cocoa beans.

5.2.2 Catechin Health Benefits-

Catechins are effective scavengers of reactive oxygen species in vitro and function indirectly as antioxidants through their effects on transcription factors and enzyme activities in our body. Tea catechins are rapidly and extensively metabolized emphasizes the importance of their antioxidant activity in vivo. In humans, modest transient increases in plasma antioxidant capacity showed by consumption of tea and green tea catechins. The effects of tea and green tea catechins as biomarkers of oxidative stress, especially oxidative DNA damage, appear very promising in animal model. Larger human studies examine the effects of tea and tea catechin intake on biomarkers of oxidative damage to lipids, proteins, and DNA. **[34]**

Figure 5.1: Foods contain Catechin (Red wine, Black tea, Green tea)

5.3 Isolation of catechin from Jute leaf (*Corchorus capsularis)*

150 g of the dried powder of jute leaf was taken to which two liters of distilled water were added

Boiled the extract solution over a hot water bath for two hour with continuous stirring & when the solution was started to boiled then the temperature was 90□ c. After the boiling period is over, removed the beaker from the heat and allowed to cool it for 45 minutes or on ice until cooled**.**

After one hour the extract was filtered through a fine muslin cloth to remove jute leaf powders and other suspended materials. Finally get 1125 ml liquid squeezed extract solution.

We filtered the solution two times through whatman's filter paper for get a clear solution. Then it was evaporated to 500 ml with a rotary Evaporator and the obtained precipitate was filtered using a filter paper. The aqueous filtrate (500 ml) was taken.

The residue (extract solution) was mixed with Dichloromethane (200 ml).Then stirred the solution continuously 20 minutes for well mixing. Then phase separation was done by using a 250 ml separator funnel. **[35]**

After separation, get two different layers. One was the aqueous layer of jute leaf (500 ml) & the other was the layer of Dichloromethane (160 ml).

With the aqueous layer of jute extract (500 ml) we were added 200 ml of pure Acetone. Stirred continuously for well mixing (25 minutes).Then with the separator funnel we separated two different layers.

Result:

After separation, get two different layers, among them one was the layer of jute leaf extract (600 ml) & other was the layer of acetone (100 ml).

Part: 2

Isolated Catechin Methods of Experiment

5.4 Thin Layer Chromatography Analysis –

Reagent Media-1.Chloroform - 5 ml

2. Ethyl Acetate - 4 ml

3. Formic Acid - 1 ml

Solvent System-1.Upper layer of Jute extract

2. Dichloromethane Layer

3. Lower Layer of Acetone (Jute extract)

4. Pure Caffeine

5. Pure Chloroform

Apparatus-1.Tlc Tank 4.Tlc plate

2. Capillary Tube 5.Pipette Pumper

3. Watch Glass

Procedure- 1.At first gave total five spots on the TLC plate.

2. One spot was from the jute extract, one was Acetone, and other was spot of Dichloromethane & other two was caffeine & chloroform's spots.

3. Then placed the TlC plate in TLC Tank.

4. After ten minutes when run of TLC solvent was completed then we placed the TLC Plate under U.V. light.

For detection of Flavanoids the plate was dipped into 0.04% DPPH Solution & allowed it to be dried while keeping in a dark place. For detection of poly phenols the plate was washed with Folin-ciocalteu reagent & then dried.

5.5 Antioxidant Tests (DPPH Test) (Isolated Catechin)

Apparatus

Test Tube	U.V Spectrophotometer
Racker	Analytical Balance
Reagent	
DPPH	L-Ascorbic Acid
Jute Leaf (Catechin extract)	Water
(Lower layer acetone fraction)	

5.5.1 Preparation of DPPH Reagent

0 .008 gm DPPH was weighed & dissolved in 20 ml distilled water. (400 µg/ ml).

5.5.2 Preparation of Extract Solution

0.02 gm of Catechin Extract was weighed in volumetric flask & the volume was made up to 50 ml with water. The concentration was 400 µg/ ml.

5.5.3 Preparation of positive Control

0.01 gm Ascorbic acid was weighted & the volume was made up to 50 ml with water. The concentration was 200 µg/ ml. Ascorbic acid was diluted with same procedure of jute catechin.

5.5.4 Procedure

1. The blank was prepared with 5ml water & DPPH solution with 100µl (control).

2. Five test tubes was marked with the following concentration as 01(40µg/ml), 02(80µg/ml), 03(120µg/ml) , 04(160µg/ml), 05(200µg/ml).

3. With this concentration plant extract solution (jute leaf lower layer) 1, 2, 3, 4, & 5 ml was transferred & the volume was adjusted up to 5 ml with water.

100 µl DPPH solutions were adjusted to each test tube. Then U.V. absorbance was taken at 517 nm.

5.6 Brine Shrimps Cytotoxicity & Lethality Test (Isolated Catechin)

Prepared Brine Shrimps (sample) with 1 liter= 38 g (Nacl Solution)

After Brine Preparation PH need 8.5(Adjusted with NaoH solution)

5.6.1 Hatching- 1.Light 3. O2 Circulation

2. Aeration 4. Nauplii /Egg (Brine Shrimp)

5.6.2 Observation Period- 48 hours

5.6.3. Shrimp Collection-

1 .Six test tubes were taken.

