Investigation of In-vitro Biological Potential of Methanol Extract of *Magnolia alba* Leaves

By

Sabrina Hossain Sarah

ID: 15146030

The thesis submitted to the department of pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

Department Of Pharmacy

Brac University

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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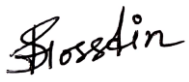

Sabrina Hossain Sarah
ID: 15146030

Approval

The thesis/ project titled "Investigation Of In- Vitro Biological Potential of Methanol Extract Of *Magnolia alba* leaves " submitted by Sabrina Hossain Sarah (15146030) of spring 2015, has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor in Pharmacy on February 2020.

Examining Committee:

Supervisor:
(Member)

17.07.2020

Shahana Sharmin
Senior Lecturer, Department of Pharnacy
Brac University

Program Coordinator:
(Member)

Professor Dr. Hasina Yasmin
Professor, Department of Pharmacy
Brac University

Departmental Head:
(Chair)

Professor Dr. Eva Rahman Kabir
Chairperson, Department of Phamacy
Brac University

Ethics Statement

No Trial Animal was harmed during this study.

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Abstract

From immemorial time medicinal plants are used as an important source of medication for ailments and diseases. Now a day it has expanded the source of income also. In this experiment some pharmacological activities were screened from methanol extracted *Magnolia alba* leaf. Antioxidant properties were identified using DPPH assay. The data from this assay proved the existence of Antioxidant in this plan. Although the IC50 level of experimental plant leaves is comparatively lower than the standard (Ascorbic Acid). Later on, thrombolytic activity also tested, by it demonstrated 19.1% clot lysis which is lower than compared with the standard clopidogrel. Furthermore, evaluation of Cytotoxicity property done by Brine Shrimp Lethality Assay (BLSA). The result showed for *Magnolia alba* leaves was 7.904% whereas the standard vincristine sulphate showed 3.324%.It has appeared a higher level of cytotoxicity in this experimental plant but the antimicrobial test did not give any significant result though. Exploring new possibilities and expectations is the beauty of research. It seems this study will help to design future researches and discovering a new source of antioxidant, cardiovascular and anticancer drug with more effectiveness and safety.

Keywords: Medicinal plants; Antioxidant; Cytotoxicity; Antimicrobial; *Magnolia alba;* Traditional medicine

Dedication

"This work is dedicated to my Family"

Acknowledgement

Initially, I would like to Thank Almighty Allah for blessing me with patience and courage to complete this project successfully. Without his blessing it would be impossible to come this far.

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List of Acronyms

M.alba	*Magnolia alba*
DPPH	2,2 Diphenyl-1-picrylhydrazyl
mg	Milligram
UV	Ultraviolet
ml	milliliter
DMSO	Dimethyl sulfoxide
µg	microgram
IC50	Median Inhibitory Concentration
LC50	Median Lethal Concentration
HCl	Hydrochloric Acid
Kg	Kilogram
gm.	Gram
FRS	Free Radical Scavengers
WHO	World Health Organization
cm	Centimeter
Conc.	Concentration
m	meter

ME	Methanol Extract
AMR	Antimicrobial Resistant

Chapter 1

Introduction

1.1 Background

There are two elements of traditional medicine are common. These elements are 1. The material used, 2. The treatment method. In ancient medication the raw materials are derived from altogether natural sources.in folk medication system, the protection from ailments and diseases are considered as necessary, because the disease will be prevented by body's natural balance. Many nutritional values of plants are mentioned in various literature. The biological active compounds present in these plants are referred to a phytochemicals. These phytochemicals are derived from each part of plants like, root, leaves, flower, seeds, stems or fruits. These phytochemical compounds are used as medicinal compound. (N. Van et al., 2008)

Who reported that 80% of population of developing world use traditional flavoring medicine as it is inexpensive and alternative source of primary health care because of cultural practice, lack of modern facilities it has become a fast growing choice. As an example in China traditional medicine produced 30-50% than that of modern medicine. In Ethiopia 80% population consume traditional medicine because of their cultural priorities and beliefs and lack of access of modern medicine. In china traditional herbal formulation account for 30-50% of total medicine. While Nigeria, Ghana, mali 60% of their children take herbal medicine as their first choice while suffering from high fever of Malaria. These data from ethno medical surveys may fill up the existing gap to discover effective drugs.(Aziz et al.,2018)

It would be helpful to conduct pharmacological properties of such plants used to treat some disease across the planet. Additionally it has become necessary to protect and cultivate

medicinal plants. Almost every plant has nutritional, esthetics or medicinal value. Traditional knowledge is essential to protect and patent. (Neha et al.,2019)

1.2 History of Medicinal Plants:

The oldest written evidence of phytochemical plants found on Sumerian Clay slab from Nagpur about 5000 years before. There were 12 recipies for 250 various plants.

The Chinese book on roots and grasses "Pen'ta Sao" written by emperor Shen Nung during 2500 BC which included 250 drugs.

The Indians holy book Vedas mentioned treatment with plant. The Ebers Pappyrus written Circa during 1550 BC which represented a collection of 800 proscriptions to 700 plants. In holy Bible the use of aromatic plants as myrtle and incense were mentioned.

Theophrast during (321-278 Bc) founded Botanical Science with his Book. The Roman physician Galen introced the list of drugs with similar and identical actionsm during 131 AD to 200 AD.

The arabs introduce various medicinal plants mostly from India. Through out the middle ages European Physicians consulted the Arab works" De Re Medica". "Liber Magnae Collectionis Simplicum Alimentorum Et Medicamentorum" by Ibn Baitar (1197-1248), in which over 1000 medicinal plants were described.

Marco Polo's journey in tropical asia, Russia , Persia ,the discovery of America resulted(1492) in medicinal plants brought to Europe.

In 18 th century species were classified and named. The polynomial system was employed. Early 19th century is a turning point for the use and knowledge of medicinal plants because of the discovery of quinine, poppy, isolation glycosides and advancement of medicinal method.

