

Phytochemical and Biological Evaluation of *Albizia richadiana* (Benth. Fabaceae family)

A project submitted

by

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Inspiring Excellence

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Certification Statement

This is to certify that this project titled ‘Analytical Method Development and Validation of a Combination Formulation’ 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 Ms. Monica Shafrin Rahman, 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

Dedicated to my parents

Acknowledgement

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Abstract

This dissertation aimed at to investigate and evaluate the possible phytochemical and biological profiles like, phytochemical screening, brine shrimp lethality bioassay and hypoglycemic activity of the medicinal plant *Albizia richardiana* (Fam. Fabaceae, subfam. Mimosoideae). Plants of the *Albizia* species are employed as traditional medicine for the treatment of anthelmentic, rheumatism, cough, diarrhea, stomach ache, wounds, etc. These plants are also recognized therapeutically for treating irritability, insomnia, wounds, as antidysentric, antiseptic, antitubercular etc. In present Phytochemical study, this plant is a great source of different group of bioactive chemical substances like- carbohydrates, saponins, glucosides, glycosides and alkaloids. Apart from this, they may show cytotoxicity as well as hyperglycemic activity. Based on the present study, we can suggest *Albizia richardiana* may be given the recognition as a medicinal plant.

Keywords: Albizia, Phenolics, Flavonoids, Terpenoids, Saponins, Cytotoxicity

CONTENTS

ABSTRACT	II
LIST OF FIGURE	VI
LIST OF TABLE	VII
LIST OF ACRONYMS	VIII

CHAPTER 1: INTRODUCTION

1.1 The Plant: <i>Albiziarichardiana</i>	4
1.2 General Botanic Data.....	5
1.3 Taxonomic Hierarchy.....	6
1.4 The Plant family: Fabaceae	7
1.4.1 Distribution and habitat.....	9
1.4.2 Description	9
1.4.3 Growth habit	10
1.4.4 Plant Physiology	10
1.4.5 Plant biochemistry.....	12
1.4.6 Pharmacological properties of the family	12

CHAPTER 2: PREPARATION OF THE PLANT MATERIAL

2.1 Extract Preparation of <i>Albiziarichardiana</i>	17
2.1.1 Collection and Preparation of the Plant material	17
2.1.2 Extraction of the Plant material	17
2.2 Design of Biological Investigations	19
2.3 Experimental Design	20

2.3.1 Investigation of chemical compounds by phytochemical screening.....	20
2.3.2 Brine Shrimp lethality Bioassay	20
2.3.3 Evaluation of hypoglycemic activity	20

**CHAPTER 3: INVESTIGATION OF CHEMICAL COMPOUNDS BY
PHYTOCHEMICAL SCREENING**

3.1 Introduction	21
3.2 Methods of the Experiment.....	21
3.2.1 Test for Carbohydrates	21
3.2.2 Test for Combined Reducing Sugar	22
3.2.3 Tests for Glycosides	22
3.2.4 Test for Glucosides	22
3.2.5 Tests for Saponins	23
3.2.6 Tests for Flavonoids	23
3.2.7 Test for Tannins	24
3.2.8 Test for Resins	24
3.2.9 Tests for Proteins	24
3.2.10 Test for Alkaloids	25
3.3 Results and Discussion.....	26

CHAPTER 4: BRINE SHRIMP LETHALITY BIOASSAY

4.1 Introduction.....	27
4.2 Materials.....	27
4.3 Experimental procedure	28
4.3.1 Preparation of seawater	28

4.3.2 Hatching of brine shrimps.....	28
4.3.3 Preparation of Test Solutions.....	29
4.3.4 Preparation of control group	30
4.3.4.1 Preparation of the positive control group	30
4.3.4.2 Preparation of the negative control group	30
4.3.5 Counting of nauplii	30
4.4 Results and Discussion	31
CHAPTER 5: EVALUATION OF HYPOGLYCEMIC ACTIVITY	
5.1 Introduction	35
5.2 Experimental Animals.....	36
5.3 Experimental Design.....	36
5.3.1 Preparation of test materials	37
5.3.2 Procedure	38
5.4 Results and Discussion	38
CHAPTER 6: CONCLUSION	
Conclusion	42
REFERENCE	43