2. 10 Nauplii were collected in every test tube.

3. Nauplii collected from brine shrimp samples.

5.6.4 Sample Preparation-

20 mg of dry drug sample of catechin was measured & dissolved in 2 ml hot water.

5.6.5 Sample Dilution

1. Stock solutions concentrations was 10 mg/ml
2. Then the stock solution was diluted with the following concentration- (Serial Dilution) 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml, 0.3125 mg/ml.(1 ml sample was taken from every test tubes & 1 ml water added.)

Pure acetone was used as standard for compare the result of catechin (present in lower layer of jute leaf acetone fraction). One blank was prepared without given any drug only brine shrimp sample (Control group). For Cytotoxicity measurement compared it with the drug sample.

5.6.6 Brine Shrimps Addition with Isolated Catechin sample-

100 µl isolated catechin was taken from every test tube. (After dilution)

Then the sample of (Isolated Catechin) was placed in Brine Shrimps Nauplii's test tubes with accurate labeling of the dilution serial of catechin sample.(was labeled with 1-6 & 6 No. was blank.)

Then the volume of every test tube was adjusted with Brine.

Same procedure of sample addition with shrimp was followed for pure acetone.

5.6.7 Observation-

Then the test tube of Brine Shrimps with drug Sample (Catechin) was kept in 24 hours for observation & after 24 hours Cytotoxicity Data of Brine Shrimp (Nauplii) was collected.

5.7 Total Phenolic Assay (Isolated catechin)

5.7.1 Salicylic Acid Preparation-

1gm salicylic acid was mixed with 100 ml distilled water.

5.7.2 Na2Co3 Preparation-

10.6 gm Na2Co3 was mixed with 100 ml distilled water.

5.7.3 Folin-Ciocalteu reagent preparation

10 ml Folin-Ciocalteu reagent was mixed with 90 ml distilled water. (Total 100 ml was prepared in an amber color bottle).

5.7.4 Blank Preparation-

5ml water was used here as a blank sample.

5.7.5 Assay Procedure-

Salicylic acid was taken in 5 test different concentrations as 5ml, 2.5 ml, 1.25 ml, 0.625 ml & 0.3125 ml. Rest of the volume of every test tubes was adjusted with water up to 5ml.(Without first one).Then 100 µl concentration was taken (with 100-1000 µl micropipette) in another 5 test tubes. 4 ml Na2Co3 was taken & then 5 ml Folin-Ciocalteu reagent was added in every test tube. Then keep the test tubes in dark place for 30 minutes. (For incubation)

5.7.6 Assay for Isolated Catechin-

100 µl sample of isolated catechin were taken. 4 ml Na2Co3 was added & then 5 ml Folin-Ciocalteu reagent was added in it. Then keep it in a dark place.

After 30 minutes the absorbance of salicylic acid & isolated catechin sample was taken in U.V Spectrophotometer. (ƛ max- 765 n.m.)

The Total content of phenolic compounds in Jute leaf Caffeine extracts in salicyic acid equivalents (SAE) was calculated by the following formula equation -

$T = (C \times V)/M$

Where:

T = total content of phenolic compounds, mg/g plant extract, in SAE;

C = the concentration of salicylic acid established from the calibration curve.

V = the volume of extract.

M= the weight of pure plant Jute Catechin extract.

5.8 Antimicrobial assay:

The antimicrobial screening is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the in vitro fungal and bacterial growth. This ability may be estimated by disc diffusion method.

5.8.1Disc diffusion method:

When a filter paper disc impregnated with a chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "zone of inhibition**". [30]** In the present study isolated Catechin from jute leaf extract were tested for antimicrobial activity of disc diffusion method.

5.8.2 Preparation of the Medium

1**.** 5.6 gm Nutrient agar powder was mixed with 200 ml distilled water**.**

2. Then the agar media was sterilized by autoclave machine

5.8.3 Sterilization Procedure

1**.** UV light was switched on, one hour before working in the Laminar Hood

2. Whatman's filter paper was punched, and 6 mm disks were collected in a beaker. Beaker Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121□C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized by autoclave machine & then kept in laminar hood.

3. Antimicrobial screening was done in Laminar Hood.

5.8.4 Preparation of the Test Plate

The test organisms were transferred from the subculture to petridish containing about 10 ml of sterilized agar medium. A sterilized cotton bud was taken and placed into the bacterial/ fungal subculture suspension. Then the bacterial/fungal sample is applied to the petridish with the help of sterilized cotton bud. All procedure was completed under laminar air flow**.**

5.8.5 Preparation of Standard Discs

Standard Discs were used as positive control to ensure the activity of standard antibiotic against the test organisms. Vancromycin(brand name Gentamycin) (30 µg/disc) used here as standard disc.

5.8.6 Preparation of Sample Discs with Test Sample

100µl upper & lower layer catechin was loaded per disc.