In late 19th and early of 20 th century there was a great danger in elimination of medicinal plants from thrapy as their shortcomings found due to destructive action of enzymes. Proposal of Fresh medicinal plants changed the situatin and the manufacturing cost of medicinal drugs was increased.

At present most of the pharmacopias proscribe plant drugs for their great value. The major producer of herbal preparation n the basis of phytochemical effectiveness whose efficiency depends on the applied dose and indentified active components. (Petrovska, 2012)

1.3Uses of Medicinal plants in Bangladesh:

It has been estimated that there are five hundred of species of medicinal plants exists in Bangladesh. Medical specialty of activities of sixty four plants has been discovered and introduced within last twenty years of Bangladesh. The pharmacological activities of those medicinal plants add notable varieties of potential and promising role in several types of folk formulations. (R.Akter et al., 2013)

Table 1: list of medicinal plant utilized in preparing traditional medicine

Plant Species	Family	Local Name	Voucher no.	Traditional Uses
Aegiceras corniculatum	myrsinaceae	kholisha	DACB 31584	B,L & S: Fish poison, asthma, diabetes, inflammation & rheumatism
Argyreia nervosa	Convolvulaceaee	Bichtarak	DACB 37954	S: Hypotensive, Psychoactive, L: Wound healing, native Stimulant, R: sickness of nervous system,

				urinary sickness & chronic lesion.
Avicennia alba	Avicenniaceae	Morcha baen	DACB 40556	Treatment reproductive disorder, skin diseases, tumors, ulcers
Clitoria ternatea	papilionaceae	Aparajita	DACB 32021	R: Laxative, Diuretic S: Cathartic, Ascites, Pharyngitis, Tumors, Skin diseases, AP: Colic, Skin Diseases, gonorrhea.
Clerodendrum viscosum	Verbenaceae	Bhant	DACB 37953	WP : hypotensive, L: Anthelmentic, Emetic, Antiperiodic In protozoal infection,Malaria, cough, asthma, snake bite, skin disorder, R: antifungal
Caesalpinia pulcherrima	Caesalpiniaceae	Krishnachura	DACB 32020	L: Purgative, anticancer, fungi poisonous, liver disorders, F: cough, asthma,

				bronchitis, malaria fever, R: Cholera, infantile, Convulsions, W: Diarrhea, infectious disease & Skin Diseases
Myrica nagi	myricaceae	kaiphal	DACB 32029	B: Astringent, antiseptic, fever, cough, asthma, ulcer, cholera, chronic dysentery, anemia, piles, tumors.
Saraca asoca	Caesalpiniaceae	Ashok	DACB 32007	B: Antitumor, bleeding, hemorrhagic infectious disease, , L: blood purification, F: hemorrhagic dysentery, syphilis, uterine tonic.
Mussaenda glabrata	rubiaceae	Patralekha	DACB 32023	R: White leprosy, L: Jaundice, F: Diuretic, chest pain, ulcer
Lannea	Anacardiaceae	Jeol/ jiga	DACB 35242	B: Astringent, mouth sores, L: local swellings

				and pain of the body
Jasminum sambac	oleaceae	Beli phul	DACB 31262	AP: CNS depressant, hypotensive, L: indolent lesion, breast tumor, R:emmenagogue
Hymenodictyon excelsun	rubiaceae	Bhui kadam	DACB 32013	B: Astringent, febrifuge, antiperiodic, antimicrobial, diarrhea, W: herpes
Gnaphalium luteoalbum	compositae	Boro Karma	DACB 37955	L: Astringent, diuretic, homeostatic, T: Counter irritant
Glycosmis pentaphylla	Ruteaceae	Daton	DACB 37931	L: fever, liver complaints, cough, jaundice, eczema and other skin disorder, R: fever, FR: infectious disease.
Ginus oppositifolius	Molluginaceae	Gima	DACB 32014	WP: CNS Depressant, diuretic, skin disease, aperients, antiseptic

Ecbolium viride	Acanthaceae	Nilkanta	DACB 32018	L: Antitumor, R: Tumors, jaundice, rheumatism, WP: Gout, Cardiovascular disorder, dysuria.
Dillenia indica	Dilleniaceae	Chalta	DACB 32019	Fr: Expectorant, Laxative, tonic, abdominal pain, L: Astringent, B: astringent, S: antifungal and bactericidal
Diospyros peregrina	Ebenaceae	Gab	DACB 30323	B: Astringent, dysentery, biliousness, Fr: astringent, Pharyngitis, ulcer, dyspnea, cough, S: Diarrhea, dysentery, SB: antiprotozoal drug, anticancer, antiviral, diuretic
Dipterocarpus turbinatus	Dipterocarpuceae	Garjan	DACB 32026	Gonorrhea, gleets, rheumatism, ring worm, lesion and skin diseases.

Here, S: Seed, Fr: Fruit, W: Wood, AP: Aerial Parts, B: Bark, F: Flowers, L: Leaves, R: Roots, RB: Root Bark, SB: Stem Bark, T: Tomentum, WP: Whole plants

1.4 Selection of *Magnolia alba*:

Many journals and publication was searched for *Magnolia alba* but no adequate information was found. So *Magnolia alba* was selected for in vitro evaluation of antimicrobial, antioxidant, thrombolytic and cytotoxic properties.

1.4.1. Introduction to selected plants:

Kingdom: Plantae

(Unranked): Angiosperms

(Unranked): Magnoliids

Order: Magnoliates

Family: Magnoliaceae

Genus: Magnolia

Subgenus: M. Subg. Yulania

Section: M. sect. Michelia

Subsection: M. subssect. Michelia

Species: M X alba

Binomial Name: *Magnolia x alba*

1.4.2 Description of *Magnolia alba*:

Magnolia alba matures to 30 meters with evergreen leaves. It is a flowering plant. *Magnolia alba* is noted for its fragrance. The flower contains about 12 petals which are white in color

and has glossy foliage. Stamens and carpels are present in flower. Leaves are green in color. This plant requires moderate care year round to keep it looking its best. It prefers to be protected sun areas but too much sunlight kills the flower. The flowering season lasts from March to November.