LIST OF FIGURE

Figure 1.1: Different parts of <i>Albizia richardiana</i>	6
Figure 1.2: Different parts of <i>Albizia amara</i>	8
Figure 1.3: Different parts of <i>Albizia odoratissima</i>	8
Figure 1.4: Different parts of <i>Albizia julibrissin</i>	8
Figure 1.5: Different parts of <i>Albizia lebbbeck</i>	9
Figure 1.6: Different parts of <i>Albizia saman</i>	9
Figure 2.1: Schematic Diagram for the Preparation of Plant Material	18
Figure 4.1: Hatching of Brine Shrimps	28
Figure 4.2: Preparation of test solutions	29
Figure 4.3: Counting of nauplii	31
Figure 4.4: Activity of sample tested and determination of LC ₅₀	33
Figure 4.5: Activity of standard and determination of LC ₅₀	34
Figure 5.1: Swiss albino mice	36
Figure 5.2: Polypropylene cages	36
Figure 5.3: Oral administration	36
Figure 5.4: Numbering of mice	37
Figure 5.5: Schematic representation for determining blood glucose level of mice after administration of bark of <i>Albizia richardiana</i> extract	38
Figure 5.6: Pricking of mice's tail	38
Figure 5.7: Plasma level of glucose of different groups of mice at different times of bark extract of <i>Albizia richardiana</i>	41

LIST OF TABLE

Table 11: Pharmacological properties of the family	12
Table 3.1: Result of phytochemical screening	26
Table 4.1: Test samples with concentration values after serial dilution	29
Table 4.2: Effect of methanolic extract of <i>Albizia richardiana</i> on brine shrimp lethality test in Artemia salina	32
Table 4.3: Result of <i>Albizia richardiana</i> against on Artemia salina	33
Table 5.1: Test materials used in the evaluation of hypoglycemic activity of crude extract of bark of <i>Albizia richardiana</i>	39
Table 5.2: Plasma level of glucose (mmol/L) of mice at different time	39
Table 5.3: % reduction of plasma glucose level by test materials	40
Table 5.4: Hypoglycemic activity of crude extract of bark of <i>Albizia richardiana</i>	40
Table 5.5: Statistical evaluation of the data	41

LIST OF ACRONYMS

AD: After Death

BC: Before Christ

DNA: deoxyribonucleic acid

DMSO: dimethylsulphoxide

GTT: Glucose Tolerance Test

ICDDR'B: International Centre for Diarrheal Diseases and Research, Bangladesh

Kg: kilo gram

LC₅₀: lethal concentration 50

mmol: mili mole

Std.: Standard

USDA: United States Department of Agriculture

WHO: World Health Organization

Chapter 1

Introduction

Chapter 1

Introduction

Staying healthy, free from diseases has always been people's prime concern since the very beginning of their existence on this earth. In an effort to accomplish this concern, human being had utilized and also made attempts to use any materials they thought to be useful in their struggle of existence.

It is always observed that natural propensity of human being is to avoid illness, physical discomfort, injuries, wounds and obviously death. They tried many things and at the end they follow nature. They found out plants with pharmacological and medicinal properties. However, early men didn't know the actual uses or pharmacological properties of those plants; they selected those plants based on trials and errors. For relieving pain and suffering caused by abnormal conditions and also for preserving health against disease and death, people became familiar with several plants containing particular chemical compounds that can be used for treating particular illness or discomfort or diseases. Therefore, there has always been an effort to discover new chemical entity that can be used for treating particular disease. (Ghani.A. 2003)

However, it is seen from the ancient period, medicinal plants have been comprehensively used for medical purposes throughout all generation and all the prehistoric society had contributed significantly for enriching the knowledge of medicinal uses of plants by mentioning the remedies for diverse diseases whether it is simple wound and skin diseases or more complicated formidable heart diseases and cancer etc. (Ghani.A. 2003)

A short history of the uses of medicinal plants is given in the following part.

