Quantification of Total Phenol, In vitro Investigation of Antioxidant Activity, Cytotoxic Effect and Phytochemical screening of the leaf Extract of *Artocarpus lacucha*

A project submitted by
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To

The Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

Dhaka, Bangladesh

Dedicated to my family.

Without them I am nothing.

Certification Statement

This is to attest that, this project titled 'Quantification of Total Phenol, In vitro Investigation of Antioxidant Activity, Cytotoxic Effect and Phytochemical screening of the leaf Extract of *Artocarpus lacucha'* 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 another.

Signed,

_____

Countersigned by the supervisor

_____

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Abstract

Artocarpus lacucha or monkey Jack fruit is mainly originated from India and it is a tropical plant. Leaves of *Artocarpus lacucha* are a great source of antioxidants. For the purpose of maintaining normal health antioxidants from plant source are important because antioxidants help to neutralize ‘’free radicals’’ which are considered as very harmful for human body. Although there are some debates on the actual affectivity of the plant. In this study, determination of antioxidant features of the extract which was acquired from the leaf of *Artocarpus lacucha* was done in the laboratory by phenol content determination and DPPH (2,2-diphenyl-1-picrylhydraziyl) a searching test for free radical. With the motivation of cytotoxic effect determination of the extract brine shrimp lethality test has been done and the value of LC50 were calculated and with standard it was compared. In this experiment, high quantity of phenol was found and also less cytotoxic effect was determined. As well as phytochemical screening of the leaf extracts has been done.

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Abbreviations

ROS - Reactive Oxygen Species

SOD - Superoxide dismutase

CME - Crude Methanolic Extract

DPPH - 1, 1-diphenyl-2-picrylhydrazyl BHT - Butyl-1- hydroxy toluene

ASA - Ascorbic Acid

DMSO – Di methyl sulphoxide

NaCl - Sodium Chloride

GAE - Gallic Acid Extract

VS – Vincristine Sulphate

TPC -Total Phenol Content

Chapter 1

Introduction

1. Introduction

1.1 Logics for the current study with objectives:

With the motivation of sustaining an appropriate life and to upgrade the grade of life, consumption of medicinal herbs has increased a lot. From the time period which is primitive, assorted herbage which comprise medicinal attributes, were used to cure human diseases. Due to the natural resistance of the organism are inundated by an enormous formation of ROS (reactive oxygen species), which is a situation 'oxidative stress' takes place and where in the body, assorted molecules which are large for instance nucleic acids, lipids, proteins etc. may influenced by decay of oxidation. As a consequence of this oxidative decay, assorted human maladies which are chronic, for instance, damaging of tissues, congestive diseases, assorted neurodegenerative disorders, mutagenesis and the aging process take place (Fontenot, 2011).

"There is no doubt in my mind that vegetal cure will persistent to grow and eventually become integrated into our material medical. If look back to the previous time, indeed there is a huge expansion has already been built. Currently we are looking ahead to the upcoming days with hope and great supposition." - Tylor V. E. *Herbal medicine: from the pastto the future,* p452

Medicine has reached heights far surpassing even the imagination of our forefathers. Which can be consider as the marvel of present medication that composite investigation subjects, for illustration, stem cell research is no more a prospect but a thriving reality. Nevertheless, with all these assists we still perceive assorted fatal diseases for instance malignancy to be on the boost, especially in third world countries. In a report, it is stated that by 2030, an estimated 13 percent of all demise in Bangladesh will be associated to malignancy with 200,000 recent occurrences outline every year, up from 7.5% in 2005 (bdnews24, 2015-12-15). This one just an example from a list and studies that provide the information about assorted after effects of synthesized and definite medicaments which have made a way for the scattering of these diseases. For instance, antioxidants which are synthesized, which most of scholars assume as more harmful than helpful for humans (Hasslberger, 2007).

One of the ancient medical practice is Herbalism, which is practicing since 33rd century BC Egypt.

With the help of modern smart technologies, we become able to understand more and explore the properties of herbs. Now we become able to analyze every small part of plants. Producing medicines from the plant source gives us several advantages like one will be their sheer verity of species. According to Botanic Gardens Conservation International (bgci.org), in the first ever checklist of the world's plants created in 2010 lists around 3,50,000 accepted varieties of plants with over 240,000 still to be confirmed. All of these plants provide different chemicals which can be use in different cases like to protect against bacterial infection as well as protect from decaying. About 12000 chemicals were isolated and only 10% of them were estimated. The purpose of this study is to looking at specific sub-study of herbology and more specifically on *Artocarpus lacucha*.

So that, developing the herbal medicine along with potential antioxidant from plants like *Artocarpus lacucha* is the rationale behind choosing such experimental work.