1.4.3. Origin:

It is a flowering plant which is considered to be a hybrid origin of Magnolia champaca and Magnolia Montana. Although exact origin is uncertain but it is commonly cultivated in Southeast Asia and tropical region East Asia. It has other names such as: *Mychelia alba*, *Mychelia champaca*

1.5 Project justification:

There is not much information has been found about chosen plant Magnolia alba. With increasing rate of diseases and emergence of microbial resistance, searching for new drugs or investigating advanced medical specialties are demands of modern time. So the true purpose of this study is to explore some pharmacological properties like antioxidant, antimicrobial, thrombolytic and cytotoxicity from the crude leaf extract of Magnolia alba. Because research is an important way of establishing development.

1.6 Aim of this project:

The Aim of this study is in vitro evaluation of pharmacological properties like antioxidant, anti-bacterial, thrombolytic and cytotoxic effects of methanol extract concentrate of *Magnolia alba* leaf *(Family: Magnoliacae)*

1.7 in-vitro evaluation of anti-oxidant property of methanol extract of *Magnolia alba* leaves:

It is suggested by growing body of evidences that cellular injury or oxidative injury arises from production of free radicals or reactive oxygen species. (M.E.H Mazumder et al., 2008).Toxic and mutagenic agent effects is caused by reactive oxygen species (ROS) on all types of cells due to oxidative damage of membrane lipids, proteins and DNA. ROS is directly or indirectly generated by either reactions of normal aerobic metabolism or exogenous substances. (Oktyabrsky O et al., 2009). ROS is chargeable for maturing and breaking down of plasma membrane, DNA transformation, impairment in membrane protein. (Rotundifolia et al., 2016)

Oxidation inhibitor compound have been found in vegetables, fruits, oil seeds, leaves, cereal crops, spices, herbs, bark and roots, crude plants etc. (M.E.H Mazumder et al., 2008) Previously It was found that medicinal plants have profound anti-oxidant potential and these plants are considered as future aspect to novel drugs development for the treatment of various varieties of neurodegenerative and inflammation related disorders associated with oxidative cellular injury. Research interest on distinguishing potential anti-oxidant property in medicinal plants is showing in recent years. As a result plant derived oxidation inhibitor like quercetin, thymol, carnosol, carnosic acid, Gallic acid, rosemarinic acid, hydroxytyrosol has come back to attention due to their extensive contribution in food preservation, dietary supplementation, treating different types of free radical mediated diseases. (M.E.H Mazumder et al.,2008) It has tested that antioxidant property is connected to polyphenol together with flavonoids and tannins. (Oktyabrsky O et al., 2009)

The purpose of this experiment was to find out plant concentrates of elements with analogous to total phenolic substances alongside cell reinforcement action and then on establish advance potentiality of normal anti-oxidant activity.

1.7.1 Screening of antioxidant Property by DPPH assay*:*

During this study, DPPH analysis was performed that may be a free radical method. It is associated anti-oxidant assay supported electron transfer which produces a violet solution in methanol and anti-oxidants is evaluated quickly by spectrophotometry. Plant extracts of *Magnolia alba* and standard was analyzed supported free radical scavenging through this strategy. Methanol was utilized as solvent and Ascorbic acid was used as a standard solution. Antioxidant property was identified by attenuated color of DPPH solution with plant concentrate and additionally compared with Ascorbic acid by UV spectrophotometer.

1.8 Thrombolytic property Evaluation:

At present, blood clot formation (Thrombus) has become a serious downside of blood circulation. Thrombosis is considered as one of the vital causes of morbidity and mortality in a extensive form of vessels diseases (Memariani Z et al., 2018). Thrombus or embolus obstructs the blood circulation by preventing the blood vessel therefore the tissues deprived of normal blood circulation and oxygen. This results in death in tissue of that part. Thrombin causes formation of blood clot from fibrinogen and is broken down by plasmin. It becomes activated from plasminogen by the tissue plasminogen activator (t-PA). Due to high occurrence of thromboembolic disorders so recent investigations are being performed on new herbal medicine as they are considered as alternative remedies.(Memariani Z et al.,2018) .fibrinoytic drugs used to dissolve thrombin in extremely blocked coronary arteries to reinforce the blood supply to limit necrosis and to facilitate prognosis. For the treatment of myocardial infarction, many thrombolytic agents are used. Among them, Streptokinase (SK) is remarkable and widely used. (Ramjan A et al., 2014)

The purpose of this experiment is to perform in vitro identification of clump lysis property from *M. alba* leaf concentration with Clopidogrel as a positive control and refined water as a negative control.

1.9 in vitro test of cytotoxic activity evaluation of methanol extract of *Magnolia alba*

Identification of medicinal plant for cytotoxicity analysis is beneficial for the treatment of cancer. The importance of development of cancer treatment is increasing with time. Within the last two decades, 64 have been studied and reported on their pharmacological activities. A number of them were known having cytotoxic property as an anticancer property. Here, in this assay the cytotoxity of methanol extract of Magnolia Alba has been identified under Brine Shrimp Lethality Assay (BLSA) to check that if this plant might contribute in the development of Cancer treatment.

1.10 Anti-microbial test of *Magnolia alba*

The emergence and rapid spread of multi and pan-drug resistant organism has placed the world in dilemma. (ANYANWU) it has been reported that a rise in pathogens multiresistance like Staphylococcus, aureus, streptococcus agalactiae, Enterococcus, Enterobacteriaceae, and Candida albicans. Available antimicrobial drug have been misused and overspreading of antibiotics is the reason to develop AMR. (Khèmiri I et al., 2019) AMR is the Capability of microorganisms such as Bacteria and fungi to sustain despite exposure to antimicrobial agent. Development of recent effective and safe antimicrobials is one amongst the way by which AMR burden can be reduced. Ethnnopharmacological researches have contributed mostly .There is renewed interest in antimicrobial activities of phytochemicals. Scientific compilation of research studies can provide helpful information on antimicrobial properties of the plants. (Ananyawu).

The purpose of this assessment is to mitigate the upcoming threat of antimicrobial resistance by indentifying a new source of it.

