

**Phytochemical and Biological investigations of
Sonneratia caseolaris Leaves**

A project submitted by

Tahera Sultana

ID:12346002

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Bachelor of Pharmacy(Hons.)



Inspiring Excellence

Dhaka, Bangladesh

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Dedicated to my parents to whom forever I owe my achievements and success. May Almighty ALLAH keep them as well as they have kept me.

Certification Statement

This is to certify that, this project titled 'Biological Investigation of Antioxidant Activity and Cytotoxic Effect of the plant Extract of *Sonneratia Caseolaris* 'submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy from the Department of Pharmacy, BRAC University constitutes my own work under the supervision of Imon Rahman , Senior Lecturer, Department of Pharmacy, BRAC University and that appropriate credit is given where I have used the language, ideas or writings of others.

Signed,

Countersigned by the Supervisor

Acknowledgement

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Abbreviations

ROS - Reactive Oxygen Species

SOD - Superoxide dismutase

CME - Crude Methanolic Extract

CCE - Crude Chloroform Extract

CNE - Crude n-hexane Extract

PPE - Petroleum Ether Extract

DPPH - 1, 1-diphenyl-2-picrylhydrazyl

BHT - Butyl-1- hydroxy toluene

ASA - Ascorbic Acid

DMSO – Dimethyl sulphoxide

NaCl - Sodium Chloride

GAE - Gallic Acid Extract

STD - Standard Deviation

VS – Vincristine Sulphate

TPC -Total Phenol Content

TFC -Total Flavonoid content

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Abstract

Sonneratia Caseolaris is also known as mangrove apple belongs to the Lythraceae family. These plants are very famous for their medicinal use worldwide especially in mangrove regions. This plant is not only famous for their medicinal use but also for cooking. In this particular study, in-vitro antioxidant activity of the methanol extract of *Sonneratia Caseolaris* leaves was determined by DPPH (2,2 -diphenyl-1-picrylhydrazyl) free radical scavenging assay. Brine shrimp lethality test was performed to determine the cytotoxic effect and the LC50 value was calculated and has been compared to standard.

Chapter One:

Introduction

1.1 Rationale and Objective

The utilization for regular items with restorative properties may be Likewise ancient administration Similarly as human progress and, to in length time, mineral, plant What's more creature results were the fundamental wellsprings from claiming pills (De Pasquale, 1984). Mixes, for example, muscarine, physostigmine, cannabinoids, colchicine. The greater part got from plants, would essential devices utilized within physiological and pharmacological Furthermore biochemical investigations (Williamson et al.,1996).

Around 25% of the pills endorsed overall turned starting with plants, 121 such animated mixes being in present utilization. Of the 252 medications acknowledged Likewise Fundamental Furthermore Fundamental Toward those planet wellbeing association (WHO), 11% are only about plant Inception Furthermore a critical number are manufactured pills gotten starting with regular precursors. Cases to basic pills procured beginning for plants might digoxin starting with digitalis spp. quinine and quinidine starting with snap spp, vincristine likewise vinblastine starting with catharanthus roseus, atropine from atropa belladonna Besides morpheus Also codeine beginning with papaver somniferum. It will a chance to be assessed that 60% regarding anti-tumor Moreover anti-infectious pills by on the business segment alternately under clinical trial compelling reason help regarding basic root (Yue-Zhong Shu, 1998).

1.2 Development of drug from bioactive natural products

Look into, and advancement of, restorative materials from plant root may be tricky Also exorbitant assignment (Borris, 1996; Willianson et al, 1996). Every new pill obliges a speculation for around US\$ 100–360 million and a base about 10 quite some time about work, for main 1 to 10,000 tried mixes being acknowledged guaranteeing and just 1 for 4 from claiming these constantly sanction Concerning illustration another medication. Any investigate under pharmacological animated characteristic mixes relies on the mix for these sciences. The lifestyle they need aid coordinated circuit and the degree of reconciliation rely on upon those targets of the study.

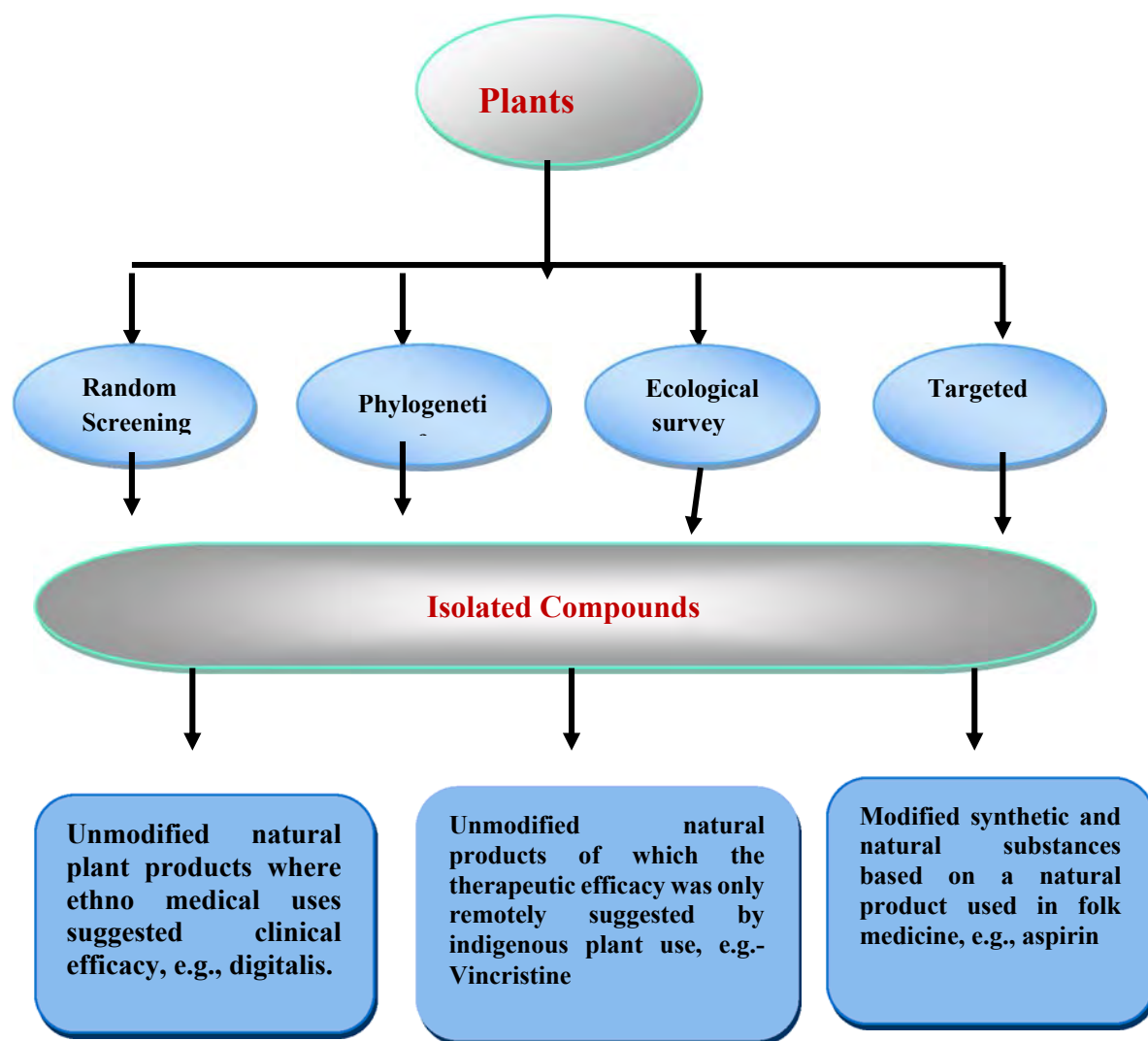


Figure 1.1: Lead material search and utilization from plants.

1.3 Photochemistry

Photochemistry may be in the strict sense of the statement the ponder about phyto chemicals. These would chemicals which determined starting with plants. For a narrower feeling those terms are regularly utilized to portray the vast number for auxiliary metabolic mixes discovered On plants. Large portions about these need aid referred to will provide insurance against creepy crawly strike Also plant illnesses. They likewise show a number from claiming

protective capacities for human buyers. Strategies regularly utilized confinement and purification Toward section chromatography, dainty layer chromatography, gel filtration chromatography and so forth and also structure explanation primarily Toward spectroscopic systems (UV, IR, NMR, impostor spectroscopy).