This study insights into the phytochemical and biological investigations of leaf extract of *Artocarpus lacucha* which includes:

- Extraction of dried powder of leaf of *Artocarpus lacucha* with methanol by cold extraction method.
- Confirmation of entire phenolic content of methanolic mixture of *Artocarpus lacucha* by Folin-Ciocalteu regent.
- In vitro assessment of crude methanolic extract of *Artocarpus lacucha* for antioxidant activity by DPPH radical scavenging assay.
- Brine shrimp lethality test to determine the cytotoxic effects of crude methanolic extract of *Artocarpus lacucha.*
- Phytochemical screening of the leaf extracts of *Artocarpus lacucha.*

1.2 Importance of potential antioxidant from natural source:

1.2.1 Antioxidants:

In daily life, from assorted of vegetal and food sources natural antioxidants can be found. Such antioxidants labor hostile to a reaction takes place in the body which is named as oxidation. As a consequence of this reaction an atom forms in the body, which is very unsteady and injurious and known as free radicals. This detrimental atom is provoked by oxygen and if they are left without control, there is a possibility that they can cause harm to the body. By contributing an electron to the molecule which is very unsteady, antioxidants make those molecules come back into stable form. Those antioxidants take part to neutralize free radicals are renewed by taking an electron from another antioxidant or it reshaped into collagen which is an elementary unit and used in repairing tissue matter. Later on, departing their electrons antioxidants which are synthesized lose their ability to be used in such way. Antioxidants have a feature to become a threatening byproduct after used up and have the ability to lead more stress on the oxidative load. Presenile dementia, malignancy, Parkinson's etc. all of these diseases are associated with the deprivation of antioxidants (Y. Feng & X. Wang,2012).

1.2.2 Protective properties of antioxidants:

For the purpose of protecting our body and fight hostile to reactive oxygen a fortification reaction which is distinctly developed and entangled has been constructed using antioxidants. It is completed by picking out exogenous as well as endogenous compounds which labor mutually to thwart free radicals. From assorted dietary sources we can get antioxidants, for instance, vitamin C and vitamin E. Vitamin C is immensely strong one as it thwart ROS in its fluid phase before lipid peroxidation. Vitamin E is a very effective antioxidant and which is known as a very efficient chain-breaker of the interior part of the cell membrane. Production of vitamin C, vitamin E assists. As a vast adjunct of vitamin C herbs as well as fruits are utilized and as a vast source of vitamin E is grains and high-quality vegetable oils (Wang & Quinn, 1999).

1.2.3 Description of free radicals:

Any atom which have at least one unpaired electron on their outer most shell is known as a free radical. When one electron leftover with newly created atom due to the breakdown of covalent bond, free radicals form. Electron of their outer shell is responsible for their highly reactive nature. They have aptitude to respond with the most molecules which located in its surrounding for illustration, proteins, assorted fatty acids named lipid, at the same time assorted carbohydrates and furthermore DNA, as they are very highly reactive in nature. With the nearest available molecule, they form bonds, taking its electron. As a result of this the attacked molecule become free radical dropping its electron, hence, a chain reaction started. As a consequence of this reaction disruption of living cells can results (Hansberger, 2007).

1.2.4 Study of cytotoxicity:

Cytotoxicity of an agent can be described as the degree at which it can provide its specific destructive action on certain cell. In a number of ways, the cells which are exposed to a cytotoxic compound can respond, for instance, autophagy, necrosis etc. For the purpose of determining the potential toxicity of a test substance like plant extract or the isolated compounds from plant which are biologically active, cytotoxic studies are helpful. For the development of a pharmaceutical or cosmetic preparation it is important to have minimal to no toxicity of the substance.

There is a number of bioassay to determine the cytotoxicity of a compound. Brine shrimp lethality assay is one of the most common modes to test cytotoxicity. Due to the swift and moreover the comprehensive instincts for the compound which are bioactive in nature originated from synthetic as well as a source which is natural, this technique is widely used. With the assistance of the current assay the feedback from the tissue of a living matter was occupied in case of natural product juice, snippet and the crude compounds as well. This assay helps in determining pharmacological activities like antimicrobial, anti-viral, pesticide and anti-tumor activities of bioactive compounds of natural and synthetic origin (Pisutthanan, 2004).

Chapter 2

Plant description

2. Plant description

2.1 Categorization scientifically:

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Rosales
Family: Moraceae
Genus: Artocarpus
Species: *A. lacucha*

2.2 Plant features:

Artocarpus lacucha is oftentimes noted as 'Monkey fruit'. It is also generally noted as 'lakuchi' in India, and known as 'lokhat' in the country Thailand and as 'dewa' in Bangali. This shrub is dispersed across the Indian landmass and the Southeast Asia too.

Having medium to huge deciduous feature, it is a tropical tree species which is evergreen in nature and contain a spreading crown. It gets bigger till 6-18 meters. It has branchlets which are 3-6 mm deep.

Blossoms are minuscule, yellowish, combined into a circular blossom figure. When fruits are not ripe enough they look green in color but when they are mature enough to be eaten they become yellow. Fruits are erratically circular and which is a syncarpous. Its leaves are cryptic, and which are 10-25 cm extended, conical and rugged. Its bark color is grey and the slit is dark red with latex which are milky. April-June is the blossoming period of it.