Chapter2

Methodology:

2.1 Steps in Preparation of Plant sample

2.1.1 Plant collection:

Plant collection was done from national herbarium. As no past examination has been done on its natural properties, so the leaves of *Magnolia alba* has been chosen for this experiment. After a complete identification of this plant and its availability, the plant has been chosen for this experiment. The leaf part of *Magnolia alba* was collected in September 2019 from Bangladesh National Herbarium.

Table 2: Analysis of *Magnolia alba:*

Name of the plant	Scientific name	Family	Part
Champaca	Magnolia alba	Magnoliacae	Leaves

2.2 Process of Extraction:

The whole process of extraction can be divided into two sections:

Plant material preparation from crude extract 2 stages

1. Washing of plant leaves
2. Air drying

Extraction process (5 Stages)

1. Size reduction by crushing
2. Extraction (maceration)
3. Filtration
4. Concentration
5. Drying of the plant concentrate
6. Plant extract

2.2.1 Preparation of Plant material for Crude Extract:

The leaves separated from the stem of the plant and washed away the plant scrap and residue particles with clean water. Then selected the spotless leaf and dried them at room temperature for couple of weeks until the leaves became crispy and fully dried. After storing the dried leaves for the subsequent stage.

2.2.2 Plant extraction technique:

Size reduction and weighing

The spotless dried and hard leaves were grinded by blending machine. Around 500 gm. of powder collected and was stored in air tight plastic container which was kept in a cool, dry and dim spot until further examination. A required step has been taken to avoid any kind of cross contamination.

Extraction of plant by solvent

The extraction methods can be divided into 2 parts according to types of solvents which has been used:

1. Extraction with aqueous solvent
2. Extraction with organic solvent

For this examination methanol was used as organic solvent. The measuring glass containing plant material of *michelia alba* was absorbed in solvent which taken two times more than the powder for 3 days in cool and dry place at room temperature with occasional stirring.

Filtration

After 3 days of maceration, the glass jar containing substance was filtrated by using a clean cotton cloth and also Whattman filter paper (pore size: 110 mm)

Concentration

After the collection of filtrated product of plant extraction was concentrated by using a rational evaporator (heidolph) at 100 rpm at 30 degree celcious. When the methanol concentrate was created, the thick concentrated potion collected in a petri dish.

Drying

Finally, the petri dish containing concentrated substance was kept under laminar air flow (LAF) to vaporize the solvent. LAF also utilized as a preventive measure which protected the concentrated product from any kind of microbial contamination while drying. After it has been dried effectively, it was kept under the refrigerator.

Table 3: The weight of methanol extracted *Magnolia alba* leaf gained after a proper extraction process

Initial weight/g	66.98
Final weight/g	104.55
Weight of extract/g	37.57

2.3 Antimicrobial Property Analysis:

Methanol extract of *Magnolia alba* leaves used as sample for this test

Table 4: Materials and Reagents for antimicrobial property analysis:

Sl. No.	Apparatus
1	Petri- dishes
2	Nutrient Agar Medium
3	Filter paper discs
4	M.H. Agar
5	Sterile forceps
6	micropipette
7	Autoclave
8	Spirit burner
9	Refrigerator
10	Screw cap test tubes
11	Incubator
12	Laminar air flow hood
13	Nose mask and hand gloves
14	Foil paper
15	Conical flask

Microorganism used in the test:

Here both gram positive and gram negative bacterial strains were collected from unadulterated culture for this trial. The list of microorganisms is given below:

Table 5: list of microorganisms used in this assay:

Gram positive	Gram negative
Staphylococcus aureus	*Salmonella typhae*
Bacillus subtilis	*Escherichia coli*

Sterilization procedure of test:

First of all every device including measuring glasses, conical flask, petri dishes, cotton and forceps had been sterilized and kept in an antiseptic spot. It was done to avoid any kind of cross contamination or microbial pollution during this procedure. All work was done in under laminar air steam hood so that the controlled condition was kept. Before starting the procedure, ultra violate light was turned on in laminar air steam hood. Micropipettes tips, cotton, forceps, blank disc were treated by UV light. Then using the autoclave machine petri dishes and different mechanical equipment were sterilized at a temperature of 121 degree Celsius and a weight of 15- lbs/sq. inch for 60 minutes. After trial all the instruments were cleaned and used bacterial strains were demolished to control all kind contamination.

Anti-microbial test procedure:

At first, in order to preparing culture 2.5 g of nutrient broth were dissolved in 100 ml distilled water. At that time 6 conical flasks were taken and each conical flask contains 10ml of stock solution including 6 distinctive bacterial strains. Then keep these conical flasks at shaking motion into incubator at temperature 37oc for 24 hours. After 24 hours these cone shaped flasks were expelled from incubator and kept under controlled condition. At this time, agar medium was prepared by using 7.6 g of Mular Hinton Agar which was dissolved in 100 ml of distilled water. Then the Mular Hinton Agar placed into petri dishes and store these petri dishes in a cool place at room temperature. At this time test solution of plant extract were diluted from

ranging 1200mg/ml to 200mg/ml and soaked the filter paper discs in it. When the Mular Hinton Agar placed in petri dishes has been cooled and strong enough, in bacterial strains placed into it by using cotton bars. Azithromycin were used as standard plates and test plant concentrated discs were placed in the petri dishes. After doing this, these petri dishes were put into incubator at 37oc for 24 hours for providing a compatible condition for bacterial growth. After 24 hours, petri dishes were collected outside from incubator and observed any restraint zone created by standard and test circles.

Determination of inhibition zone for test:

Antimicrobial property of *Magnolia alba* was assessed due to ensure that if the plant has any ability to stop the microorganism growth around the petri dishes. Counteractive action of microbial growth plates give strong spot of restraint. At this point, when the incubation is done antimicrobial property of the sample were assessed by computing the distance across the restraint territory with a clear zone.

2.4 In vitro thrombolytic property analysis:

Thrombolytic property can be determined by a simple tactic where plant extract was used as test sample, clopidogrel which is anti-platelet specialist as positive standard and water as negative standard.