- ✓ In 3000 BC: It is observed from different studies that a large number of medicinal plants were used by the Babylonians. Of course they were aware of their properties. Nevertheless, early people like Assyrians, Babylonians, Egyptians and ancient Hebrew, were also recognized for knowing the properties of many medicinal plants. However, the use of some medicinal plants that were used by Babylon still exists today with same manner and purpose. (Ghani.A. 2003)

- ✓ In 4500-1600 BC: Huge information about the uses of medicinal plants in the Indian subcontinent during this period is mentioned in *Rig Veda*, the oldest book in library of man. For example, the use of Soma plant (*Amanita muscaria*) which is used as a medicinal agent as narcotic and hallucinogenic agent and Sarpagondha (*Rauwolfia serpentina*) which is used as healing plant are mentioned in this book. *Susruta Samhita* and *Charaka Samhita* are the two other oldest book from which information about the Indian medical systems and medicinal plants can be obtained. (Ghani.A. 2003)
- ✓ In 1500 BC: *Papyrus Ebers*, contains a good knowledge about the properties of hundreds of medicinal plants. It is also seen from different observations that today's most important plants, were also used in Egypt about 4500 years ago (Hill, 1972), such as henbane (*Hyoscyamus* spp.), mandrake (*Mandragora officinarum*), opium (latex of the mature but unripe fruits of *Papaversomniferum*), pomegranate (fruits of *Punicagranatum*), castor oil (oil of *Ricinus communis* seed), aloe (juice of leaves of *Aloe* spp.), onion (*Allium cepa*), hemlock (*Conium* spp.), cannabis (*Cannabis sativa*), senna (*Cassia senna*) and many others. However, since they had the vast knowledge about the properties of medicinal plants and human anatomy, they use plant parts and extracts also for the preservation of dead bodies as mummy, (Ghani.A. 2003)
- ✓ In 1200 BC: Ayurvedic system described the medicinal uses of about 127 plants (Obianwu, 1984).
- ✓ After 131 AD: Galen (131-200 AD), the great Greek pharmacist-physician, was the first to include the procedures and methods for preparing therapeutic recipes. He attempted to include ingredients of both plant and animal origins (Claus & Tyler, Jr., 1965). He wrote about 500 volumes of books, describing a large number of medicinal plants and animal products with hundreds of recipes and formulations. (Ghani.A. 2003)

But, before going to further discussion, one must be very much clear about the medicinal plants. Medicinal plants are plants with therapeutic properties and may use for beneficial pharmacological effects on animal body. These plants are not morphologically different from other plants but may have various special qualities which make them medicinally important than others. However, it is observed from different studies, plants that are naturally synthesized and contain secondary metabolites, more instance alkaloids, sterols, terpenes, flavonoids, saponins, glycosides, cyanogenics, tannins, resins, lactones, quinones, volatile oils, etc as well as minerals

and vitamins, may have medicinal properties. With this continuation, World Health Organization (WHO) consultative group had given a definition on medicinal plants in the following way:

“A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drugs.” (Sofowora, 1982).”

However, early man didn't have any special knowledge about medicinal plants. They discovered plants with medicinal properties not only from their intuition, guesswork or trial and error approach, but also from their curiosity and searching trend for food. Sequentially they gather knowledge about medicinal plants. However, now-a-days, with the advancement of technology, people are using modern techniques to discover new drugs. For this reason, they are working on different unknown plants for enlisting them to the list of medicinal plants.

In country like Bangladesh, there are about 6500 plant species which may include plants like bryophytes, pteridophytes, gymnosperms, and angiosperms. This large diverse plant features is available in the country due to its favorable agroclimatic circumstance with productive soils, a tropical climate, and seasonal diversity. However, among the large diversity, about 500 plant species contain medicinal values. These medicinal plants are normally found to grow as native, naturally occurring, or cultivated plants in the country's forests, wetlands, homestead forests, and also even roadside areas. However, in country like Bangladesh, around 75% (10 million households in over 85,000 villages) of the country's total population lives in rural areas and almost 80% of them are reliant on natural resources (e.g., medicinal plants) for their primary healthcare as herbal medication.