Broad phytochemical examination Furthermore seclusion of animated parts in the immaculate manifestation get important on dodge untoward impacts. Those reasonability of the display contemplate may be seclusion and purification about concoction constituents & assessment of could reasonably be expected living exercises of the separate extractives from claiming *Sonneratia caseolaris*.

In this investigation, we used the leaf of *Sonneratia caseolaris* and our aim was

- Isolation of the secondary metabolites from the plants
- Determination of bioactivities such as cytotoxicity, antimicrobial, thrombolytic and antioxidant activity.

1.4 The plant family Lythraceae

Lythraceae otherwise called mangrove tree fruit or crabapple mangrove tree. It may be a species about plant in the lythraceae gang. The apples and oranges will be noted to its outward comparability of the persiflage apples and oranges. This tree will be kind for mangrove tree Experiencing childhood on 20 m in stature What's more with a trunk arriving at a most extreme breadth for 50 cm. It might make present done tropical tidal mud flats beginning with Africa with Indonesia, southwards down will northeast Australia in addition California and northwards subordinate upon Hainan island on china and the Philippines. The tree developed sustenance's from asserting this tree might make the individuals liable of a legend regarding Maldivian folklore, Kulhlhavah Falhu rani. Furthermore, tree may be associated for congregating fireflies the sum around Southeast Asia in addition it might a chance to be those sustenance wellspring of motel room and separate insects.

1.5 Some Genera of the family

Lythraceae will a chance to be group about sprouting plants also it's including 32 genera for around 620 species from asserting herbs, shrubs Also trees. The greatest genera fuse Cuphea (275 spp.), Lagerstroemia (56), Nesaea (50), Rotala (45), Besides Lythrum (35). It similarly incorporates the pomegranate (*Punica granatum*, when with Punicaceae) and the water caltrop Lythraceae require around the globe distribution, for an extensive part species in the tropics, at any rate setting off under quiet Ecological Locale and blacks. The pack might a chance to be named then afterward the individuals kind genus, Lythrum, the individuals loosestrifes (e. G. *Lythrum salicaria* purple loosestrife) also incorporates henna (*Lawsonia inermis*). It presently incorporates the individual's pomegranate, once classed for differentiate family Punicaceae. The pack also incorporates the by and large develop crape myrtle trees. Botanically, the abandons require support regularly through pairs (opposite), and the sprout petals create from the individuals edge of the calyx tube. Those petals consistently appear collapsed.

1.6 Characteristics

Lythraceae species might herbs and regularly shrubs alternately tree; the shrubs Furthermore trees need flaky bark. Qualities imparted Toward species inside the Lythraceae that perceive them beginning for Hosting a spot to different plant aggregations would the individual's petals constantly collapsed in the bud and the many-layered outside integument of the seed.

1.7 Taxonomy

Inside the request for Myrtales, those Lythraceae crew may be A large portion nearly identified with the Onagraceae, for the Combretaceae sister should both groups. Atomic phylogeny worth of effort need prompted those considerations of the in the past distinguished groups Duabangaceae, Punicaceae, Sonneratiaceae and Trapaceae.

1.8 Uses as food

Those leaves and the apples and oranges would eatable Also acknowledged Similarly as nourishment Previously, specific areas, for example, such that Maldives. Over Sri Lanka, the place the apples and oranges may be known as *kirala gédi* in Sinhala, those mash of the tree grown foods may be blended with coconut milk extricate and settled on under a milk shake. A significant number visitor resorts arranged in the south of Srilanka the place the trees develop abundantly close by rivers, the table new tree grown foods drinks produced starting with the apples and oranges. In the Maldives those fruits need aid utilized as invigorating drink What's more also consumed with scratched coconut & sugar fruit.

1.9 Some reported species

1. *Sonneratia acida*

2. *Sonneratia alba*

3. *Sonneratia caseolaris*

4. *Sonneratia evenia*

5. *Sonneratia griffithii*

6. *Sonneratia gulngai*

7. *Sonneratia hainanensis*

8. *Sonneratia lanceolata*

9. *Sonneratia neglecta*

10. *Sonneratia obovate*

1.10 The plant *Sonneratia caseolaris*

Common names

Those *Sonneratia* would called berembang Previously, Malaya, mangrove apple done English, and Mangrove Enapfel alternately Holzapfel mangrove On German. In the Philippines, they would have called Pagatpat.

Synonyms

- *Blatti acida*
- *Rhizophora caseolaris*
- *Sonneratia acida*
- *Sonneratia evenia*
- *Sonneratia neglecta*
- *Sonneratia obovata*
- *Sonneratia ovalis*

Scientific Classification

Kingdom : Plantae

Order : Myrtales

Family : Lythraceae

Genus : *Sonneratia*

Species : *S. Caseolaris*

Distribution

Grows well along bank of streams, rivers, Common all over the bank of river but mostly in mangrove forest of Bangladesh, India, parts of the far East, in primary rain forests or mixed deciduous forests throughout the Africa and in tropical Asia. Lythraceae is broadly distributed, yet all the for the vast majority species tropical and a few calm. They need aid

absent in Sahara and most of the areas of Australia. Large portions species happen done oceanic and nearly-aquatic natural surroundings.

1.11 Description of the plant

It will be a sort about tree that grows On salt-marsh forests along the coasts for numerous nations. The mangrove tree fruit tree grows essential On salty areas along littoral riverbanks the place the tides climb and fall. Its abandons would oval. Those mangrove tree fruit tree blooms after those dry season only in front of the blustery season. The parts that would regularly utilized should settle on nourishments would the abandons Furthermore fruits. Two predominant sorts of the apples and oranges On Vietnam need aid those saucer mangrove tree fruit (*Sonneratia caseolaris*) and the guava mangrove tree fruit (*Sonneratia giffithii*). Saucer mangrove tree apples develop along riverbanks, looking into islands, what's more at waterway ramifications. Its fruits are extensive What's more round like a saucer, Consequently its name. With respect to the guava mangrove tree fruit tree, it is planted close homes on facilitate picking its fruit, which is consumed seasonally. This apples and oranges is medium over size. As far as compound composition, mangrove apples tree holds the shade operators archin (emodin) Also archicin (chrysophanic acid), which bring anti-oxidizing, laxative, also detoxifying impacts. They also reinforce blood vessels, cure stomach ulcers, lessen absorption about sugars Furthermore fat, also lessen pulse. As stated by accepted oriental medicine, the mangrove tree fruit need cooling properties, quells inflammation, furthermore diminishes agony. Those abandons bring harsh taste What's more styptic properties. Over India, individual's utilization those tree grown foods juices Toward aging them to make a solution that inhibits dying. To Malaysia, individuals bawl those abandons with rice to make pharmaceutical that remedies pee maintenance Also murders intestinal parasites. They consume ready mangrove apples tree on treat hacking. Done Myanmar, individuals utilization mangrove tree by grinding them under a glue mortar or glutinous paste, which would that point blended for salt and connected should creepy crawly bites, same time to congested bruises they mature those soil grown foods juice Similarly as homeostatic. For numerous nations basic society often grind those leaves, include salt, what's more utilized for wounds.

Despite the mangrove tree fruit might appear to be a touch plebeian, it will be element that is committed under a number wide open claim to fame dishes: mangrove tree fruit soup, mangrove tree fruit shoots singed with field mice, ready mangrove tree apples braised for fish, mangrove tree apples Also fish hotpot, What's more mangrove tree fruit jam, and so on. The simplest will be to lift mangrove apples tree and dip them to salt Likewise a snake.

Leaves

The leaves by bring inverse arrangement, in any case at times would exchange. They are straightforward for smooth birch edges and pinnate venation. Stipules would normally reduce, showing up Likewise column for moment hairs, alternately absent.

Flowers

The blossoms from claiming this plant would bisexual, radially alternately Sporadically bilaterally symmetric, Hosting a well-developed hypanthium. The blossoms would A large portion usually four-merous at could a chance to be six-merous, for four will eight sepals and petals. The sepals might make distinct, incompletely combined to structure a tube, or touching without covering. The petals would have folded in the bud and wrinkled toward maturity, also would normally dissimilar What's more overlapping; they need aid Sporadically absent. There need aid normally twice as huge numbers stamens as petals, orchestrated over two whorls, and the stamens are often unequal long. Occasionally, those stamens need aid decreased to one whorl, alternately need aid more various with numerous whorls. That ovary may be normally superior, rarely semi-inferior, or infrequently mediocre. The two with Numerous carpels could be combined together with two will various ovules On every locule, for axile placentation of the ovules.