Materials and reagents

Table 6: list of materials and reagents used for In-vitro thrombolytic test:

Sl. No	Name of the materials
1	Blood
2	Plant extract
3	Clopidogrel (anti-platelet agent)

4	Micro centrifuge tube
5	Distilled water

Test sample preparation

For preparing test sample, a test tube which contained refined water of 10ml at that point 200 mg plant concentrate was suspended in it. Then the test tube was kept in dry, dim place for overnight so that the solvent supernatant turned in to solution and after that filtrated the solution.

Standard solution preparation

Clopidogrel as an antiplatelet specialist used as a standard for this test. 75 mg of clopiogrel was dissolved in 10 ml of distilled water and blended appropriately at this point this suspension kept as a stock solution from which 100 µl preparations was used in thrombolytic action assessment.

Blood sample preparation

5 healthy volunteers (n=5) who did not take any anticoagulant treatment in the past, blood sample collected from them under aseptic condition. From collected blood sample 1 ml of blood was move into pre weighted ependroph tube and kept them for incubation at 37 °C for 45 minutes for exaggerate clump formation.

Thrombolytic property test process

At first, collection of 5ml blood sample from each volunteer was done. Collected the samples in pre weighed sterile ependroph tubes and incubate for 45 minutes at 37 °c. After formation of coagulation, the upper liquid portion was separated from smaller tubes. The weight of coagulation was taken by subtract the weight of eppendroph tube before the formation of

coagulation. At this point, 100 µl of Clopidogrel used as positive control whereas 100 µl of distilled water were used as a non-thrombolytic negative control with 100 µl of each sample was added from each test-tube. Eppendroph tube was incubated at 37°c for 90 minutes so that the precipitation of clump lysis can be formed. After a short time, when the incubation was finished, the fluid was separated from cluster again weighted the tube to observe the weight contrast after the coagulation diversion.

Finally, the percentage of clot lysis can be measured below,

Percentage (%) of clot lysis= (released clot weight/ clot weight) *100

2.5 In - vitro anti-oxidant property analysis:

Anti-oxidant activity assayed in terms of:

Determination of anti-oxidant properties by performing DPPH Assay

2.5.1. Screening of free radical scavenging DPPH Assay:

Anti-oxidant activity of plant extract and also the standard was determined by radical scavenging impact of 2,2Diphenyl 1- picryl hydrazyl (DPPH) free radical action with slight alteration. The test extract was diluted as serial dilution in methyl alcohol. Ascorbic acid was used as reference compound (100µg/ml arrangement). DPPH of 0.004% was dissolved in methyl alcohol solvent and 3 ml of this solution was mixed with 2 ml of test solution and standard solution on an individual basis. The mixtures were kept in dark place for 30 minutes at room temperature. Once the incubation has been done, the absorbance was estimated by spectrophotometric machine at 517 nm against blank solution which is mainly methanol. In DPPH test, DPPH radical was used mainly as a constant free radical in order that the reducing substances or anti-oxidant activity of plant extracts can be estimated. The anti-oxidant property of plant extract that contains poly phenol compound in order that they can donate hydrogen

particles or electrons to catch the free radicals. The purple color of 2,2 diphenyl 1-picrylhydrazyl (DPPH) will turn out to be yellow shaded complex. This DPPH test is observed as most outstanding system for the in vitro evaluation of antioxidant activity.

Materials and Reagents:

Table 7: List of the materials and reagents for DPPH assay:

Materials	Reagents
Test tubes	Methanol
Volumetric flask	DPPH (2,2 diphenyl l- Picrylhydrazyl)
UV spectrophotometer	Ascorbic acid(ASA)
Pipette & micropipette	Extracts of the plant
Beaker	Distilled water

Control Preparation for Evaluation:

In this test, Ascorbic Acid played a role as positive control. Estimated quantity of ascorbic acid was dissolved within the methyl alcohol solvent to acquire a solution which concentration was 1200µg/ml. Then the serial dilutions of solution were done to get four different concentrations ranging from 1200 to 200µg/ml.

Table 8: Ingredients and amount used in preparing control:

Name of chemicals	Calculated Amount
Methanol	10ml
Ascorbic Acid	120 mg

Test Sample Preparation for Evaluation:

120 mg of *Magnolia alba* leaves extract was placed in a clean test tube. So as to preparing the test sample 10 ml of methanol added to the test tube to get concentration of 12 mg/ml. after the preparation of test sample the sample solution was diluted by serial dilution technique 1200µg/ml, 800µg/ml, 400µg/ml, 200µg/ml and stored in dry dark place with imprint.

Preparation of DPPH solution For Evaluation:

For DPPH solution preparation, two mg of DPPH powder were weighed accurately and then dissolved it in fifty ml of methanol to achieve 20µ g/ml concentration. At this time the solution was placed in dark place which was wrapped by aluminum foil paper.

Table 9: name of the components with quantity used in DPPH solution preparation:

Name	Calculated quantity
DPPH	2 mg
Methanol	50 ml

Assay Of DPPH Free Radical Scavenging Activity:

Test solution in each tube having completely different concentration starting from 1200µ g/ml, 800µ, 400µ, 200µg/ ml were mixed with 2 ml of DPPH solution of concentration 20µg/ml. At this time, this mix is kept in a dark place for half an hour to get response. After 30 minutes the absorbance of mixtures were calculated by UV spectrophotometer at 517 nm wavelength where

Methanol used as a blank.

Calculation:

Inhibition in percentage (1%) of free radical DPPH was estimated as given below:

Inhibition (1%) = (1-A Sample / A blank) * 100

Where, a blank stands for absorbance of the control reaction

Afterwards, 50% inhibition (IC 50) was provided by methyl alcohol plant extract concentration and also the value was obtained from the graph where plotted inhibition percentage (1%) against the concentration of plant extract (μg/ml)

2.5 In-vitro cytotoxicity analysis:

2.5.1. Procedure of Brine shrimp lethality assay (BLSA):

Materials for Test:

Table 10: Name of the apparatus used in Brine Shrimp Lethality Assay:

Sl. No.	Name Of materials
1.	Brine Shrimp
2.	Spherical Glass Jar
3	NaCl
4	Micropipette, pipette
5	Dimethyl Sulfoxide (DMSO)
6	Test tube
7	Lamp Attract to shrimp
8	Magnifying Glass
9	Glass vial
10	Plant extract

Preparation of sea Water:

38 g of salt (pure NaCl) was weighted and dissolved in 2L of distilled water and then filter the water to induce clear solution.