Herbal medication represents a well known and accepted form of treatment among the rural people in Bangladesh. This is may be due to the capability of those people to recognize many species of plants in their areas from which various products like food, firewood, medicine, forage, etc. can be obtained. Apart from this, the traditional homestead tree production system serves as a source of plant products and remedies in the country (Rahman M.H., 2013). But it is quite unfortunate for us that between the 25 000 and 75 000 plant species that are used as traditional medicine, only 1% are known by scientists and accepted for commercial purposes (Aguilar, 2001). Nevertheless, to meet the increasing demand, about 500 companies are established in the country, but still more are needed to fulfill the demand of herbal medicine. For

this purpose, more research are needed to be conducted so that we can compete with this never-ending require. (Ghani.A. 2003)

The work elucidated in this dissertation is an attempt to evaluate the possible biological profiles of the medicinal plant *Albizia richardiana* (Fam. Fabaceae, subfam. Mimosoideae). The genus of the plant is Albizia. The other plants of this genus have various significant medicinal properties for instance they are recognized to treat asthma, arthritis, antiseptic, burns, antidysentric, allergic rhinitis etc. But no significant amount of work has been done on the plant *Albizia richardiana* of this genus. So the main objective of our dissertation is:

- ✓ To find out and evaluate the phytochemical and biological profiles of the medicinal plant *Albizia richardian*, so that it can be given the recognition of medicinal plant.

1.1 The plant: *Albizia richardiana*

The plant under investigation, *Albizia richardiana* is locally known as Raj koroï and the synonym for the plant name is *Albizia niopoides*. The plant belongs to the family Fabaceae and the subfamily Mimosaceae. However, the plant is also known as *Albizia paludosa*. The plant is generally recognized as the ornamental plant in tropical areas of USDA Zones 10 and above. It is a fast growing plant, normally found in Bangladesh (Das and Alam, 2001). The species is usually found to grow in completely hot and humid environment like Asia, Africa, Madagascar, North America and Australia, but mostly in the old world tropics (Azad et al. 2010). In Bangladesh, this species is usually found to grow in the forest of Sunamgonj, in the forest of Chittagong Hill Tracts (Kaptai) and also in the village of northern districts. (Rahman. M. and Kumar Das.A.2014)

Albizia richardiana is a large, dry tree with contrastive characteristics like temporary and quick growing nature. It contains an open, spreading, beautiful, light green, very finely pinnate and crown like leaves and powder-puff like flowers. (Rahman. M. and Kumar Das.A.2014)

Considering the usage of this plant, this plant is known as an ornamental plant in general. However, it is an important component of the village and society of our country as being an important part of wood based industry. In Bangladesh, the timber of *Albizia richardiana* is

generally used for construction purposes. However, medicinal use of this plant has not been extensively studied. (Rahman. M. and Kumar Das.A.2014)

1.2 General botanic data

The plant is an anise. It is a fast growing tree with pseudo dichotomously branching habit and straight bole, which can form a beautiful crown. Structural features of the plant are given below:

Leaves: Leaves are bipinnated compound. Rachis are about 12 cm long, channeled above, usually with a cup-shaped gland on the petioles near the base and 1-3 small similar glands between the bases of distal pairs of pinnae, usually 8-14 pairs, 4.5-7.5 cm long with pulverous base, softly white tomentose along the upper sides, leaflets 60-100 pairs, 6 x 1 mm, linear-falcate, sessile, acute, entire, base unequal, lower side auricled, midrib closer to upper margin, glabrous, smooth and dark green leaflets in very close set. Inflorescence axillary or terminal panicles are shorter than the leaves, in short pedunculate, globose heads, and peduncles. Usually 1.0-1.4 cm long and head many flowered. The tree sheds leaves at the end of winter and leaves are replaced during early summer.

Flowers: Flowers are small, greenish white and sessile. Calyx is funnel-shaped, tomentose from outside, and in very minute amount. Corollas are campanulated with 5 lobes, oblong, lanceolated and acute. Stamens contain 25-30, filiform, exserted and small anthers. Ovaries are directly attached to the stem.

Fruit: Fruit contains a pod, 8-10 x 2.0-2.3 cm, and thin firm, flat and strap-shaped, shortly beaked, dull grayish brown.