Fruits and seeds

Those fruits need aid Typically a dry, dehiscent capsule, sporadically might a chance to be a berry. The seeds need aid normally smoothed, with more layered external integument and epidermal hairs extend Also ended up adhesive The point when wet would found done regarding half the genera.

Flower:

Flowers are seen in March-June months.

Propagation

Seed - it has a low viability of less than three months.

1.12 Chemistry

Fruits yield 11% pectin

Wood yields 52.7% tan mash (lignin, pentosan).

Emodin and chrysophanic corrosive might be the coloring matter

Tannin, archin ($C_{15}H_{10}O_5$) and archinin ($C_{15}H_{14}O_{12}$).

1.13 Usage**Medicinal**

The plant is said to be homeostatic. Also used as folk remedy for swellings, and worms. The old fruit walls are used as a treatment for worms. Half-ripe fruits are a treatment for coughs. The fruits are used to make poultices. The pounded leaves are used as a treatment for haematuria, and smallpox. The leaves are crushed, mixed with salt and applied as a poultice onto cuts and bruises. All in all, it is also a very good antioxidant for the body and can help slow down the age related changes in the body.

Folk Medicine

Appeared for be hemostat, crabapple mangrove tree will be society cure for swellings, and worms. Malayans utilization old tree grown foods dividers to worms, fruits to cough and smallpox.

Agroforestry Uses:

A very important tree in the coastal swamp community, helping to protect the soil from erosion and providing an important habitat for wildlife.

Other Uses

Those pneumatophores (vertical bases climbing over ground) need aid utilized as floats to fish nets and, being corky on texture, need aid utilized in the fabricate of inward soles for shoes. Furthermore, camwood a chance to be utilized Likewise substitute to plug alternately pith. The bases would have bubbled preceding constantly utilized. Those bark is a hotspot from claiming tannins.

Those heartwoods will be light tan should dark chocolate, the sapwood light greyish tan Also 3 - 8cm thick. At wet alternately under varnish, the heartwood of old develop trees takes a gander very nearly bootleg. Those grain may be straight or extremely marginally crossed; those compositions fine, altogether homogeneous, smooth, not glossy; it need a different salty taste and a fishy alternately swampy odor, particularly new. The wood may be reasonably difficult and reasonably overwhelming with overwhelming. It is not difficult to work; keeps up great in the ground Furthermore actually those sapwood is infrequently struck by insects.

Those wood holds a little sum from claiming salt, settling on the utilization for copper nails Also screws important. It may be utilized for piles, posts, poles, track ties, clearing blocks; ship, bridge, furthermore wharf building; general solid construction; entryways; siding, sheathing, ceiling, flooring, also various sorts about inner part finish; boat planking Also decking; furniture What's more cabinetwork; What's more musical instruments. The wood will be utilized for fuel, however just when better forested areas need aid not accessible.

1.14 Cultivation Details

A plant of coastal areas in the tropics. It grows best where the mean annual minimum and maximum temperatures are within the range 20 - 30°C, though it tolerates 10 - 38°C. It prefers a mean annual rainfall of 1,500 - 2,500mm, tolerating 1,000 - 3,000mm.

Edible Uses

Young berries - raw or cooked. They can be eaten as tasty fruit and cooked as vegetable. A sour flavor. A cheese-like flavor. The fruits are a source of pectin. Young leaves – raw.

Ecology

Evaluated should go from tropical damp on sprinkle through subtropical damp on sprinkle timberland term Zones, crabapple mangrove tree may be assessed should endure twelve-month precipitation for 10 with 80 dm, yearly temperature about 20 should 27°C and PH for 6. 0 to 6. 5.

Cultivation

As stated by those NAS (1980), planting is as a rule not necessary a result common recovery will be something like that fruitful. For Avicenna Furthermore Rhizophora, regulate seeding brings about ca 90% survival.

1.15 Harvesting

Reaped as required starting with wild remained. Trees recuperate quickly following limbs would cut off to fuel. Since this mangrove tree could regrow quickly from buds underneath the bark along those trunk and branches, it may be said will middle of the road minimal starting with evacuation for considerably of the branch wood (NAS, 1980).

1.16 The Plant view



Figure 1.2: Whole Parts of *S.caseolaris*

**Chapter Two:
Methodology**

2.1 Chemical Investigation of the Experimental Plants

A plant species representing to the family **Combretaceae** was investigated in this study.

Name of Plant	Family	Plant part
<i>Sonneratia caseolaris.</i>	Lythraceae	Leaves

2.2 Chemical work

The following processes roughly show the chemical work done on the *Sonneratia caseolaris.*

1. Firstly, need to collection and identification of the plant sample.
2. Then preparation of the plant sample.

3. After that methanol Extraction of the plant part and with chloroform and n-hexane separately.
4. Then Solvent evaporation that will produce yield crude methanolic extract (CME), crude chloroform extract (CCE) and crude n-hexane extract (CNE).
5. Then Chemical tests or determination of total phenol content and flavonoid content of the three partitionates-CME, CCE and CNE) are performed.
6. Antioxidant assay of the all the fractions of the extract.

2.3 Collection and Identification of the Plant Sample:

Sonneratia caseolaris were taken from the local plant in Pirojpur city, Bangladesh during March, 2017.

2.4 Preparation of Plant Samples:

After collecting, the leaves were comprehensively cleaned with tap water and were dried out under sunlight for 21 days. These samples were made into a powder in a grinding mill. The powder then stored in air tight container and kept in cool and dry place.

2.5 Extraction and Solvent Evaporation:

The powdered plant materials were concentrated Eventually by cold extraction methodology. Pounded plant material (leaves) were made clinched alongside a reagent container What's more doused with methanol (500ml), chloroform(500ml) and n-hexane (500ml) independently. The substance might have been held done a container for a time for seven days, sporadically being blended What's more shaken. Those whole blend might have been after that separated through cotton et cetera through Whitman no. 1 channel paper et cetera might have been committed concentrated with rotating evaporator under decreased weight during

raised temperature will get rough extricate known as rough methanolic extricate (CME), rough chloroform extricate (CCE) and rough n-hexane extricate (CNE).

2.6 Chemical tests (total phenolic and flavonoid contents) of CME, CCE, CNE:

Determination of Total Phenolic:

Plant polyphenols, a diverse group of phenol compounds have a good structural chemistry for free radical hunting (Ahmed et al., 2014).

Principle:(Ahmed et al., 2014)

The content of the phenolic compounds of the various extracts in the plant was found using Folin–Ciocalteu Reagent (FCR). The complete chemical components of the FCR is not known but is thought to have hetero poly phosphotungstates – molybdates. A series of reversible one or two electron reduction reactions result in a blue species. Molybdenum is easily reduced in the complex reaction.

Reagents & chemicals

- Folin-ciocalteu reagent
- Sodium carbonate
- Methanol
- Gallic acid
- Distilled water

Experimental procedure:

0.5 ml of plant extract placed in a test tube which contains 2.5 ml of Folin-ciocalteu reagent.

And that was diluted for 10 times with water. Then added to the mixture. 2.5 ml of Sodium Carbonate was added and the solution was incubated for 20 minutes at 24°C temperature. Every reagent should be prepared freshly. The absorbance was then fixed at 760nm using a spectrometer against a blank. The standard blank solution had all the reagents with the exceptions of plant extract or standard solutions. The below equation is used to calculate the content of phenolic compounds and in polyphenolic fractions in GAE,

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

Where,

C = phenolic compounds content

c = Gallic acid concentration

V = Volume of the sample

m = sample weight

All samples were analyzed three times and averaged results are taken

2.7 Determination of Total Flavonoids

Principle (Kadam et al., 2013)

Those substance downright flavonoids in distinctive fractionates from claiming plant extract might have been controlled Eventually by aluminum chloride colorimetric strategy. In this strategy aluminum chloride types intricate for hydroxyl Assemblies of flavonoids exhibit in the specimens. Complex need that greatest absorbance of 420 nm (Kadam et al., 2013).