Hatching of Brine shrimp's eggs for test:

Brine Shrimps (Artemia Salina) eggs were collected from BRAC University, Department Of Pharmacy which was employed in this experiment. A spherical shaped tank was taken and filled with seawater and also the nauplii were placed in it for 48 h at room temperature under constant supply of oxygen and illumination to make sure survival and maturity before use. Just about 20 nauplii collected with the aid of pateur pipette and added to each test tube containing 5ml of seawater.

Preparation of test solution for experiment

Test solution was taken in a test tube and disintegrated in DMSO. After serial dilution distinctive concentration starting from 1200µg/ml to 200µg/ml. first 50µl of sample which concentration was 400 µg/ml placed into test tube holding 5 ml of DMSO additionally with 20 nauplii. Then 50µl DMSO was added to test tube to dilute the solution by this method diverse concentration acquired.

Table 11: Plant sample with different concentration after serial dilution:

Test tube no	Concentration (µg/ml)
1	1200
2	800
3	400
4	200

Preparation of control group for experiment:

In cytotoxicity study to certify the assessment methodology and assurance that the results accomplished were equivalent to the performance of the test administrator and also the potential effects different potential stoppages control group is principal. Generally 2 types of control groups are used; they are positive and negative control.

Preparation of Positive Control:

In cytotoxicity test positive control commonly referred to as a cytotoxic compound which facilitate in test appeared differently in relevance o the results of positive control. Here, vincristine sulphate is a cytotoxic compound that was used as a standard. The portion of vincristine sulphate is blended in DMSO to gain the principal portion of 20µg/ml at the moment by sequential dilution different concentration of standard solution were gained 10µg/ml, 5µg/ml,2.5µg/ml and 1.25µg/ml. finally, standard incorporated the test tube holding 5 ml seawater with regarding 10 nauplii.

Preparation Of Negative Control:

In order to obtaining negative control, 3 test tube were taken and 100µl of DMSO was added to the all of the test tube each of that contain 5 ml of sea water with regarding 20 nauplii. At that moment, if the downfall of nauplii is fast, which shows the test is unsuitable and the nauplii terminated because of some unfortunate reason.

Nauplii Counting

Result was collected following 24h , by the help of a magnifying glass and the quantity of survivors was estimated decisively altogether the test tube. From each dilution, the extent of mortality was set by direct relapse of IBM-PC program which is employed to which is used to survey the mortality data. Besides, the concentration versus mortality relationship of plant concentrate is expressed by LC50 regard which implies average dangerous core esteem. At

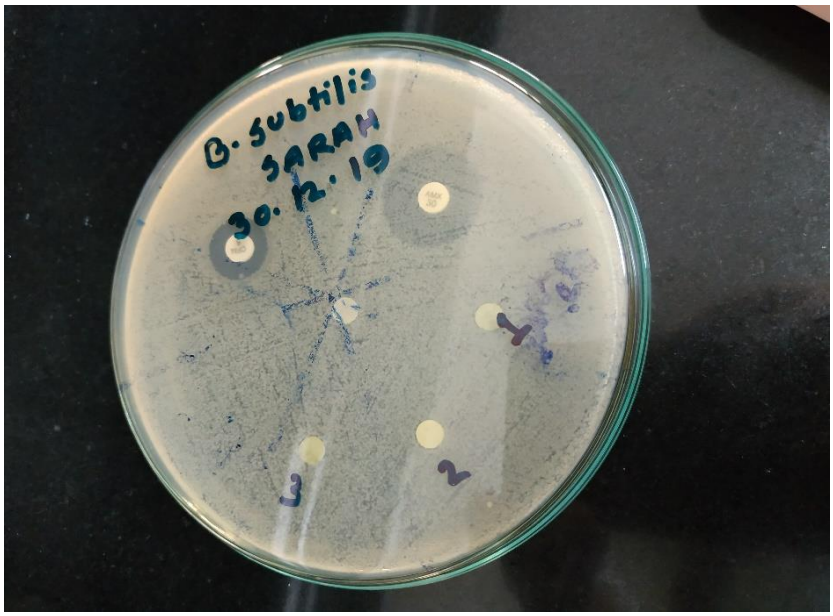
now concentration of the prepared compound is responsible for the downfall in half of the test nauplii after a particular time period.

Chapter3

Observation and Result:

3.1 Antimicrobial Property Analysis:

Different ranges of concentration from 1200 µg/ml to 200 µg/ml of Methanol Extract of *Magnolia alba* leaves has been prepared to perform this experiment. With each of this concentration every types of bacterial strain were observed. There was very little potential outcome was observed for some of concentration which can be negligible. So it did not demonstrate any significant result for Antimicrobial property. In this assay streptomycin antibiotic was used as a standard so that the obtained result of sample can be compared with that of standard and also used both Gram Positive and Gram Negative Bacteria


B. subtilis
SARAH
30.12.19
3
2
1


S. typhae
SARAH
30.12.19
3
2
1

Figure1: Antimicrobial Property of Plant *Magnolia alba* leaves analysis on petri-dishes

3.2Anti oxidant Property Analysis:

3.2.1 Evaluation of free radical scavenging assay:

Table 12: IC50 Value (µg/ml) of Ascorbic acid (ASA):

Conc.(µg/ml)	Absorbance Of Standard(ASA)	% of inhibition	IC50(µg/ml)
1200	0.005	99.18962723	82.53283
800	0.011	98.2171799	
400	0.067	89.14100486	
200	0.404	34.52188006	
Blank	0.617		