5.8.7 Test Organisms

Different bacterial strains of gram positive, gram negative bacteria and fungi were used to carry out this assay. Table: 5.1 list of microorganisms

Gram positive Bacteria	Gram negative Bacteria	Fungi
1.*Streptococcus pyrogen*	1.*Pseudomonas aureus*	1.*Candida albicans*
2.*Staphylococcus aureus*	2.*Kleb siella*	2.*Saccharomyces cerevisiae*
3.β *hemolytic streptococcus*	3.*E.coli*	3.*Aspergillus niger*
4.*Bacillus cereus*	4.*Salmonella P.typhi* 5.*Shigella dysentry*	

5.8.8 Procedure:

1. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts were placed on nutrient agar medium, contained the test microorganisms.

2. Standard antibiotic discs were used as positive control.

3. The plates are then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms.

4. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as zone of inhibition. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter.

Part: 3

Isolated Catechin

Result & Discussion

5.9 TLC of Isolated Catechin from Jute Leaf *(Corchorus capsularis*)

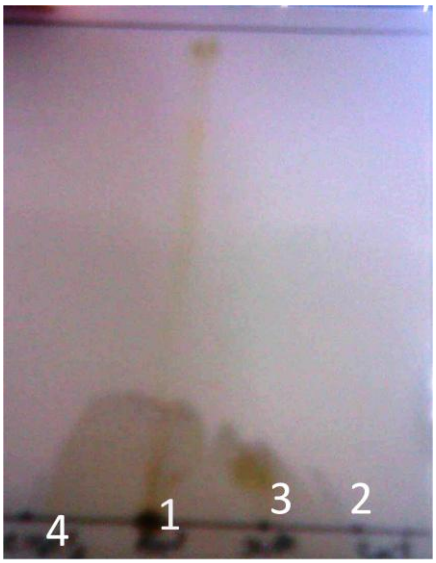


Fig 5.2: TLC plate under open eye

Fig 5.3: TLC plate under UV light

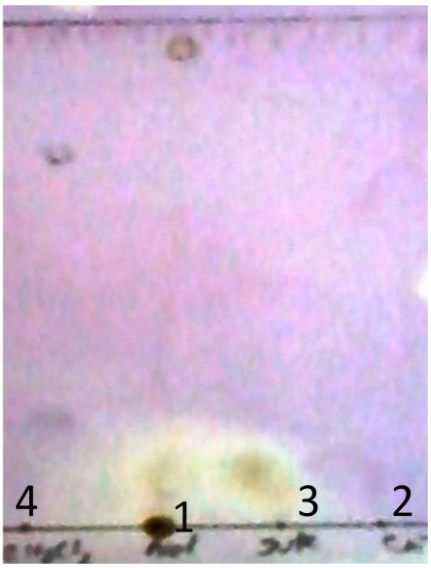


Fig 5.4: TLC plate after dipped in DPPH solution

Fig 5.5: TLC plate after charring

TLC plate 1=Lower layer jute extract (Isolated catechin), 3=Upper layer of jute Extract, 2=Pure Caffeine, 4=Dichloromethane

5.9.1 R.F (Retardation Factor) Value Calculation:

R.F value: Distance traveled by solute (Extract spot phase)

Distance traveled by solvent (Mobile phase)

R.F value for Isolated Catechin = 6.1 c.m = 0.94 c.m

6.5c.m

R.F value for Pure Caffeine = 4.3 c.m = 0.66 c.m

6.5c.m

R.F value for Dichloromethane= 5.0 c.m = 0.77 c.m

6.5c.m

5.9.2 Result:

Open Eye Observation- Showed peak of isolated catechin, lower layer of jute extract, Light green color, in spot 1(more well) then upper layer of jute extract in spot 3.

Observation under U.V light-

1. Under U.V. light we show three individual peaks. One was the peak of pure Caffeine (Brown Color in spot 2), one was the peak of isolated catechin (Lower Layer Jute Extract, Red Color in spot1), and one was the peak of Dichloromethane, (White Color in spot 4).

Staining with DPPH (2, 2-diphenyl-1-picrylhydrazyl)-

For detection of Flavanoids the plate was dipped into 10% DPPH Solution & allowed it to be dried while keeping in a dark place. In dark we showed a yellow peak of isolated catechin in spot 1 indicates the presence of Anti-Oxidant compound in Jute-leaf Catechin extract.

Charring of T.L.C Plate-

We again showed the peak of isolated catechin (Lower Layer Jute Extract) in spot 1 more well then before with brown color after charring.