Reagents and chemicals

- Aluminium chloride
- Sodium nitrite
- Catechin as standard
- Methanol
- Distilled water

Experimental Procedure:

0.5ml of plant extract or standard were taken in test tubes. Then 2.5 ml of distilled water added. Then 0.15 ml of 5% sodium nitrite added. After 5 minutes 0.3 ml of 10% aluminum chloride were added following the addition of 0.55 ml distilled water to the mixtures. Again, after 5 minutes- about 1ml of 0.001M sodium hydroxide was added. All the reagents must be freshly prepared.

The absorbance was then fixed at 517nm using a UV-spectrometer against a blank. The standard blank solution had all the regents with the exceptions of plant extract or standard solutions. The total content of flavonoids of the partitionates were shown as catechin equivalents after calculation.

The following equation is:

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

Similar to determination of phenol content, here again all the samples were analyzed trice and averaged results are taken.

2.8 Antioxidant Assays:

DPPH Radical Scavenging assay:

DPPH is most well-known and acceptable reagents used to hunt free radicals of plant extracts of both natural and synthetic origin (Marinova, 2011).

The antioxidant activity of different fractions and isolated compounds determined by their hydrogen donating capacity and using DPPH process with a minor modification.

Principle (Marinova, 2011)

This technique may be in view of the diminishment for DPPH in methanol result in presence of hydrogen giving cell reinforcement due to shaping of non-radical structure DPPH-H. A newly arranged DPPH result exhibits profound purple shade. Those change brings about shade transform from purple should yellow, which will be measured spectrophotometrically. Accordingly, those antioxidant particles will kill DPPH spare radicals through converting them under colorless results bringing about diminishing absorbance. Therefore, the potency for cell reinforcement/ antioxidant action may be conversely proportional of the rate from claiming diminish On wavelength. In this experiment, BHT (butyl-1- hydroxy toluene) and ASA (ascorbic acid) are utilized Likewise sure/positive control.

Experimental procedure:

At first, 2ml of sample extracts in methanol solution and standard were prepared at different concentrations. Then, freshly prepared DPPH solution (3ml) were added to all test tubes. After that, the test tubes were kept for 30 minutes in dark place for the reaction. Lastly, at 517 nm wavelength absorbance is measured using a UV spectrophotometer.

$$\%I = \{(A_0 - A_1) / A_0\} \times 100$$

Where,

A₀ = absorbance of control

A₁ = absorbance of extract or standard

Finally, %I of inhibition were designed against concentration (µg/ml). Then from the graph IC₅₀ value is calculated.

2.9 Brine Shrimp Lethality Bioassay

2.9.1 Principle (Olowa & Nuneza, 2013)

Brines shrimp bioassay may be the practically distinguished Furthermore acknowledged system for figuring out cytotoxic impact of extract from claiming whatever plant constitute. Toward so as with get nauplii, those salt water shrimp eggs are incubated On ocean water. At that point obliged measure from claiming dimethyl sulphoxide (DMSO) were included that those wanted fixation of the test tests might make ready. When those bring forth might have been done, then nauplii were counted Eventually by inspecting visually. They were made under vials holding water (5ml). At that point specimens for diverse focuses were included of the pre-labelled vials through micropipette. Following that those vials were kept for 24 hours and survivors need aid counted then afterward 24 hours.

2.9.2 Materials

Brine shrimp eggs (<i>Artemia salinaa</i> leach)	Lamp to attract shrimps
Magnifying glass	Test tubes
Pipettes	Small tank
Test samples of experimented plants	Micropipette
Glass Vials	Sea salt (NaCl)

Table 2: Test samples of experimental plant

Plant part	Sample code	Test sample	Calculated amount(mg)
<i>Plant leaves of Sonneratia caseolaris</i>	ME	Methanolic extract partitionate	4.0
<i>Plant leaves of Sonneratia caseolaris</i>	AE	Acetone extract partitionate	4.0

2.9.3 Experimental Procedure

Preparation of seawater

At first, 38g of salt NaCl (pure sea salt) was weighed. It is dissolved in one liter of distilled water. After that the solution was filtered to get clear solution.

Brine shrimps Hatching

From close-by pet shops, those salt water shrimp eggs of the test living being named *Artemia salina* drain were gathered Furthermore included of the little tank holding ocean water. Whatever remains part of the tank might have been secured. After that the eggs were permitted with get incubated and developed under nauplii Eventually quit offering on that one day. All around the bring forth time, steady oxygen supply might have been given of the tank. Those tank might have been under the light through those punctured dam attracting the salt water shrimp eggs Furthermore they were made to doing those test. Over ten existing shrimps were included will every one of the test tubes holding separate test focuses with the assistance of Pasteur pipette.

Counting of nauplii

After a day the number of survivors were counted through inspection of the vials by magnifying glass. For each diluted concentrations, the percentages of mortality were counted. Statistically Data were analyzed by using linear regression.

2.10 Modified Kupchan Partition (Solvent-Solvent Partition of Crude Extract)

Solvent-solvent parceling might have been done utilizing the protocol planned Toward Kupchan and altered Eventually by van Wagenen et al (1993). The rough extract (5 gm) might have been dissolve for 10% watery methanol. It might have been concentrated with petroleum ether, that point with carbon tetrachloride and last for Di-chloromethane.

2.10.1 Partitioning with Pet-Ether

For this purpose, mother solution result might have been made in dividing funnel. After that 100 ml of the petroleum ether might have been included and the funnel might have been shaken and kept undisturbed. The natural parcel might have been gathered. That procedure might have been rehashed thrice and the portions gathered were dissipated together for rotating evaporator.

2.10.2 Partitioning with Chloroform

In the mother solution result exited after partitioning petroleum ether, 13.5ml about refined water might have been included and blended. Those mother solution result might have been after that taken clinched alongside dividing pipe What's more concentrated with chloroform (100 ml X 3). Those carbon tetrachloride portions were gathered together Furthermore evaporated. The watery portion might have been safeguarded to those following venture.

2.10.3 Partitioning with Di-chloromethane

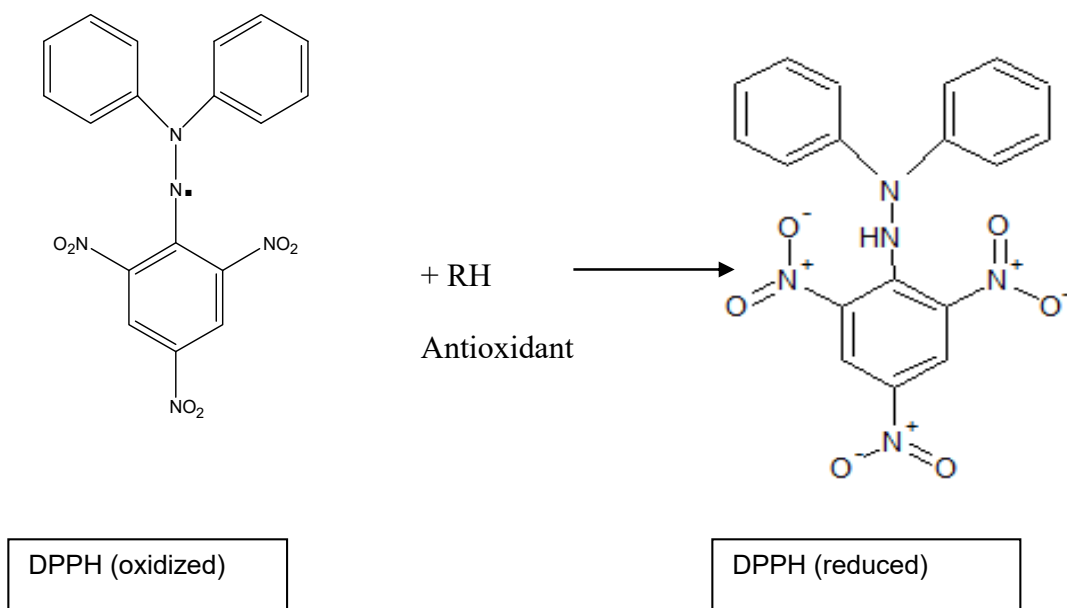
The mother solution result that left accompanying washing with petroleum ether in addition carbon tetrachloride, 16 ml from guaranteeing refined water might bring been included and Additionally mixed uniformly. Those mother solution result might have been that point taken done a dividing pipe Furthermore concentrated with Di-chloromethane (100 ml X 3). Those Di-chloromethane dissolvable portions were gathered together Furthermore evaporated. Those watery methanolic portion might have been safeguarded Similarly as watery portion.