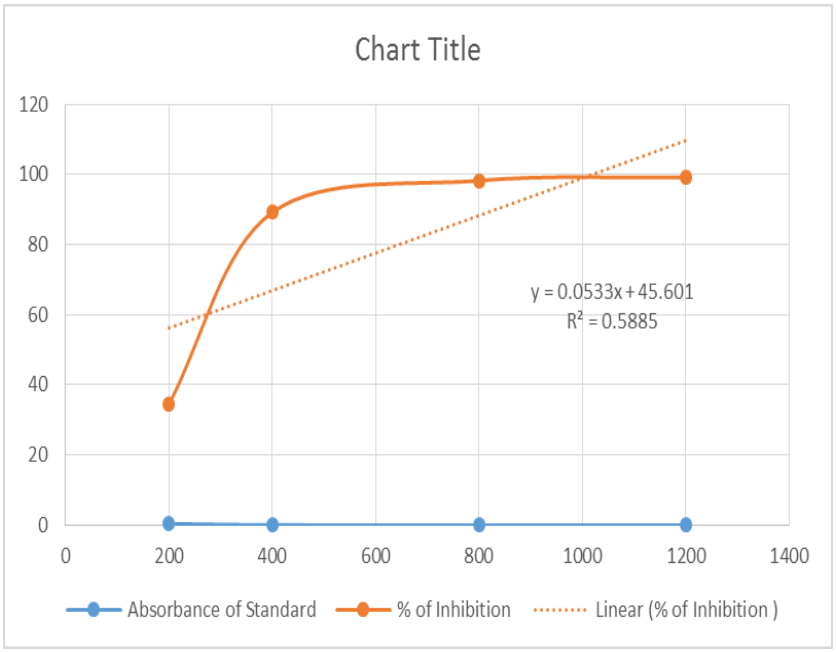


Figure 2: Inhibition vs. Concentration Curve of Ascorbic Acid

Table 13: IC50 Value of Methanol plant Extract of *Magnolia alba*:

Conc.(µg/mL)	Absorbance of Methanol plant extract	% of inhibition	IC50(µg/ml)
1200	0.053	91.41004862	37.8734177
800	0.094	84.7649919	
400	0.167	72.93354943	
200	0.323	47.64991896	
Blank	0.617		

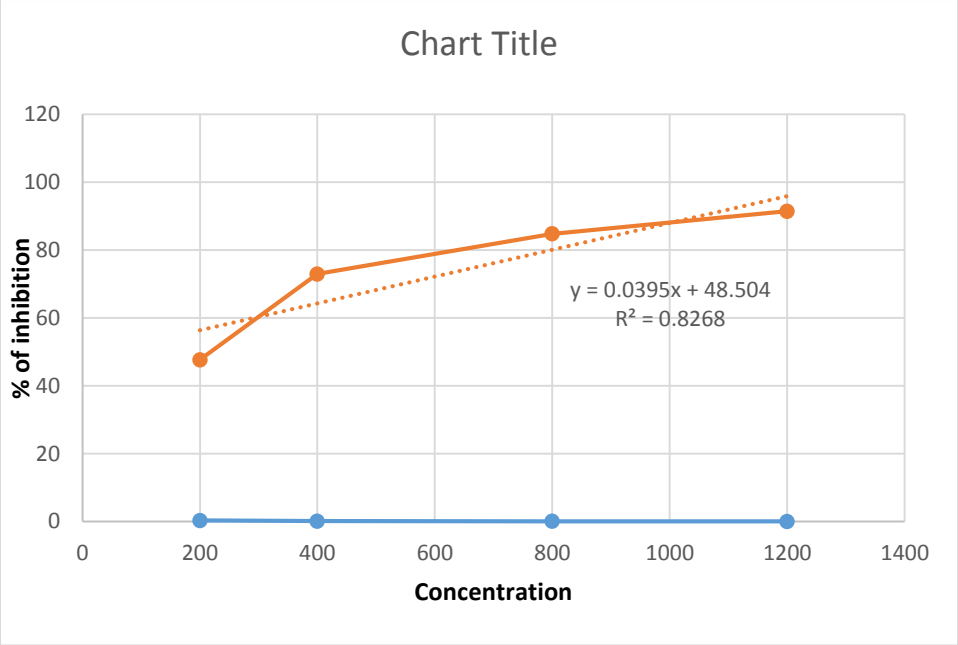


Figure 3: Inhibition vs. concentration curve of methanol extract of Magnolia alba

After observing the above analysis, it can be said that, the rate (%) limitation of free radical scavenging of *Magnolia alba* concentrate was lower inhibition at certain applications than the equivalent concentration of Standard (Ascorbic Acid). Moreover, IC50 calculation of Methanol Extract of *Magnolia alba* is lower than that of Ascorbic Acid.

3.3 Thrombolytic property Analysis:

Table 14: Thrombolytic property assay of Magnolia alba

Name 0f sample	Weight of empty ependroph, W1	Clot with weight of ependroph W2	Weight of clot, W3=	Weight of ependorph after clot lysis	Weight of released clot,	% of clot lysis

			(W2-W1)	W4	W5= W4-W2	
Methanol extract of *Magnolia alba*	0.805	1.321	0.516	1.222	0.099	19.1
Clopidogrel (Anti-Platelet Agent) as Standard	0.805	1.329	0.524	1.189	0.14	26.72
Blank	0.805	1.343	0.538	1.254	0.089	16.54

From This investigation it might be said that Methanol Extract of *Magnolia alba* Leaves has showed moderate effect on clump lysis, yet appear differently in relation to Clopidogrel group lysis rate was lower.