2.3 Medicinal importance and traditional use of *Artocarpus lacucha*:

There are vast conventional apply of the herb *Artocarpus lacucha*. Its fruits are consumed freshly. Its tuber is constricting and utilize as a laxative. For the motivation of healing the ache of the head, its bark is utilized. Its bark is also chomped in the similar manner of betel nut, moreover, it is occupied to cure dermal aliments. Its latex which are milky in nature and kernel are used as laxative. As the seeds and leaves have the property of hemagglutination, that means for the property of blood cells clumping they are conventionally utilized. Its juice from fruits are traditionally utilized for treating the loss of hair. Due to the motivation of healing the swelling of arthritis, cleansing of the wounds as

well as for treating dysentery they are used. For the purpose of fight against the worms its extracts from leaves are used. At the same time from the wood chips the aqueous extract is collected and from that powder which are brown in color are gathered, these powder are utilized to fight against fluke of intestine (Lakoocha: A Multipurpose Tree of Warm Climate, 2002).

(a)

(b)

(c)

Figure 2.1: *Artocarpus lacucha* (a) Plant, (b) Leaves, (c) Fruits.

Chapter 3

Methodology

3. Methodology

3.1 Chemical work:

The chemical labor done on the leaves of Artocarpus lacucha has represented beneath roughly:

- Gathering and recognition of plant sample properly.
- Development of plant samples.
- Extraction of the part of the plant with methanol.
- Evaporation of solvent that will yield crude methanolic extract.
- Chemical tests or determination of total phenolic content has done.
- Antioxidant assay of the extract has done.

3.1.1 Gathering and recognition of plant samples:

Artocarpus lacucha leaf were gathered from a park inside Dhaka city, Bangladesh during October, 2016.

3.1.2 Development of plant samples:

After gathering the leaves of the plants, those were scoured with water from tap and cleansed. After that they were dried out by sun light for a few days. Later on, parching appropriately these test materials were converted into powdered form with the help of grinding mill. The rough powder later on stockpiled appropriately in a container which is closed well so that air cannot enter into it, and preserved in a cool and dry place.

3.1.3 Extraction and evaporation of solvent:

Utilizing cold extraction method, the plant sample which was in the form of powder, was extracted. Test sample of the herb which was in the form of powder, was poured into a reagent bottle and was immersed in methanol (600ml). For the interval of seven days the test material was preserved in the bottle and it was being stirred and shaken occasionally. Later on, with the help of whitman no.1 filter paper as well as cotton, filtering of the mixture was done. Right after the filtration process, by utilizing of rotary evaporator beneath

reduced stress at raised temperature it was clustered for the purpose of obtaining crude extract, which is known as methanolic extract (CME).

3.1.4 Chemical tests:

3.1.4.1 Determination of total phenolic content:

For the motivation of countervailing free radical's vegetal polyphenols, which is an assorted group of phenol composite, have a good structural chemistry (Ahmed et al., 2014).

Due to the presence of different properties such as less side effects, potent antioxidant characteristics and economic attainability, the medicinal activities of herbs are exploring in the contemporary augment of science right through the planet.

3.1.4.1.1 Principle:(Ahmed et al., 2014)

With the utilization of Folin–Ciocalteu Reagent (FCR) the finding of the elements of all the phenolic compounds of the various extracts in the plant can be done. The fact that, still the entire chemical elements of the FCR is unrevealed but the presence of heteropolyphosphotung states – molybdates is assumed. In a species of blue, a sequence of versatile reduction reactions of one or two electron take place. A reaction which is complex in nature, take place in the middle of Mo(VI) and reductants, where Molybdenum is easily reduced.

3.1.4.1.2 Used chemicals as well as reagents:

- Folin-ciocalteu reagent
- Sodium carbonate
- Methanol
- Gallic acid
- Distilled water
- Beaker (100 & 200ml)
- Test tube
- Pipette (1ml)

- Pipette (5ml)
- Micropipette (50-200 µl)

3.1.4.1.3 Experimental procedure:

At the beginning, a test tube which can hold 2.5 ml of a reagent which is named Folin-ciocalteu (which is mixed well with water to make it diluted 10 times) was taken, as well as 0.5 ml of extract of the test material was put into the tube. After that, mixing of 2.5 ml of a chemical which is named Sodium Carbonate was done to the tube, as well as it was incubated at 24^{o}C temperature for 20 minutes. All the reagents have to be produce newly. Consequently, against a blank an absorbance of 760nm was set with the help of a spectrometer. The blank solution, which is known as a standard carries all reagents except the extract of the plants.

The phenolic elements which is exist in the test material was indicated as mg of GAE (gallic acid equivalent)/gm of the extract.