Chapter Three:
Antioxidant Screening

3.1 Antioxidant activity: DPPH assay

3.1.2 Principle

That framework free of charge radical looking exercises (antioxidant capacity) of the plant extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were assessed Eventually Tom's perusing that framework for Brand-Williams et al., 1995. 2. 0 ml of a methanol consequence of the extricate Throughout diverse centralization were mixed for 3. 0 ml of a DPPH methanol bring about shortages (20 μ g/ml). The cell reinforcement possibility might have been assayed starting with those bleaching about purple hued methanol result for DPPH radical Eventually Tom's perusing those plant extricate Concerning illustration contrasted with that from claiming tert-butyl-1-hydroxytoluene (BHT) and ascorbic corrosive (ASA) Eventually Tom's perusing UV spectrophotometer.



*DPPH= 1, 1-diphenyl-2-picrylhydrazyl

3.1.3 Materials

Chloroform	Pet ether	Micropipette
Ascorbic acid	Light-proof box	Distilled water
Methanol	<i>tert</i> -butyl-1-hydroxytoluene (BHT)	1,1-diphenyl-2-picrylhydrazyl
Di-chloromethane	UV-spectrophotometer	Beaker
Amber reagent bottle	Test tube	Pipette

Control preparation (antioxidant activity measurement)

Ascorbic corrosive and *tert*-butyl-1-hydroxytoluene might have been utilized as positive control. Computed measure of ASA and BHT were dissolved over methanol will get mother solution result Hosting a concentration 1000 µg/ml. Serial dilution might have been aggravated utilizing those mother solutions answer for get diverse fixation going starting with 500. 0 with 0. 977 µg /ml.

3.1.4 Test sample preparation

Determined measure from asserting notable extractives were measured Additionally broken down done methanol to get those mother solution result (Conc. 1000 µg/ml). Serial weakening of the mothball result aftereffect advertised notable obsession setting off starting with 500. 0 once 0. 977 µg /ml which were held in the checked flasks.

3.1.5 DPPH solution preparation

20 mg DPPH powder might have been weighed Besides deteriorated completed methanol ought to kick DPPH outcome facilitating obsession 20 µg /ml. The result might have been ready in the golden reagent jug Also held in the light confirmation box.

3.1.6 Assay of free radical scavenging activity

2. 0 ml of a methanol result of the test (extractives/ control) Throughout notable centralization (500µg/ml with 0. 977µg/ml) were mixed for 3. 0 ml of a DPPH methanol come about (20µg/ml). Following 30 min reaction period at room temperature finished dull set the absorbance may have been measured during 517 nm against methanol Concerning outline plain in the end Tom's examining uv spetrophotometer. Restraint of free radical DPPH On percent (I%) might have been computed.

3.2 Results and discussion

3.2.1 Total phenolic content (TPC)

That methanol extract of *Sonneratia caseolaris* (MESF) also different partitionates such as Pet ether (PESF), chloroform (CHLSF), Di-chloromethane (DCM) Furthermore watery (AQSF) dissolvable partitionates were subjected ought to absolute phenolic content determination. Over light from claiming the individual's absorbance qualities of the diverse remove solutions, reacted to Folin-Ciocalteu reagent Moreover compared to those standard outcomes about Gallic destructive equivalents, results of the colorimetric examination of the aggravator phenolic have support given to secured close. Absolute phenolic substance of the tests might need communicated Concerning illustration mg starting with guaranteeing GAE (Gallic destructive equivalent)/ gm about extractives. The measure from claiming absolute phenolic content differed Previously, notable extractives also went beginning for 3. 67 mg from guaranteeing GAE /gm from asserting extractives with 13. 24 mg to GAE /gm around extractives to *Sonneratia caseolaris*. "around the sum extractives from claiming *Sonneratia caseolaris*, the most noteworthy phenolic content might have been found for MESF (13. 73 mg of GAE /gm of extractives) accompanied Eventually Tom's perusing DCMSF (12. 07 mg from claiming GAE /gm about extractives), PESF (8. 92 mg for GAE /gm about extractives), CHLSF (7. 92 mg for GAE /gm for extractives) Furthermore AQSF (3. 67 mg for GAE /gm about extractives).

Table 3: Standard curve preparation (Gallic acid)

SL. No.	Concentration of the Standard (µg / ml)	Absorbance	Regression line	R ²
1	100	1.620	y = 0.0162x + 0.0215	0.9985
2	50	0.866		
3	25	0.450		
4	12.5	0.253		
5	6.25	0.120		
6	3.125	0.059		
7	1.5625	0.034		
8	0.78125	0.022		
9	0.3906	0.020		
10	0	0.011		

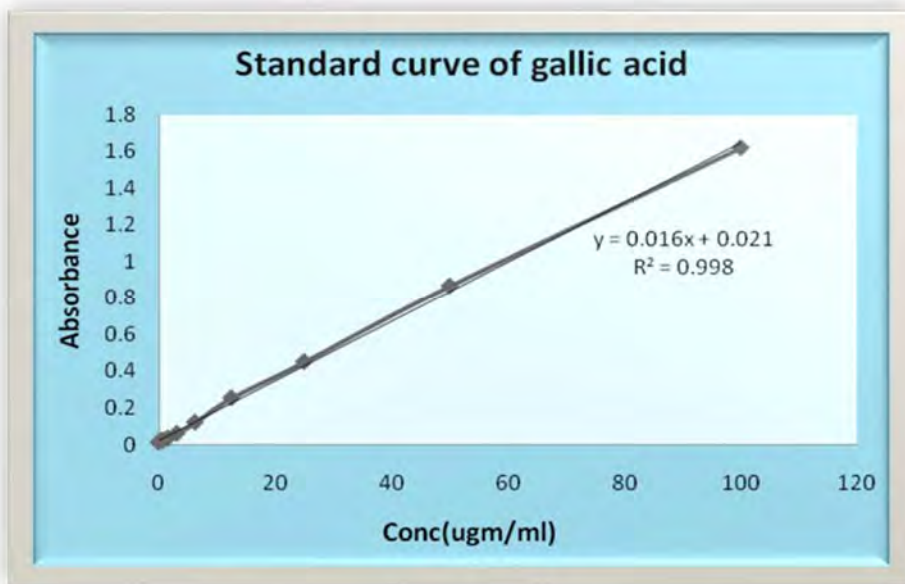


Figure 3.1: Standard curve of Gallic acid

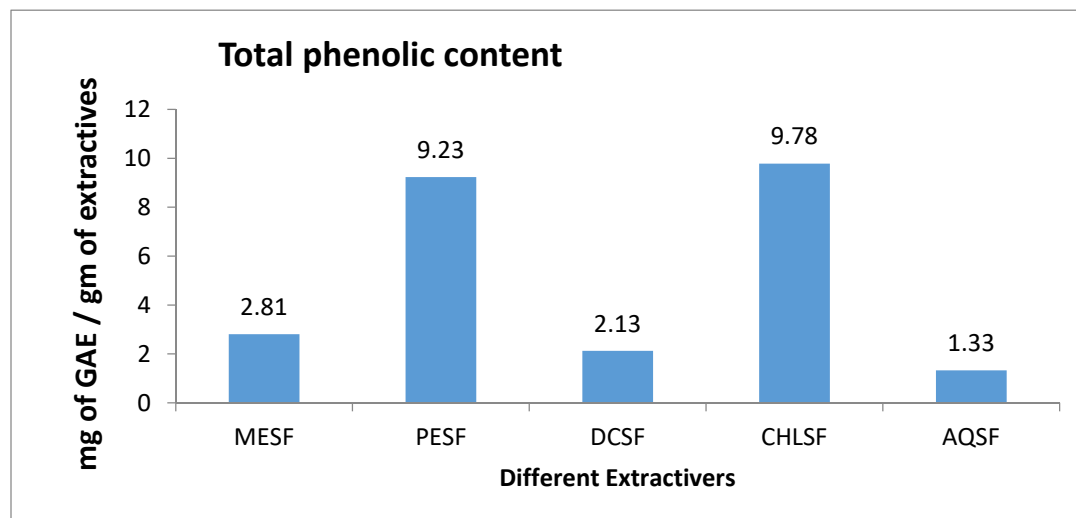


Figure 3.2: Phenolic content of different extractives of *Sonneratia Casiolaris*

3.2.2 Free radical scavenging activity

The methanol extricates from claiming abandons for *Sonneratia caseolaris*(MESF), Furthermore diverse partitionates such as PESF, CHLSF, Di-chloromethane(DCM) Also watery (AQSF) solvent partitionate of the methanol extricate from claiming entire plant about *Sonneratia caseolaris* were subjected to free radical searching action by that system. Here, BHT and ascorbic corrosive might have been utilized Likewise reference standard. The cell reinforcement movement of IC50 values to DPPH strategy would differed in distinctive extractives Furthermore went starting with (2. 16 µg/ml) to (5760. 62 µg/ml). "around at extractives about *Sonneratia caseolaris* the most noteworthy free radical searching action might have been provided for by CHLSF (2. 16 µg/ml) took after Toward MESF (3. 7 µg/ml), DCMSF (4. 57 µg/ml), AQSF (14. 37 µg/ml) and PESF (5760. 62µg/ml).