Seeds: Seeds are 8-12 per pod, and 6-8 mm long, oblique towards the tip near attachment with the funicle. Funicle is yellow in color and as long as seed.

Flowering and fruiting time: The flowering and fruiting time period is between August-December of the year.



Figure 1.1: Different parts of *Albizia richardiana*

1.3 Taxonomic Hierarchy

Kingdom :	Plantae
Division:	Magnoliophyta
Subdivision:	Magnoliophytina
Class:	Rosopsida
Subclass:	Rosidae
Superorder:	Fabanae
Order:	Fabales
Family:	Fabaceae
Subfamily:	Mimosaceae
Tribe:	Ingae
Genus:	<i>Albizia</i>
Species:	<i>Albizia niopoides</i>

1.4 The plant family: Fabaceae

The name 'Fabaceae', which is now included in *Vicia*, comes from the extinct genus *faba*. The term "faba" is a Latin word and represents simply "bean". Leguminosae is an older validated name, comes from its fruits name which are called legumes.

The Fabaceae or Leguminosae family is also known as legume, pea, or bean family. This large family is usually known for economical importance because of flowering plants. However, the family is rich with trees, shrubs and herbaceous plants. The plants of this family are either annuals or perennials. They can be easily identified by their fruit (legume) and specified leaves. According to the number of species, the family is the third-largest land plant family and extensively distributed. This family contains about 630 genera and over 18,860 species. They are commonly found in the tropical rainforests as well as in the dry forests of Americas and Africa.

The history of this family is closely related to the human evolution, since along being used as cereals. Their some fruits and tropical roots in a few number of Leguminosae have been used as staple human food for millennia.

However, Fabaceae is a single monophyletic family, which is established by the molecular and morphological evidences. It is also supported by both the degree of interrelation of different groups within the family and phytoogenetic studies based on DNA sequences. These studies has also confirmed that Leguminosae is closely associated with Polygalaceae, Surianaceae and Quillajaceae families which are belonging to the order Fabales, a monophyletic group.

The family is rich with a number of significant agricultural and food plants, which include *Glycine max* (soybean), *Phaseolus* (bean), *Pisum sativum* (pea), *Cicer arietinum* (chickpeas), *Medicago sativa* (alfalfa), *Arachis hypogaea* (peanut), *Lathyrus odoratus* (sweetpea), *Ceratonia siliqua* (carbo) and *Glycyrrhiza glabra* (liquorice). A number of species are also found as weedy pests in different parts of the world, which includes *Cytisus scoparius* (broom), *Robinia pseudoacacia* (black locust), *Ulex europaeus* (gorse) and *Pueraria lobata* (kudzu).



Figure 1.2: Different parts of *Albizia amara*



Figure 1.3: Different parts of *Albizia odoratissima*



Figure 1.4: Different parts of *Albizia julibrissin*



Figure 1.5: Different parts of *Albizia lebbek*

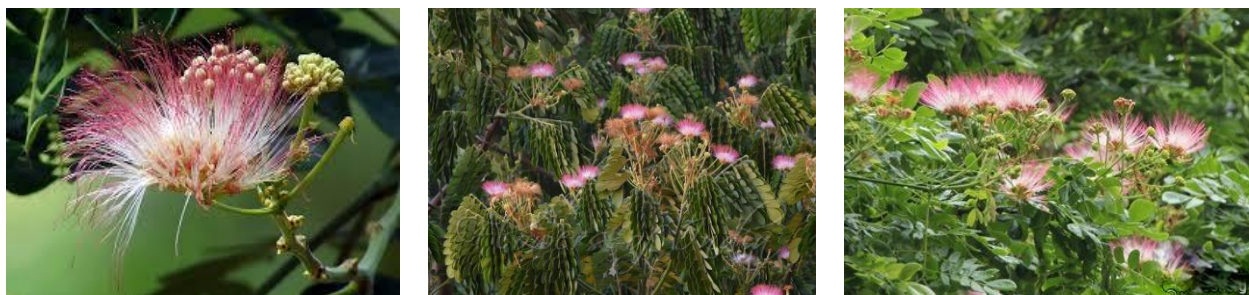


Figure 1.6: Different parts of *Albizia saman*

Some of the features of this family are given below:

1.4.1 Distribution and habitat:

The Fabaceae have been found almost everywhere except Antarctica and the high arctic. Whereas, the herbaceous plants and shrubs mainly predominant in extratropical areas, with main occurrence in tropical regions.