Table3.1 Folin-Ciocalteu reagent constituents:

SL. No.	Constituents	Percent
1	Water	57.5
2	Lithium Sulfate	15.0
3	Sodium Tungstate Dihydrate	10.0
4	Hydrochloric Acid>=25%	10.0
5	Phosphoric Acid 85 % solution in water	5.0
6	Molybdic Acid Sodium Dihydrate	2.5

3.1.4.1.4 Development of standard curve:

Here, Gallic acid was utilized and which is considered as a standard. The solution of gallic acid was made up with Folin-Ciocalteu reagent (which is mixed well with water to make it diluted 10 times) and also 2.0 ml of a chemical named Na_2CO_3 (7.5 % w/v) solution was mixed with gallic acid solution of 0.5 ml. After that, the combination was incubated at normal temperature for the time interval of 20 minutes. Consequently, at 760nm the absorbance was determined. A linear relationship was obtained subsequently graphong the absorbance in hostile to the concentration in abscissa, which was utilized as a curve, which is considered as a standard with the motivation of finding out the entire phenolic constitutes of the sample which was tested.

3.1.4.1.5 Development of sample:

With the motivation of getting a sample, which have the concentration of 2 mg / ml, extractives amount of 2 mg was taken subsequently that was dissolved using water which was distilled.

3.1.4.2 DPPH (1, 1-diphenyl-2-picrylhydrazyl) a searching test for free radical:

3.1.4.2.1 Principle:

On the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) the antioxidant capacity or the free radical scavenging activities were determined by the technique of Brand Williams *et al.,*1995.

In this method, different concentration solution of methanol was taken at the amount of 2.0 ml and were mixed up with DPPH methanol solution of 3.0 ml (20µg/ml). Methanol mixture of DPPH which has the appearance of purple was bleached by the extract of plant as contrasted to that of BHT or in broad *tert*-butyl-1-hydroxytoluene as well as ASA by utilizing UV spectrophotometer the assaying of potential antioxidant was done.

3.1.4.2.2 Materials & Methods:

For the evaluating purpose of searching test for free radical (potential of antioxidant) of assorted constituents as well as herbs DPPH was used (Choi *et al.,* 2000; Desmarchelier *et al.,* 1997).

3.1.4.2.3 Materials:

- 1,1-diphenyl-2-picrylhydrazyl *tert*-butyl-1-hydroxytoluene (BHT)
- Ascorbic acid
- Distilled water
- Methanol
- Chloroform
- Carbon tetra chloride
- n-hexane
- UV-spectrophotometer
- Beaker (100 & 200ml)
- Amber reagent bottle
- Test tube
- Light-proof box
- Pipette (5ml)
- Micropipette (50-200 µl)

3.1.4.2.4 Preparation of control group:

Utilization of ASA as well as BHT was done as a positive control. For obtaining a mixture which is consider as mother solution that have a density of 1000 µg/ml of ASA and BHT. The quantity of those chemicals was calculated, consequently in methanol they were dissolved. Utilizing the mixture which is called as mother solution, with the motivation of obtaining assorted density, ranging from 500.0 to 0.977 µg /ml, a sequence of dilution was generated.

3.1.4.2.5 Preparation of test sample:

For obtaining a mixture which is consider as mother solution that have a density of 1000 µg/ml the calculated quantity of assorted extractives was gauged and subsequently in methanol it was dissolved. Afterward assorted density ranging from 500.0 to 0.977 µg /ml were made by the process of serial dilution of the mother solution. They were kept in flakes with marked.

3.1.4.2.6 Preparation of DPPH solution:

To acquire a mixture of DPPH that have a density of 20 µg/ml20mg DPPH powder was weighed and dissolved in methanol. In a bottle of reagent which appears as amber, the solution was prepared and kept in a box which was light proof.

3.1.4.2.7 Free radical scavenging activity assay:

In this method, different concentration solution of methanol was taken at the amount of 2.0 ml and were added with DPPH methanol solution of 3.0 ml (20µg/ml). Methanol mixture of DPPH which has the appearance of purple was bleached by the extract of plant as contrasted to that of BHT or in broad *tert*-butyl-1-hydroxytoluene as well as ASA by utilizing UV spectrophotometer the assaying of potential antioxidant was done.

Inhibition of free radical DPPH in percent *(I%)* was determined as follows:

$$(I\%) = (1 - A_{sample}/A_{blank}) \; X \; 100$$

Where A_{blank} is represented as the control reactions absorbance, which consist of all reagents but the meterial to be tested.

From the diagram where percentage of inhibition was plotted hostile to the density of the extract, calculation of the density of extract that gives 50% inhibition *(IC_{50})* was done.