Table 3.3: IC₅₀ values of the standards and partitionates

Plant part	Sample Code	IC ₅₀ (µg/ml)	Test Sample
Leaves of Plant <i>Sonneratia</i> <i>caseolaris</i>	BHT	21.2	
	Ascorbic Acid	3.05	
	MESF	3.7	Methanol Soluble Fraction
	PESF	5760.62	Pet-Ether Soluble Fraction
	CHLSF	2.16	Chloroform Soluble Fraction
	AQSF	14.37	Aqueous Soluble Fraction
	DCMSF	4.57	Dichloromethane Soluble Fraction

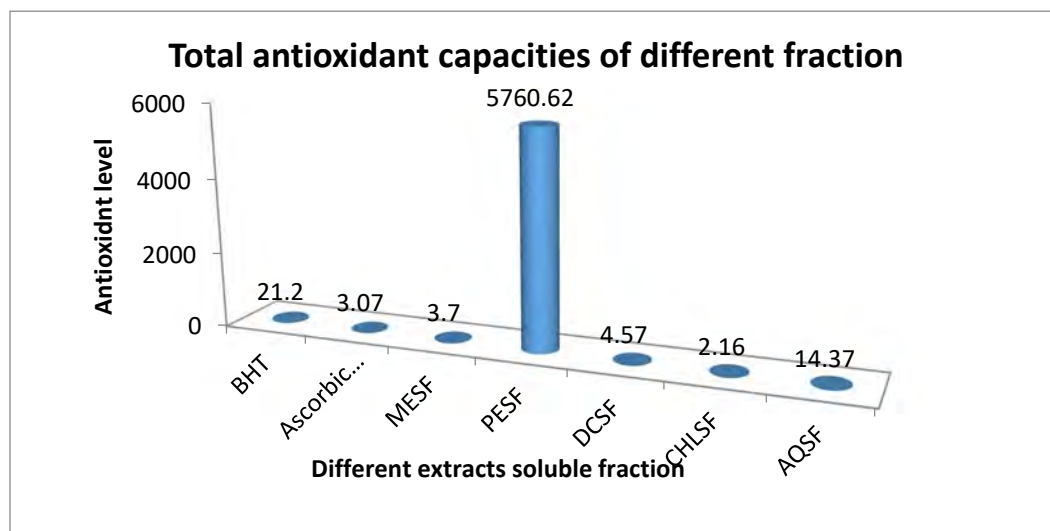


Figure 3.3: IC₅₀ value of the standard and partitionates of Leaves.

Chapter: Four

Brine Shrimp Lethality Test

4.1 Introduction

The mixes which are bioactive would generally harmful should existing figure at a few higher doses Also it supports the proclamation that 'Pharmacology may be basically toxicology toward higher doses Also toxicology is basically pharmacology during bring down doses'. Salt water shrimp deadly gene bioassay (McLaughlin et al, 1998) will be fast and far reaching bioassay for those bioactive compound of the common Furthermore engineered root. By this method, regular item extracts, portions and additionally the immaculate exacerbates camwood make tried of bioactivity. In this process, in vivo deadly gene done straightforward zoological living being (Brine shrimp nauplii) may be utilized Likewise positive position screen for screening. Salt water shrimp deadly gene bioassay procedure stands better than different cytotoxicity trying methods a direct result it will be fast to process, modest and obliges no uncommon gear or aseptic technobabble. It uses an extensive number for creatures for Factual Acceptance Furthermore a generally little amount for test. Furthermore, Dissimilar to different methods, it doesn't oblige creature serum.

4.2 Principle

Salt water shrimp egg need aid incubated over recreated ocean water on get nauplii. By s were as for computed add up of dimethyl sulphoxide (DMSO), fancied focus of the test example will be readied. Those nauplii would counted by visual review Furthermore are made On vials holding 5 ml of recreated ocean water. Then specimens for different focuses are included of the pre-marked vials through micropipette. Those vials need aid that point exited for a day. Survivors need aid counted then after a day.

Table 4: Test sample of experimental plants part

Sample code	Test sample	Amount
PESF	Petroleum ether partitionate	4 mg
DCMSF	Dichloromethane soluble partitionate	4 mg
CHLSF	Chloroform soluble partitionate	4 mg
AQSF	Aqueous soluble partitionate	4 mg
MESF	Methanolic soluble fraction	4 mg

4.3 Experiment Procedure

4.3.1 Seawater preparation

38 gm NaCl) was measured and dissolved in one liter of distilled water. After that it is filtered to get solution.

4.3.2 Hatching

Artemia salina gathered from BRAC University laboratory might have been utilized Concerning illustration the test living being. Seawater might have been made in a small tank. Then shrimp eggs included on the one side of that small tank. This side might have been secured. One day might have been permitted on hatch the shrimp What's more will be developed Concerning illustration nauplii. Steady oxygen supply might have been conveyed crazy through those bring forth the long haul. Those incubated shrimps were pulled in of the light through those punctured dam and they were made for analyze those by a Pasteur pipette. Then 10 living shrimps were included in the test tubes which takes 5 ml of ocean water.

4.3.3 Preparation of test samples (experimental plant)

Every last one of test specimens taken in vials Furthermore disintegrated in 100ml from claiming immaculate dimethyl sulfoxide (DMSO) should get stock results. Afterward 50 µl about result might have been taken in the principal test tube holding 5ml of recreated seawater also 10 shrimp nauplii. Thus, last centralization of the arranged result in the to begin with test tube might have been 400 µg/ml. After that, an arrangement from claiming results for changing focuses were ready from the stock result Toward serial weakening strategy. Previously, each case, 50 µl specimens were included to test tube and new 50µl DMSO might have been included with vial. Therefore, separate focuses were found in the diverse test tubes.

Table 4.1: Concentration values after serial dilution (test sample)

Test tube number	Concentration(µg/ml)
1	0.78125
2	1.5625
3	3.125
4	6.25
5	12.5
6	25.0
7	50.0
8	100.0
9	200.0
10	400.0

4.3.4 Control group preparation

Control Assemblies would have utilized within cytotoxicity consider should accept the test technique Also guarantee that the effects acquired need aid best because of those action of the test agenize and the impacts of the other conceivable factors are invalidated.

4.3.5 Preparation of the positive control group

Control Assemblies are utilized within cytotoxicity investigation should accept those test techniques and guarantee that the comes about got would best because of those action of the test agenize and the impacts of the other could reasonably be expected variables would have invalidated. Certain control in cytotoxicity consider will be generally acknowledged cytotoxic agenize and the come about of the test agenize will be compared with those come about gotten to those certain control. In the display consider vincristine sulfate might have been utilized Similarly as the sure control.

4.3.6 Negative control group preparation

100 µl about DMSO might have been included to every for 3 pre-marked glass vials holding 5 ml about ocean water and 10 shrimp nauplii to utilized like control gatherings. In salt water shrimps to those vials demonstrate fast mortal sin rate, afterward those test will be recognized as invalid Similarly as the nauplii kicked the bucket because of a portion reason than those cytotoxicity effect of the mixes.

4.3.7 Nauplii counting

Afterward a day, the vials inspected utilizing amplifying glass. The amount of survivor counted. The percentage (%) mortal sin might have been computed to each weakening. The concentration-mortality information was investigated statistically Eventually by utilizing straight relapse utilizing PC. Viability/those concentration mortality association of crude item is generally communicated Likewise average disappointment focus (LC50) worth.