1.4.2 Description:

The plant of Fabaceae family range from enormous trees (like *Koompassia excelsa*) to minute annual herbs, with the greater part of the plants being perennials. Plants, which contain imprecise inflorescences, occasionally reduced to a single flower. The flowers, which have a short hypanthium and a solitary carpel with a short gynophore, produce fruits after fertilization called legumes.

1.4.3 Growth habit:

The Leguminosae have a wide diversity of growth forms which includes trees, shrubs or herbaceous plants and even vines or lianas. This type of plants can be annuals, biennials or perennials. The plants can be without basal or terminal leaf aggregations. They are vertical plants, epiphytes or vines. The latter support themselves by means of shoots which twist around a support or through cauline or foliar tendrils. However, these plants can be heliophytes, mesophytes or xerophytes.

1.4.4 Plant Physiology:

Leaves: The leaves are generally alternate and compound. Frequently they are even and odd pinnate (e.g. *Caragana* and *Robinia* respectively), often trifoliate (e.g. *Trifolium*, *Medicago*) and seldom palmately compound (e.g. *Lupinus*). But in the Mimosoideae and the Caesalpinioideae subfamilies, they are usually bipinnate (e.g. *Acacia*, *Mimosa*). They always have stipules, which can be leaf-like (e.g. *Pisum*), thorn-like (e.g. *Robinia*) or can be rather unremarkable. Leaf borders are entirely or, rarely serrate. Both the leaves and the leaflets generally have wrinkled pulvini to allow nastic movements. However, in a few species, leaflets have developed into tendrils (e.g. *Vicia*).

It is also observed that in various species, they have leaves with structures which magnetize the ants which may protect the plants from herbivore insects (a form of mutualism). It is also observed that extrafloral nectaries are familiar among the Mimosoideae and the Caesalpinioideae, and may also be found in several Faboideae (e.g. *Vicia sativa*). However, in a few *Acacia*, the customized hollow stipules are inhabited by ants, known as domatia.

Roots: Various Fabaceae host bacteria in their roots, called root nodules. These bacteria, known as rhizobia, contain the capability to take nitrogen gas (N_2) out of the air and convert it to a form of nitrogen, which is then further used by the host plant (NO_3^- or NH_3). The process is known as nitrogen fixation. In this way, the legume, acting as a host, and rhizobia, acting as a provider of usable nitrate, form a symbiotic relationship.

Flowers: The flowers of this family usually contain five fused sepals and five free petals. Generally they are hermaphrodite. The flowers contain a petite hypanthium, frequently cup shaped. There are also ten stamens and one extended superior ovary which occur in curved style. They are arranged in indeterminate inflorescences. Fabaceae are characteristically entomophilous plants (i.e. they are pollinated by insects), and their flowers are habitually showy to catch the attention of pollinators.

In the Caesalpinioideae, the flowers are repeatedly either zygomorphic, as in *Cercis*, or almost symmetrical with five identical petals as in *Bauhinia*. The upper petal is the deepest one, except in Faboideae. A few species, like some in genus *Senna*, include asymmetric flowers and this style twisted to one side. The calyx, corolla, or stamens can also be showy in this group.

In the Mimosoideae, flowers are actinomorphic and arranged in globose inflorescences. The showiest part of the flower is petals, which are minute and the stamens, which can be more than just 10, have long, coloured filaments. However, all the flowers open at once.

In the Faboideae, the flowers are zygomorphic, and also contain a specialized structure. The upper petal is large and encloses, called the banner and the remaining petals often reflexing in bud when the flower blooms. The two adjacent petals, called the wings, surround the two bottom petals. These two bottom petals are fused together at the apex (remaining free at the base) and forming a boat-like structure called the keel. The stamens are always ten in number, can be often in a group of nine stamens plus one separate stamen. The filaments of stamens can be fused in various configurations.