3.1.4.3 Brine Shrimp Lethality Bioassay:

3.1.4.3.1 Introduction:

Toxic effects of compounds which are bioactive are present on the body which is living at a heavy dose. The bioassay of brine shrimp which utilized to determine the lethality (McLaughlin, 1998) is an assay done utilizing living cells, is utilized for the compounds which are bioactive originated from synthetic source, furthermore from natural sources is a comprehensive and rapid process. For the purpose of testing the bioactivity of the extracts of a product which is considered as natural, fractions at the same time the crude compounds this method is use. According to this method a zoological organism that is the nauplii of Brine shrimp is utilized for suitable supervision for the purpose of screening furthermore fractionation so that new bioactive natural products can be discovered.

Along with pharmacologic features of vast range, for example antimicrobial, antiviral, pesticidal & anti-tumor etc. this bioassay indicates cytotoxicity of the compounds (Meyer, 1982; McLaughlin, 1998).

This method is preferred than the others because of its simplicity, less cost, rapid process and no requirements of special equipment or aseptic technique. In this process for the statistical purpose a large number of organisms are used and the amount of sample is small and it doesn't require animal serum like the other methods.

3.1.4.3.2 Principle (Meyer *et al.*,1982):

Due to the purpose of getting nauplii the hatching of Brine shrimp eggs is done. To prepare the test sample with desired concentration, calculated amount of di-methyl-sulphoxide (DMSO) have to add. With the help of visual inspection, the nauplii are counted subsequently they are kept in vial that contain 5 ml of water of sea. After that into the premarked vials the samples of test material at assorted density are mixed with the help of micropipette. Next the vials have to kept for a time period of 24 hours. After passing the period of 24 hours, the survivors are counted.

3.1.4.3.3 Materials:

- *Artemiasalina* leach (brine shrimp egg)
- Sea salt (NaCl)

- For hatching the shrimp, a perforated dam, which is diving in a tank, which is small in size.
- For enticing the shrimps, a light.
- Pipettes
- Micropipette
- Glass vials
- Magnifying glass
- Test tubes
- Test samples of experimental plants

3.1.4.3.4 Experimental Procedure:

3.1.4.3.5 Preparation of seawater:

After weighing of 38 gm crude NaCl which is also known as sea salt, was dissolved in distilled water of 1 liter and after that for obtaining a solution which appears clear, it was filtered off.

3.1.4.3.6 Hatching of brine shrimps:

As the test organism *Artemiasalina* leach (brine shrimp eggs) was used. At first in a small tank seawater was taken and then the eggs of brine shrimp were added to only a side of the tank, at the same time the side was unexposed. A period of 24 hours was given so that the shrimp can be grow up as nauplii. During the hatching time, the oxygen supply was constant. Also, there was lamp attached to the hatched shrimps. After that 10 shrimps were taken to each of the test tube utilizing Pasteur pipette, where every test tube individually contained seawater amount of 5 ml.

3.1.4.3.7 Development of samples for testing the experimental plant:

Experimental sample was taken in the vials and to get stock solution 100 µl of DMSO was dissolved. In the first test tube 50 µl of solution was taken, which consist of 5 ml of seawater which is simulated furthermore 10 shrimp nauplii. So, in the first test tube the final concentration of the prepared solution was 400 µg/ml. After that, from the stock solution by sequence dilution technique a sequence of solutions of varying density were produced. Each time fresh 50 µl DMSO and 50 µl sample was mixed to vial. As a result, in the individual test tubes various concentrations were found.

Table 3.2: Test samples with concentration values afterward series dilution

Test Tube No.	Concentration (µg/ml)
1	400.0
2	200 .0
3	100 .0
4	50 .0
5	25 .0
6	12.5
7	6.25
8	3.125
9	1.5625
10	0.78125

3.1.4.3.8 Control group development:

For the purpose of validating the test technique as well as ensuring that gathered result are for the property of the material to be tested and the outcome of the possible other factors are nullified control groups are used. There are two types of control groups are used and they are:

a. Positive control
b. Negative control

3.1.4.3.9 Development of the positive control group:

In the study about cytotoxicity positive control is an agent, which is cytotoxic in nature, that is widely accepted and the comparison between the outcome of agent, which is tested with the outcome of positive control is done. In this technique, vincristine-sulphate was utilized as positive control. For getting a primary density of 20 µg/ml dissolving of the calculated quantity of vincristine sulphate in the DMSO was done. For getting 10 µg/ml, 5 µg/ml, 2.5µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.078125

µg/ml, 0.0390 µg/ml serial dilutions are made using DMSO. After that, to obtain the groups of positive control, the addition of the positive control solutions was done in to the premarked vials and where in 5 ml of simulated water of sea contains 10 brine shrimps.

3.1.4.3.10 Preparation of the negative control group:

As a group known as negative control, each of three premarked vials prepared by glass, holding 5 ml of simulated sea water as well as 10 shrimp nauplii100 µl of DMSO was added. If there is rapid mortality rate of nauplii in the vials then the test is considered as invalid because owing to assorted causes rather than the cause of cytotoxicity of the test material the nauplii died.