5.10 Chemical analysis by UV spectroscopy

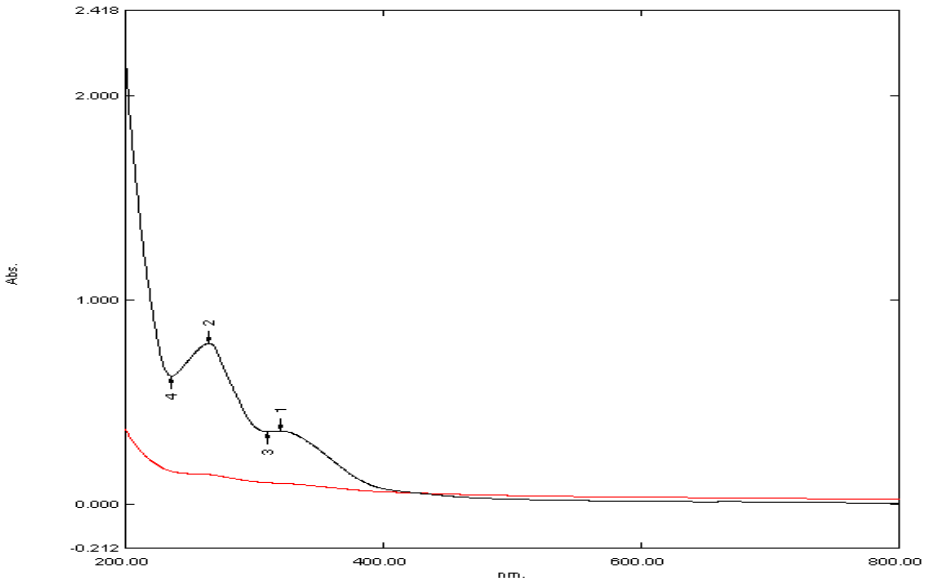


Figure 5.6: Chemical analysis of U.V spectroscopy

Result- U.V spectrophotometer give peak on ƛmax=264 n.m & ƛmax=320 n.m for isolated catechin (Lower layer acetone fraction) isolated catechin sample was 0.002 µg/µl (Dissolved in hot water).

Explanation: Catechin is an ester of gallic acid and epigallocatechin's combination. This is a very potent antioxidant & usually found in green tea. From the scanning curve the λ max for catechin was found in 264nm. Using the Woodward" s rules we can find an approximate estimate of the λ max that matches the actual value. Catechin has an ester link in its molecule and it is an oxyalkyl benzoyl derivative. Using this portion as the chromophore, and considering the substituents attached to this we can calculate the value for λmax-

Ring system	**Wavelength**
Oxyalkyl benzoyl derivative	230nm
One para hydroxyl substitution	25nm
Two meta hydroxyl substitutions (2X7)	14nm
Total	269 nm

The value obtained practically was 264nm, which is very close to the calculated value, 269 n.m.

5.11 Antioxidant Test

Table: 5.2 Determination of Free Radical Scavenging Capacity of Ascorbic Acid

Name	Concentration µg/ml	Absorbance	% Scavenging
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	52.38
Ascorbic Acid (S5)	200	0.098	53.33
Blank	0	0.210	0

Table: 5.3 Determination of Free Radical Scavenging Capacity of Isolated Catechin

Name	Concentration µg/ml	Absorbance	% Scavenging
Isolated Catechin (C1)	40	0.175	13.51
Isolated Catechin (C2)	80	0.169	19.52
Isolated Catechin (C3)	120	0.164	21.90
Isolated Catechin(C4)	160	0.154	26.66
Isolated Catechin (C5)	200	0.151	28.09
Blank	0	0.210	0

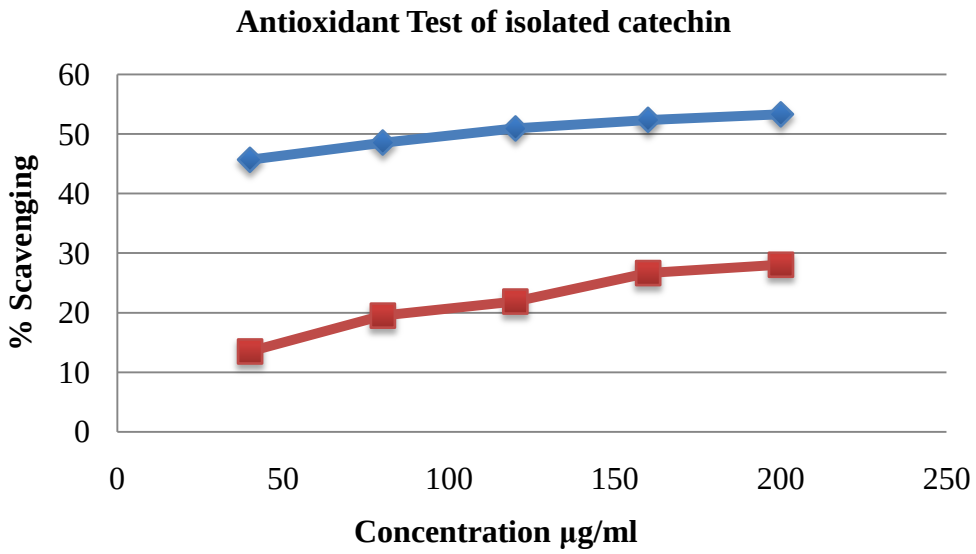


Figure: 5.7 DPPH Scavenging potential of Corchorus capsularis (Isolated Catechin-Red line) & (Ascorbic acid- Blue line)

Result-

Corchorus capsularis (Jute leaves) Isolated Catechin extract was subjected to free radical scavenging (antioxidant)activity, & it showed an IC 50 value of 0.432 mg/ml.(432.77 µg/ml),by using the equation of $y=0.090x+11.05, R^2=0.961$.