Fruit: Usually the ovary builds up into a legume. A simple dry fruit like legume habitually dehisces (opens along a seam) on two sides. They have a common name called "pod". This name can also be utilized to a few other fruit types. From the basic legume fruit a small number of species have developed for instance samarae, loments, follicles, indehiscent legumes, achenes, drupes, and berries.

1.4.5 Plant biochemistry:

Sometimes the Leguminosae are cyanogenic and their cyanogenocity is derived from tyrosine, phenylalanine or leucine, and frequent presence of alkaloids in them. Proanthocyanidins also be able to present either as cyaniding or delphinidine or both at the same time. As well as Flavonoids like kaempferol, quercitin and myricetin are often found to be present. In any of the genera or species, which are analyzed, Ellagic acid has never been found. In the form of sucrose, sugars are transported within the plants. C3 photosynthesis has also been found in a wide variety of genera. However, Pterocarpan (derivatives of isoflavonoids) are found only in the Fabaceae.

1.4.6 Pharmacological properties of the family:

Various plants like *Albizia lebeck*, *Albizia julibrissin*, *Albizia saman*, *Albizia inundata*, *Albizia odoratissima*, *Albizia mollis*, *Albizia procera*, *Albizia gummifera*, *Albizia amara*, *Albizia schimperiana* are included in this family. All those plants contain different kinds of pharmacological properties.

Table 1.2: Pharmacological properties of different plants of Albizia species

Name of the plant	Pharmacological properties	Literature review	Reference
<i>Albizia lebeck</i>	Used in traditional and folk medicine and also used for treating several inflammatory pathologies for example, asthma,	USDA forest service work on the physical properties of this plant.	JOHN A. PARROTTA. International Institute of Tropical Forestry, USDA Forest Service.

	<p>arthritis, antiseptic, burns, antidysentric, allergic rhinitis, learning of mice, bronchitis, leprosy, paralysis and helminth infections and anti-tuberculosis. The alcoholic extract of this plant has antihistaminic property, by neutralizing the histamine directly or by corticotrophic action as evidenced by raising cortisol levels in plasma.</p>	<p>Concentration of heavy materials present in this plant depends on the environment of the locality.</p>	<p>Muhammad Ishaq, Ali Rahman, Muhammad Adnan, Najeeb Ullah, Ijaz Ahmed, Muhammad aamir. Department of Chemistry, kohat University of Science & Technology (kust), kohaqt-26000, Pakistan.</p>
<p><i>Albizia julibrissin</i></p>	<p>Used to treat bruises, ulcer, abscessed, boils, hemorrhoids and fractures. However, they may show cytotoxic activity.</p>	<p>Few cases are reviewed and the result shows that this plant has an effective natural approach for the treatment of mild states of depression and anxiety.</p> <p>A research has done on physical properties of this plant.</p>	<p>Michael Tierra L.Ac., O.M.D., Founder of the American Herbalists Guild.</p> <p>Miller, J.H. 2003. In: Nonnative invasive plants of southern forests: a field guide for identification and control. Asheville, NC: U.S.Department of Agriculture, Forest Service,Southern Research Station: 93p.</p> <p>SE-EPPC. 2003. Southeast Exotic Pest</p>

			Plant Council Invasive Plant Manual.
<i>Albizia saman</i>	Show good anti-plasmodial activity as well as anti-candida.	A review of pharmacological properties of this plant.	Karuppannan Kokila, Subramanian Deepika Priyadharshini and Venugopal Sujatha. Department of Chemistry, Periyar University, Salem, Tamil Nadu, India.
<i>Albizia inundata</i>	Show good anti-plasmodial activity as well as anti-candida.	A review of pharmacological properties of this plant.	Karuppannan Kokila, Subramanian Deepika Priyadharshini and Venugopal Sujatha. Department of Chemistry, Periyar University, Salem, Tamil Nadu, India.
<i>Albizia odoratissima</i>	Used to treat leprosy, ulcers and cough.	A review of pharmacological properties of this plant.	Karuppannan Kokila, Subramanian Deepika Priyadharshini and Venugopal Sujatha Department of Chemistry, Periyar University, Salem, Tamil Nadu, India.