3.1.4.3.11 Counting of nauplii:

The counting of shrimps which are still alive were calculated after 24 hours period by utilizing a magnifying glass. For each dilution, the percent (%) mortality was calculated. By utilizing linear regression, utilizing a IBM-PC program, which is not complicated, the data of concentration-mortality were analyzed statistically. As a median lethal concentration (LC_{50}) value the relationship between concentration-mortality furthermore the effectiveness generally explored in case of the products of the plant. After a certain exposure period, the density of the compound which caused the death of test subjects about half of their number, was represented by this.

Chapter 4

Results and Discussion of Antioxidant Assay

4. Results and Discussion of Antioxidant Assay

4.1 Results & discussion of total phenolic content:

The methanolic extract (ME) of leaves of *Artocarpus lakoocha* was experiment for entire content of phenols. For that purpose, a reagent named as Folin-Ciocalteu was used. On the basis of the values of the absorbance of the plant extract the entire content of phenols of the extract was measured and with the solutions of gallic acid, which is considered as a standard, it is compared. The entire contents of phenols of the test material are explored as mg of GAE (gallic acid equivalent)/ gm of extract.

Table 4.1: Standard curve prepration by using gallic acid:

Sl. No.	Conc. Of the Standard (µg / ml)	Absorbance	Regression line	R^2
1	100	0.800	**y = 0.0081x - 0.0007**	**0.9975**
2	50	0.423		
3	25	0.215		
4	12.5	0.123		
5	6.25	0.047		
6	3.125	0.007		
7	1.5625	0.003		
8	0.78125	0.000		
9	0.3906	0.000		
10	0	0.000		

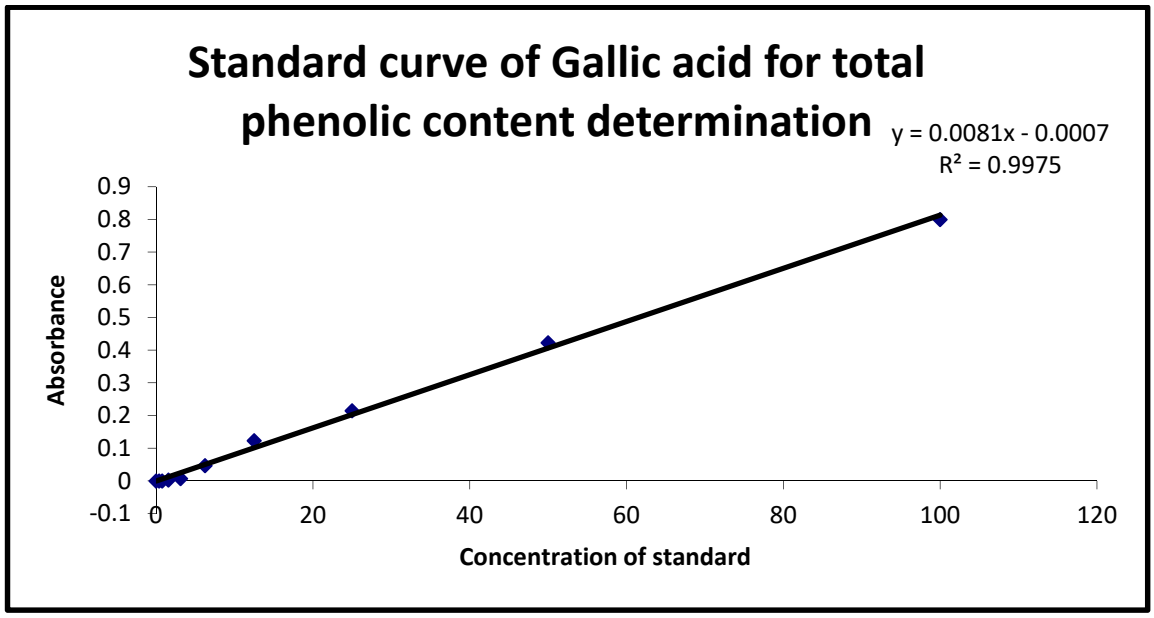

Figure 4.1: Standard curve of Gallic acid for total phenolic determination.

So, entire constitute of phenol: $x = (y + 0.0007)/0.0081$

$x = 24.78$ (mg of GAE / gm of extractives)

4.2 Outcome of DPPH experiment with discussion:

The leaves extract which is methanolic of *Artocarpus lakoocha* was placed for determining the existence of the properties of free radical by the technique of Brand-Williams *et al.,* 1995. Here, as reference standards BHT as well as ASA were utilized.