Here, Standard Ascorbic acid, showed free radical scavenging activity with an IC 50 value of 0.117mg/ml.(117.65 µg/ml),by using the equation of $y=0.047x+44.47, R^2=0.956$

5.12 Brine shrimp lethality test (Isolated Catechin)

Table 5.4: Percentage of Nauplii Alive

Sample	Concentration (mg/ml)	No. of nauplii alive	% alive
Pure Acetone	5	3	33.3
	2.5	4	44.4
	1.25	4	44.4
	0.625	5	55.5
	0.3125	6	66.6
	Control	10	100
Isolated Catechin	5	1	11.1
	2.5	3	33.3
	1.25	3	33.3
	0.625	4	44.4
	0.3125	5	55.5
	Control	10	100

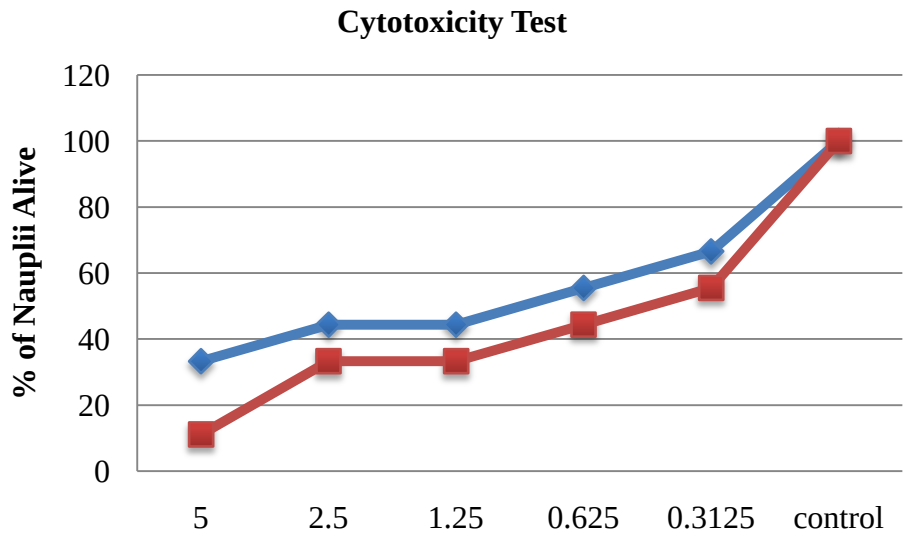


Figure 5.8: % of Nauplii alive (Isolated Catechin-Red Line) (Acetone-BlueLine)

Table 5.5: Percentage of Lethality Test

Sample	Concentration (mg/ml)	No. of nauplii dead	% of lethality	LD_{50} (mg/ml)
Pure Acetone	5	7	70	**0.625**
	2.5	6	60	
	1.25	6	60	
	0.625	5	50	
	0.3125	4	40	
	Control	0	0	
Isolated Catechin	5	9	90	**0.3125**
	2.5	7	70	
	1.25	7	70	
	0.625	6	60	
	0.3125	5	50	
	Control	0	0	

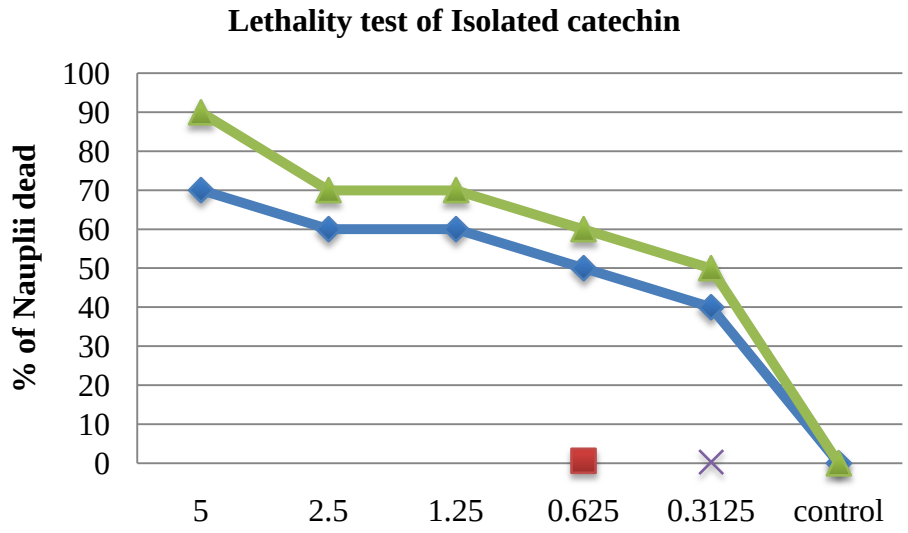


Figure 5.9: % of Lethality test (Isolated Catechin-Green Line) (Acetone-BlueLine)