3.4 In Vitro Cytotoxicity Activity Evaluation:

3.4.1 Evaluation of Brine Shrimp Lethality Assay:

Table 15: Effect of Vincristine sulphate (positive control) on shrimp nauplii:

Concentration(µg/ml)	Log Concentration	Nauplii Taken	Nauplii Dead	Nauplii Alive	%of mortality	LC 50
10	1.00	10	8	2	80%	

5	0.69	10	7	3	70%	3.324
2.5	0.39	10	5	5	50%	
1.5	0.18	10	3	7	30%	

Effect of Vincristine Sulphate on Nauplii

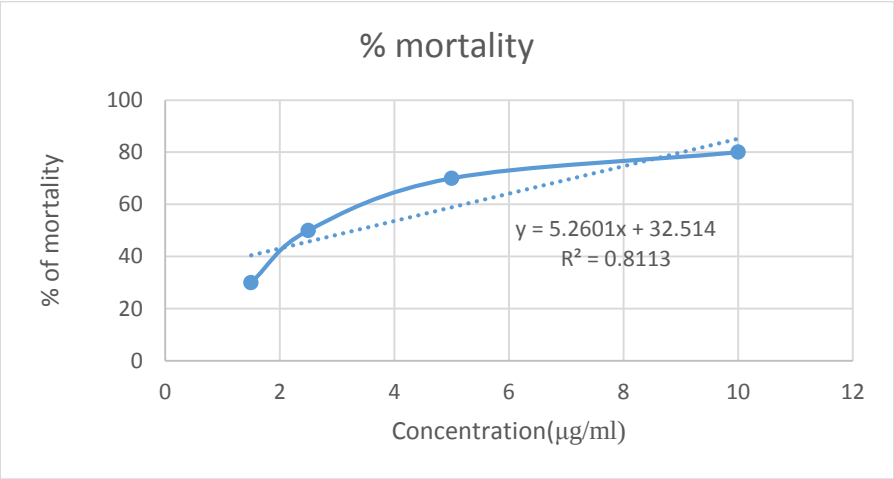


Figure 4: percentage of mortality and predicted regression line of Vincristine Sulphate

3.4.2 Evaluation of Cytotoxic Activity in Methanol Extraction of *Magnolia alba* leaves:

Table 16: Effects of Methanol concentrated extract of Magnolia alba leaf:

Concentration(µg/ml)	Log concentration	Nauplii taken	Nauplii Dead	Nauplii Alive	% of Mortality	LC50 (µg/ml)
12	1.08	20	12	8	60	7.904
8	0.903	20	11	9	55	

4	0.602	20	9	11	45	
2	0.301	20	4	16	20	

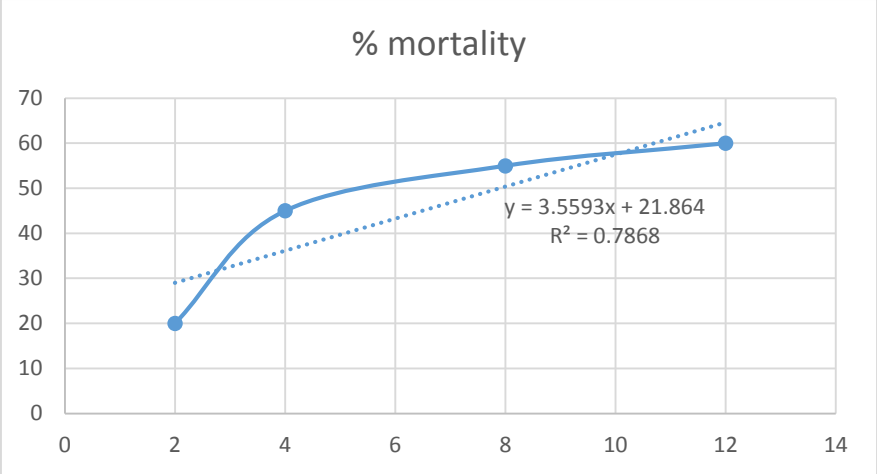


Figure 6: Percentage of Mortality and predicted Regression Line of Methanol extract of Magnolia alba

Chapter 4

Discussion:

The data got by screening process of Methanol extract of *Magnolia alba* leaf can contribute to the medication world . the methanol extract of *Magnolia alba* was analyzed by DPPH method to investigate the antioxidant property of the concentrated extract. The reference standard used in this experiment was Ascorbic Acid for which IC50 result was 82.53µg/ml and the result of IC50 for methanol concentrated extract of *Magnolia alba* leaves acquired 37.87µg/ml.

Methanol concentrated *Magnolia alba* leaves extract gave a remarkable outcome on thrombolytic Activity Screening. In this experiment, a drug called "Anclog" under Clopidogrel group was used as reference standard

As a positive control group for which outcome got approximately 26% clump lysis activity. Here, distilled water used as a negative control and the result for this was seen approximately 17% clot lysis activity. The methanol concentrated *Magnolia alba* leaf extract was given 19.1% lysis of blood Coagulation. Considering the percentage of clot lysis calculation of Methanol extract with the positive control, the plant did not cover practicable thrombolytic activity.

Brine shrimp Lethality Assay (BLSA) was performed to survey the cytotoxicity activity of methanol concentrated extract of *Magnolia alba* leaves. The graph plotted the level of mortality (nauplii) versus the concentration of the plant to determine the result of LC50 of the analyzed sample. Here, Regression method was used to predict the best fitted line from the graph using the given data. Vincristine sulphate was used as a positive control in this experiment which gave the outcome for LC50 was 3.324 µg/ml whereas LC50 estimated for Methanol concentrated extract of *Magnolia alba* leaf was 7.904 µg/ml. therefore, the result for *Magnolia*

alba was higher than that of the positive standard. So further research should be done to ensure that, this plant contains cytotoxic property.

Antimicrobial test also done for this sample plant solution. But unfortunately it did not give any significant result for this experiment. So it seems, this plant might not contain any antimicrobial property or May some error occurred during the trial period. So, further experiment is required to confirm the presence of antimicrobial property in this plant.

Conclusion:

In this experiment it was tried to investigate the presence of any organic compound in methanol concentrated extract of *Magnolia alba*. It has demonstrated some existence of Antioxidant, moderate level of thrombolytic property and critical level for cytotoxic property. But the outcome on Antimicrobial for this plant was not significant. More Advanced level of experiment recommended for exploring better pharmacological properties for this plant so that our health sector can be more enriched with ethno botanical knowledge and utilization of it.

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