Table 4.2: IC_{50}value of Methanolic extract (ME) of leaf's of *Artocarpus lakoocha*:

Absorbance of the blank	Conc. (µg/ml)	Absorbance of the extract	% inhibition	IC_{50} (µg/ml)
0.426	500	0.094	77.93	160.0
	250	0.136	68.08	
	125	0.198	53.52	
	62.5	0.233	45.30	
	31.25	0.270	36.61	
	15.625	0.299	29.81	
	7.813	0.310	27.23	
	3.906	0.314	26.29	
	1.953	0.317	25.59	
	0.977	0.326	23.48	

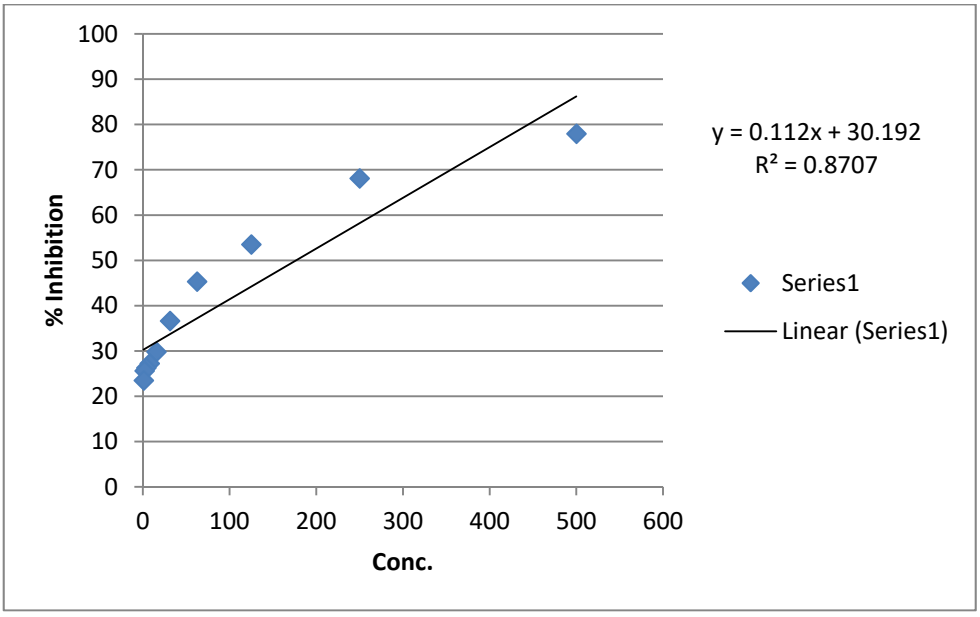

Figure 4.2: IC_{50} value of Methanolic extract (ME) of leaf's of *Artocarpus lakoocha*

4.3 Results and Discussion of Brine Shrimp Lethality Bioassay

4.3.1 Leaves of *Artocarpus lacucha*:

The extract which is methanolic (ME) of leaves of *Artocarpus lacuch* was tested for bioassay, where, brine shrimp is used according to the Meyer *et al.,* (1982) procedure. Finishing a time period of 24 hours the lethal concentration (LC_{50}) of the material to be tested was gained by a plotting the percentage of how many shrimps died hostile to the logarithm of the density of the tested material (toxicant density). As well as from the graph data for regression analysis the best-fit line was obtained.

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

Concentration (micro gram per ml)	Log10 conc	% of mortality	LC_{50} value (µg/ml)
0	0	20	0.451
0.0390	-1.4089	20	
0.078125	-1.1072	30	
0.15625	-0.8061	30	
0.3125	-0.5051	40	
0.625	-0.2014	50	
1.25	0.09691	70	
2.5	0.39794	80	
5	0.6989	80	
10	1.00	90	
20	1.3010	100	

Figure 4.3: Effect of Vincristine sulphate (positive control) on shrimp nauplii

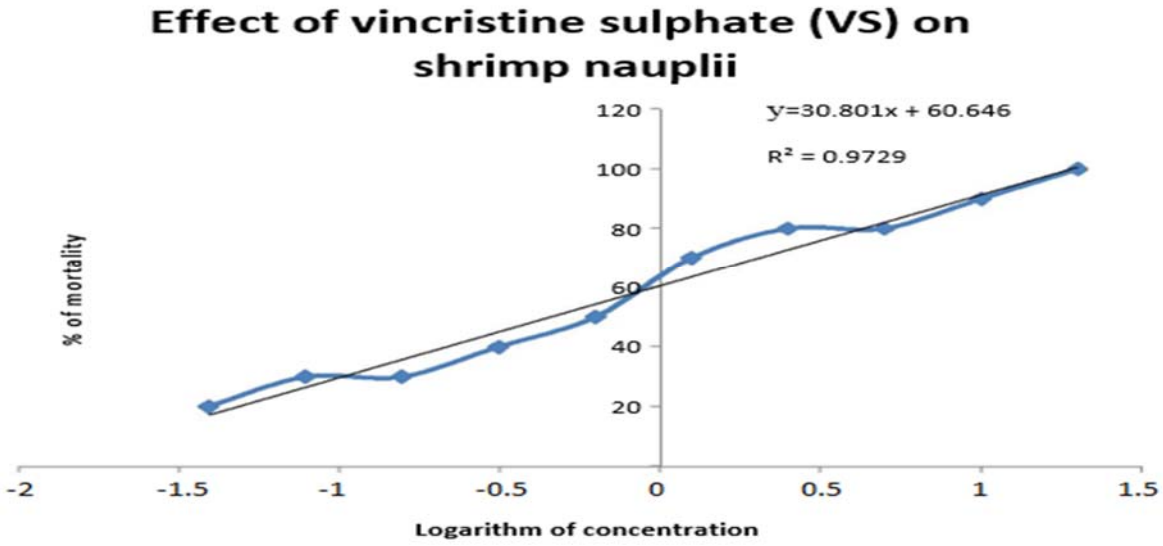
Effect of vincristine sulphate (VS) on shrimp nauplii
y=30.801x + 60.646
R² = 0.9729
% of mortality
120
100
80
60
40
20
-2
-1.5
-1
-0.5
0
0.5
1
1.5
Logarithm of concentration