5.13 Determination of Total phenolic content of Isolated Catechin

Table 5.6: Assay values of standard solution of salicylic acid

Serial no.	Concentration (mg/ml)	Absorbance (nm) at 765 nm
01	5	0.653
02	2.5	0.467
03	1.25	0.281
04	0.625	0.161
05	0.3125	0.117

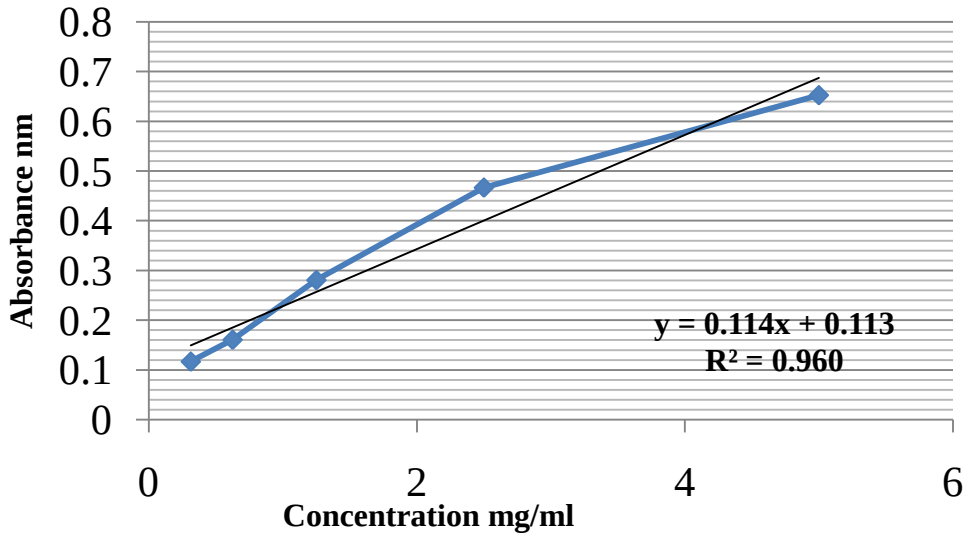


Figure 5.10: Standard curve of salicylic acid

Table 5.7: TPC (Total Phenolic Content) of Isolated Catechin

Sample name	Absorbance at 765 nm	Concentration (100µl) [x= y-0.113/0.114]	TPC (mg/g) [T=CxV/M)	Average with SD
Isolated Catechin	5.153	44.12 (4.412/ml)	44.12	**44.25±0.11**
	5.159	44.26 (4.426/ml)	44.26	
	5.173	44.38 (4.438/ml)	44.38	