Table 4.2: Test samples LC₅₀ values of *S.caseolaris*

Test samples	Regression line	R2	LC50 (mg/ml)	logx	x (LC50)
VSSF	$y = 28.188x + 69.64$	0.972	0.203	-0.345	0.203
MESF	$y = 28.99x + 25.83$	0.957	6.82	0.834	6.82
PESF	$y = 26.57x + 34.84$	0.663	3.72	0.571	3.72
DCMSF	$y = 29.99x + 35.58$	0.736	3.03	0.481	3.03
CHLSF	$y = 26.97x + 46.34$	0.735	1.37	0.136	1.37
AQSF	$y = 26.97x + 12.34$	0.844	23.60	1.373	23.6

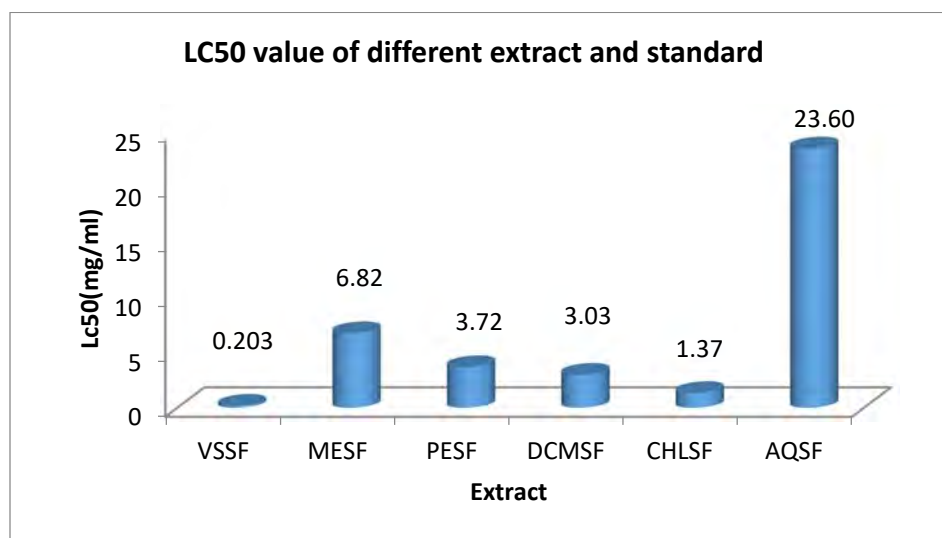


Figure 4: LC₅₀ values (different extractives of *S. caseolaris*)

Table 4.3: Vincristine sulphate effects (positive control) on shrimp nauplii

Conc. (µg/mL)	Log ₁₀ Conc.	% Mortality	LC ₅₀
00			0.203
0.0390	-1.4089	20	
0.078125	-1.1072	40	
0.15625	-0.8061	50	
0.3125	-0.5051	60	
0.625	-0.2014	70	
1.25	0.09691	70	
2.5	0.39794	80	
5	0.6989	90	
10	1.00	100	
20	1.3010	100	

Figure 4.1: % plot of mortality and predicted regression line of VS

Table 4.4: Methanol soluble fraction effect of the extract (MESF)

SI.	Conc. ($\mu\text{g/ ml}$)	Log ₁₀ C (Conc.)	% Mortality	LC50	logx	X
1	0.78125	-0.107	20	6.819	0.834	6.819
2	1.5625	0.1938	30			
3	3.125	0.4949	50			
4	6.25	0.7959	50			
5	12.5	1.0969	50			
6	25	1.3979	60			
7	50	1.699	80			
8	100	2	90			
9	200	2.301	90			
10	400	2.6021	100			

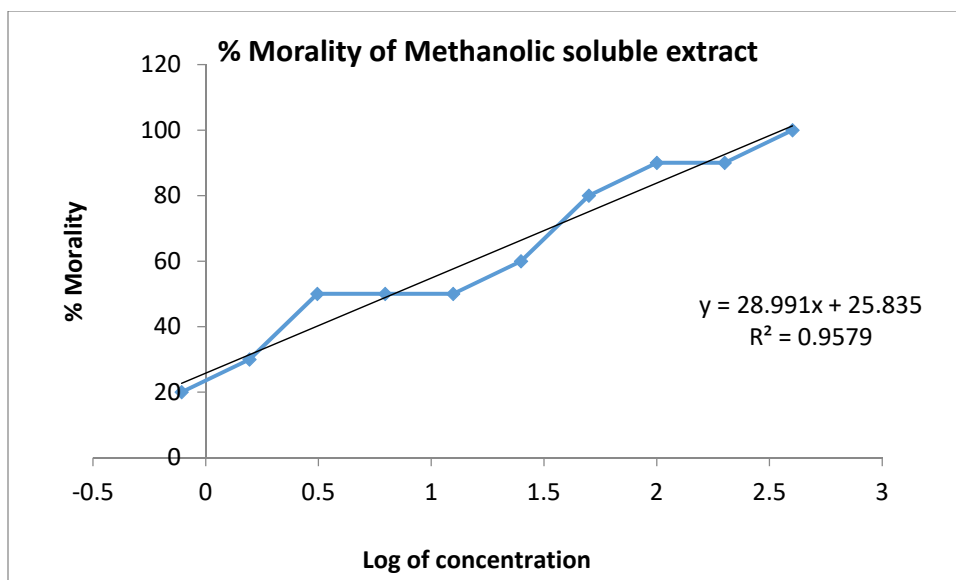


Figure 4.2: % plot of mortality & predicted regression line of MESF

Table 4.5: Pet-ether soluble fraction effect of the extract (PESF)

Sl.	Conc. (µg/ml)	Log ₁₀ C (Conc.)	% Mortality	LC50	logx	X
1	0.78125	-0.107	30	3.720	0.571	3.720
2	1.5625	0.1938	40			
3	3.125	0.4949	70			
4	6.25	0.7959	20			
5	12.5	1.0969	60			
6	25	1.3979	80			
7	50	1.699	100			
8	100	2	100			
9	200	2.301	80			
10	400	2.6021	100			

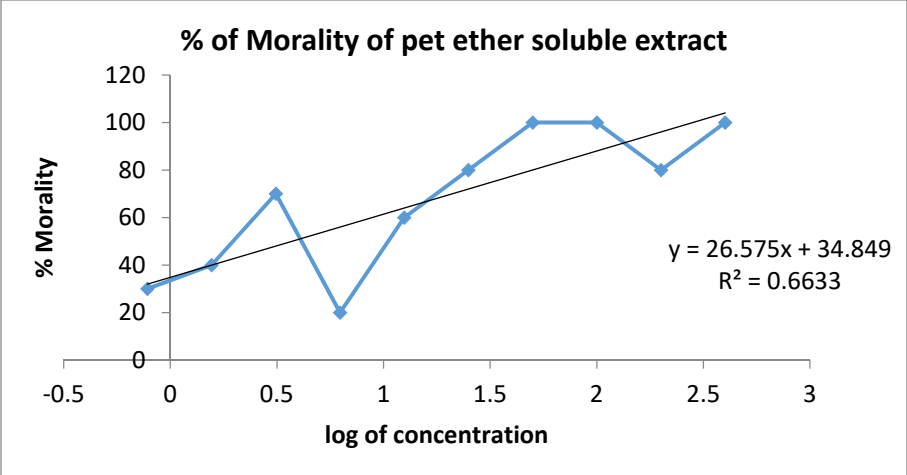


Figure 4.3: % mortality and regression line(predicted) of PESF

Table 4.6: Dichloromethane soluble fraction effect on shrimp nauplii.