Table 4.4: Effect of the Methanolic extract (ME) of leaves of *Artocarpus lacucha* on shrimp nauplii.

Conc. (µg/mL)	Log_{10} conc.	% of mortality	LC_{50} (µg/mL)
0		0	72.663
0.78125	-0.10720997	20	
1.5625	0.193820026	30	
3.125	0.494850022	40	
6.25	0.795880017	50	
12.5	1.096910013	50	
25	1.397940009	60	
50	1.698970004	70	
100	2	70	
200	2.301029996	80	
400	2.602059991	80	

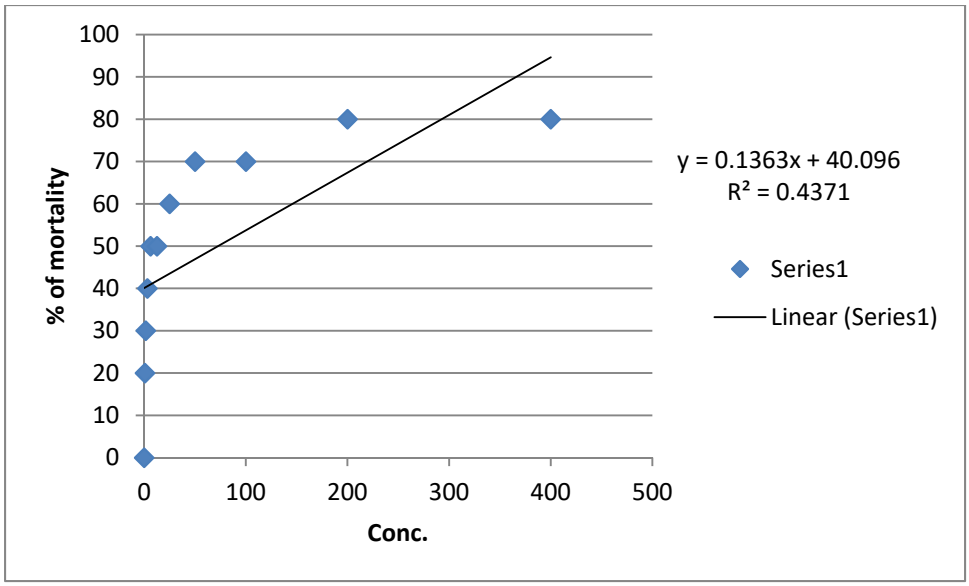

Figure 4.4: Effect of the Methanolic extract (ME) of leaves of *Artocarpus lacucha* on shrimp nauplii.

Chapter 6

Phytochemical screening from the leaves extract of *Artocarpus lacucha*

6 Phytochemical screening from the leaves extract of *Artocarpus lacucha*:

The following chemicals has been found after the phytochemical screening:

1. Alkaloids are present.
2. Phenols are present.
3. Flavonoids are present.
4. Tannins are present.
5. Lignins are present.
6. Sterols are present.
7. Glycosides are present.
8. Saponins are present.

Chapter 7

Conclusion

Conclusion

The Crude extracts of *Artocarpus lacucha* leaves was subjected for different phytochemical and biological tests. The investigations involved determination of total phenol content, which is a very important phytochemical compound of the plant. The result of total phenol content was 24.78 (mg of GAE / gm of extractives). Which shows a good amount of phenols was present on the plant sample.

After that, through DPPH test the antioxidant features of the extract which is methanolic, of the plant was investigated in laboratory. Where, IC50 value of the extract which was methanolic, was calculated.

Furthermore, the features like cytotoxic of the extract which is methanolic of the plant leaves was also experimented through brine shrimp assay. Where, LC50 of the extract was calculated and it was compared with the control.

Finally, the phytochemical screening of the leaves extract of *Artocarpus lacucha* was done. Where, the presence of different important chemical compounds like alkaloids, flavonoids, tannins, sterols etc. was found.

So, it can be said that this study states that the extract of *Artocarpus lacucha* is very important as natural source of potential antioxidant. It has a less cytotoxic effect on the living organism and there are a number of important chemical compounds are present on it.

Chapter 8

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