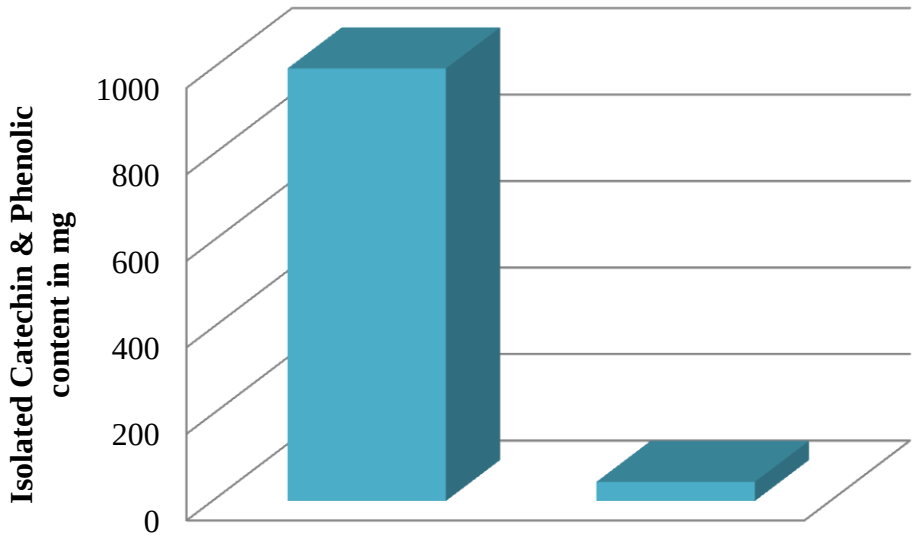
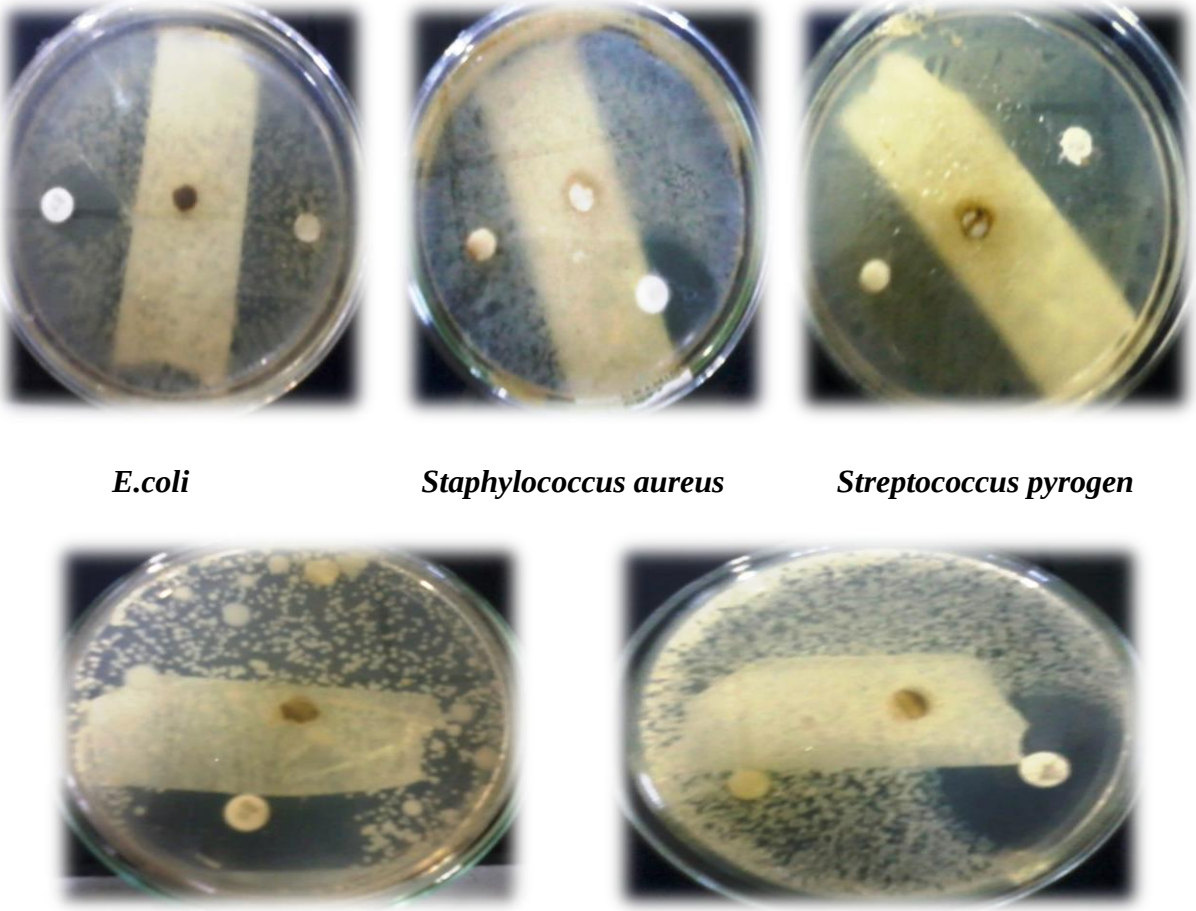


Figure 5.11: Graph of TPC of isolated catechin (44.25 mg in 1000 mg)

Result: From the standard curve, the total phenolic content as Salicylic acid equivalent (SAE) of the isolated catechin was 44.25 mg/g respectively.

5.14 Antimicrobial assay (Isolated Catechin)



E.coli ***Staphylococcus aureus*** ***Streptococcus pyrogen***

Kleb siella ***Salmonella p.typhi***

Figure5.12: Antimicrobial assay of isolated catechin

Table 5.8- Antimicrobial screening of Corchorus capsularis (Jute Leaf) (Isolated Catechin)

Type	Species	Zone of inhibition (mm)		
		Upper layer Jute leaf extract (100µg/µl)	**Lowerlayer Acetone fraction (100µg/µl)**	**Vancomycin (30µg/ml)**
Gram (+)	***Streptococcus pyrogen***	-	-	25 mm.
	Staphylococcus aureus	-	-	20 mm.
	β *hemolytic streptococcus*	-	-	19 mm
	Bacillus cereus			22 mm
Gram (-)	***Salmonella P typhi***	-	-	22 mm.
	E.coli	-	-	12 mm.
	Pseudomonas aureus	-	-	20 mm.
	Kleb siella		-	15 mm.
Fungi	***Candida albicans***	-	-	22 mm.
	Saccharomyces cerevisiae	-	-	20 mm.
	Aspergillus niger	-	-	17 mm.

Result: Catechin has no antimicrobial activity

Discussion Isolated Catechin:

Antioxidant Test-.

Catechins are a type of flavonoid found & are potent antioxidants that may help to protect us from potentially damaging chemicals called free radicals**. [32]**

Phytochemicals screening of *Corchorus capsularis* leaf demonstrated the presence of flavonoids, saponins, triterpenes and steroids, but not alkaloids. 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 flavanoids type. Methanolic extract of *Corchorus capsularis* showed DPPH Scavenging potential & IC 50 value of 0.301 mg/ml**. [34]**

From experiment DPPH Scavenging potential & IC 50 value of Isolated Catechin was 0.432 mg/ml. Because of presence of flavonids in *Corchorus capsularis* leaf & because of antioxidant activity is present in catechin, isolated catechin of *Corchorus capsularis* leaf extract showed antioxidant activities.