Sl.	Conc. (µg/ml)	Log ₁₀ C (Conc.)	% Mortality	LC50	logx	x
1	0.78125	0.10721	20	3.026	0.481	3.026
2	1.5625	0.19382	40			
3	3.125	0.49485	60			
4	6.25	0.79588	70			
5	12.5	1.09691	40			
6	25	1.39794	100			
7	50	1.69897	100			
8	100	2	100			
9	200	2.30103	100			
10	400	2.60206	100			

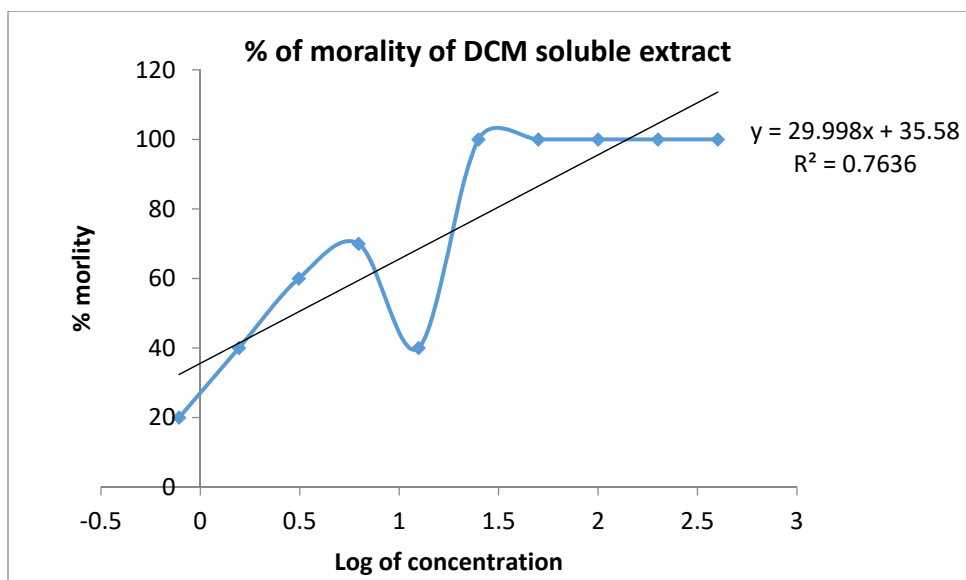


Figure 4.4: % mortality and regression line(predicted) of DCMSF

Table 4.7: Chloroform soluble fraction effect (CHLSF)

Sl.	Conc. (µg/ml)	Log ₁₀ C (Conc.)	% Mortality	LC50	logx	x
1	0.78125	0.10721	30	1.367	0.136	1.367
2	1.5625	0.19382	50			
3	3.125	0.49485	40			
4	6.25	0.79588	80			
5	12.5	1.09691	100			
6	25	1.39794	100			
7	50	1.69897	100			
8	100	2	100			
9	200	2.30103	100			
10	400	2.60206	100			

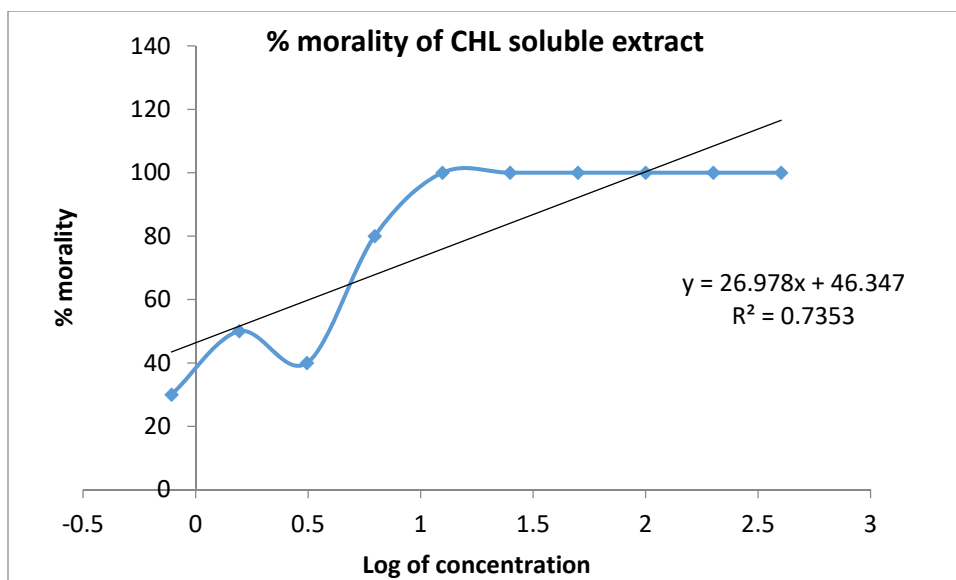


Figure 4.5: % mortality and regression line(predicted) of CHLSF

Table 4.8: Aqueous soluble fraction effect of the extract (AQSF)

SI.	Concentration(μg / ml)	Log ₁₀ C (Concentration)	Percentage(%)Mortality	LC50	logx	x
1	0.78125	-0.10721	10	23.605	1.373	23.605
2	1.5625	0.19382	10			
3	3.125	0.49485	50			
4	6.25	0.79588	20			
5	12.5	1.09691	40			
6	25	1.39794	50			
7	50	1.69897	50			
8	100	2	70			
9	200	2.30103	70			
10	400	2.60206	90			

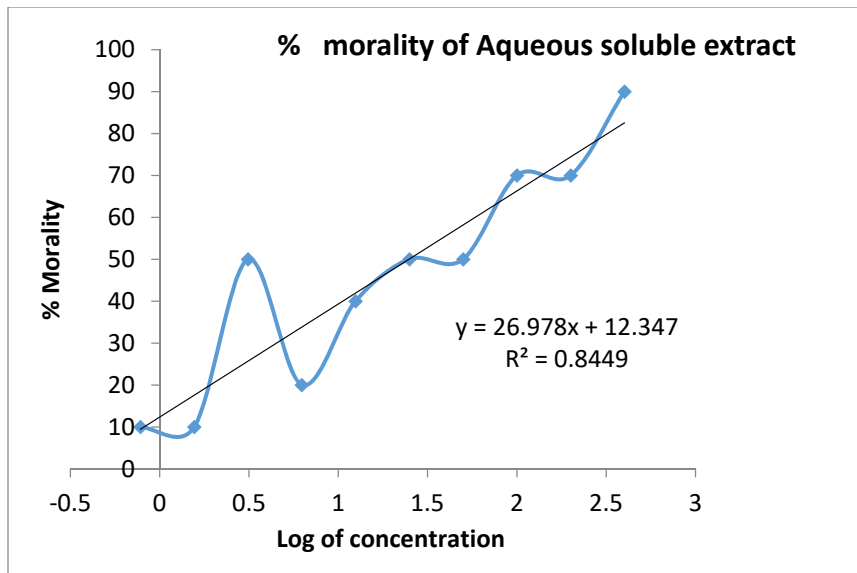


Figure 4.6: % mortality and regression line(predicted) of AQSF

4.4 Results and discussion

The individual's frustration obsession LC50 of the test tests following 24 hr. Could have been procured Eventually Tom's perusing plot starting with guaranteeing rate of the shrimps passed looking into against the logarithm of the test center (toxicant concentration) and the best-fit transport may need been got starting with that twist majority of the data to system for backslide examination. Vincristine sulfisoxazole (VS) may have been used Likewise Concerning illustration specific control and the LC50 might need been ran across 0. 203 mg/ml to VS. Compared for the individuals negative control VS (positive control) advertised gigantic mortal adversary and the LC₅₀ values of AQSF, MESF, PESF, DCMSF and CHLSF were found to be 23.60mg/ml, 6.82mg/ml, 3.72mg/ml, 3.03 mg/ml, and 1.37mg/ml respectively.

Chapter: Five
Conclusion

Conclusion

Crude extracts of *S. caseolaris* can be a source of produce new and efficient herbal medicine beside the synthetic antioxidants to treat oxidative stress related disorders. In this study, those different partitionates from claiming encountered with urban decay because of deindustrialization, engineering imagined. Caseolaris were subjected to phytochemical also living examination. Living examination were done uncommonly will focus the in-vitro cell reinforcement and cytotoxic exercises from claiming abandons extracts about encountered with urban decay because of deindustrialization, engineering imagined. Casiolearis through DPPH test, to evaluate the free radical searching limit.

And brine shrimp assay respectively. IC₅₀ value and LC₅₀ value of different fractions of extracts were determined and compared with the control. Therefore, this study clearly indicates that the extracts of *S. caseolaris* may be a very important contributor in various drug discoveries especially as a natural source of potential antioxidant with less cytotoxic effect. However, in vivo pharmacological evaluation needs to be done to promote the traditional use and to explore other therapeutic activity along with their side effects to gather substantive understanding about the safety and efficacy of mangrove apple. Next, phytochemical analysis of the extract will be helpful for isolation and characterization of its active compounds.

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