Cytotoxicity Test-

Phytochemicals screening of *Corchorus capsularis* leaf contains HCN, which may responsible for cytotoxicity. The LD50 of tissue extracts to mice report that the "lethal dose" of Corchoroside A to mice are 0.053-0.0768 mg/kg and Corchoroside B 0.059-0.1413 **[15].**

Isolated catechin sample from *Corchorus capsularis* leaf extract showed lethality with an LD50 values of 0.3125 mg/ml, which was compared with pure acetone, LD50 values of 0.625 mg/ml. Cytotoxicity test was compared with acetone because, catechin was present in jute leaf lower layer acetone fraction.

Total Phenolic test

Resulting data showed considerable variability in both total phenols [80.5-134.9 mg/g of dry matter (DM) in black teas and 87-106.2 mg/g of DM in green teas] and catechins (5.6-47.5, 51.5-84.3,and 8.5-13.9 mg/g of DM in black, green, and fruit teas, respectively); Indicates the presence of polyphenols in catechin**.[35]**

Isolation of *Corchorus capsularis* gives characterizaton of cyanidin glucoside, indicates the presence of phenolic compounds.The phenolic content found in the crude methanolic extract of *Corchorus capsularis* (leaves) was 0.226 mg equivalent of Tannic acid (TAE) per mg of dried extract.The phenolic content found in *Corchorus capsularis* (leaves) isolated catechin extract was 44.25 mg with salicylic acid equivalent (SAE) per gram of dried extract.

CHAPTER 6

CONCLUSION

Conclusion:

Extraction of caffeine & catechin from corchorus capsularis are two different, simplified processes. We need different solvent such as chloroform & NaOH for caffeine & dichloromethane, acetone for catechin extraction in laboratory.

Isolated caffeine sample after five days open air drying, was used for various kinds of experiment, such as T.L.C (thin layer chromatography technique), showed R.F value 0.98 c.m.& in same experiment R.F value of pure caffeine was 0.66 c.m this value indicated a high amount of caffeine present in *corchorus capsularis* leaf extract. After get isolated caffeine's absorbance in U.V. spectrometer,272 n.m which was similar with pure caffeine & the amount of caffeine in *corchorus capsularis* was higher than pure caffeine make sure us about isolated caffeine from jute leaf, concentration of the isolated caffeine sample was 0.1mg/ml. Then the caffeine content was determined with U.V spectrometer & detected that 1mg *corchorus capsularis* leaf extract contain 0.234 mg caffeine.

Isolated caffeine showed it's cytotoxic property with brine shrimps, LD 50 value was 0.3125 mg/ml. Pure caffeine was used here as a standard & showed LD 50 value of 0.9375 mg/ml. This value indicates higher cytotoxicity of isolated caffeine sample than pure caffeine.

Free radical scavenging; Antioxidant values of isolated caffeine sample was 0.285 mg/ml.(I.C 50), was compared with pure caffeine 0.342 mg/ml. Indicates better antioxidant property then pure caffeine. Ascorbic acid was here used here as standard. (0.117 mg/ml) (I.C 50).

In our isolated caffeine sample total phenolic content was 50.26 mg/g. pure caffeine used here as standard & showed total phenolic content of 38.20 mg/g.

Isolated sample from *corchorus capsularis* indicates the presence of high amount of caffeine which characteristics & experiment results were similar sometimes better than pure caffeine as antioxidant & polyphenolic content. Caffeine is the most widely used psychoactive, stimulant substance in the world. We can use *corchorus capsularis* as a new source of caffeine.

Isolated catechin sample was used for various kinds of experiment, such as T.L.C (thin layer chromatography technique), showed R.F value 0.94 c.m. (Lower layer acetone fraction) this value indicated a high amount of catechin present in *corchorus capsularis* leaf extract.

Isolated catechin showed it's cytotoxic property with brine shrimps, LD 50 value was 0.3125 mg/ml. Pure acetonene was used here as a standard & showed LD 50 value of 0.625 mg/ml. This value indicates higher cytotoxicity of isolated catechin sample than pure acetone.

Free radical scavenging; Antioxidant property of isolated caffeine sample was 0.432 mg/ml.(I.C 50), Ascorbic acid was here used here as standard. (0.117 mg/ml) (I.C 50).

After get isolated catechin's absorbance in U.V. spectrometer, 264 n.m which was similar with pure catechin (269 n.m) indicates catechin isolated from jute leaf, concentration of the isolated catechin sample was 0.002 μg/μl.

Isolated sample from *corchorus capsularis* indicates the presence of catechin. Catechins are a type of flavonoid & are potent antioxidants that can help to protect us from potentially damaging chemical, free radicals. Polyphenoic compounds are present in catechin also. In our isolated catechin sample total phenolic content was 44.25 mg/g. We can use *corchorus capsularis* as a new source of catechin, besides black tea, green tea & other sources of catechin.

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