<i>Albizia mollis</i>	Contains sedative and sleeping pill properties.	A review of pharmacological properties of this plant.	Karuppannan Kokila, Subramanian Deepika Priyadharshini and Venugopal Sujatha Department of Chemistry, Periyar University, Salem, Tamil Nadu, India.
<i>Albizia procera</i>	Wildly used to treat variety of wounds and considered to be used in the pregnancy and stomachache.	A review of pharmacological properties of this plant.	Karuppannan Kokila, Subramanian Deepika Priyadharshini and Venugopal Sujatha Department of Chemistry, Periyar University, Salem, Tamil Nadu, India
<i>Albizia gummifera</i>	Very important for its anti-trypanosomal activity. The plant is used in the indigenous medical system for a variety of ailments, bacterial infections, skin diseases, malaria and stomach pains. Lipophilic extracts of this plant revealed very promising anti-trypanosomal activity.	A review of pharmacological properties of this plant.	Karuppannan Kokila, Subramanian Deepika Priyadharshini and Venugopal Sujatha Department of Chemistry, Periyar University, Salem, Tamil Nadu, India.

<i>Albizia amara</i>	Seeds of this plant are used as an astringent, treating piles, diarrhea, gonorrhoea, leprosy, leucoderma, erysipelas and abscesses. The leaves and flowers have been used to treat boils, eruptions, and swellings, and also used for the remedy of coughs, ulcer, dandruff and malaria. The plant also contains emetic properties.	A review of pharmacological properties of this plant.	Karuppannan Kokila, Subramanian Deepika Priyadharshini and Venugopal Sujatha Department of Chemistry, Periyar University, Salem, Tamil Nadu, India
<i>Albizia schimperiana</i>	The plant is used as traditional medicine and normally used to treat bacterial and parasitic infections, particularly pneumonia and malaria, respectively.	A review of pharmacological properties of this plant.	Karuppannan Kokila, Subramanian Deepika Priyadharshini and Venugopal Sujatha Department of Chemistry, Periyar University, Salem, Tamil Nadu, India University, Salem, Tamil Nadu, India.

The plants of this family contain a various kind of important pharmacological properties. Our experimental plant *Albizia richardiana* is unknown as not much work has been done even now for this plant. So we are working on this plant with the hope to get some new constituents.

Chapter 2

Preparation of the Plant Material

Chapter 2

Preparation of the Plant Material

In the present study, plant *Albizia richardiana*, belonging to the Fabaceae family, was investigated.

Name of plant	Family	Plant part
<i>Albizia richardiana</i>	Fabaceae	Bark

2.1 Extract preparation of *Albizia richardiana*

2.1.1 Collection and Preparation of the Plant material

Bark of *Albizia richardiana* was collected from the Chittagong hill area, Bangladesh, as plant sample. The sample was then identified by an expert taxonomist at S.K.H.D.U (Acc. No.10778). Then it was cut into small pieces for better drying as we know evaporation increases with the incensement of the surface area. After that it was sun dried for several days which is then followed by oven drying. The dried bark was then crushed into coarse powder by a high capacity grinding machine, which was well cleaned before grinding to avoid cross contamination.

2.1.2 Extraction of the Plant material

Two amber containers (2.5) liters were used for soaking 500 g powdered plant materials (bark) in 1.2 liter of methanol as solvent. These two containers together with its contents were sealed by cotton plug and aluminum foil. Then these two containers were reserved like this in a dry place for about a period of two weeks with occasional shaking and stirring. After two weeks whole mixture was filtered, at first through cotton and after that through Whatman No. 1 filter paper. From this filtration, about 525ml filtrate was obtained. The filtrate volume was then reduced using a Buchii Rotavapour at low temperature and pressure, after which, the final volume of the filtrate was 100 ml. Then the filtrate was kept in a beaker and sealed with aluminum foil with

several pores on it. After that the filtrate was kept for drying to become crude dry extract. The weight of final extract was 12 g.

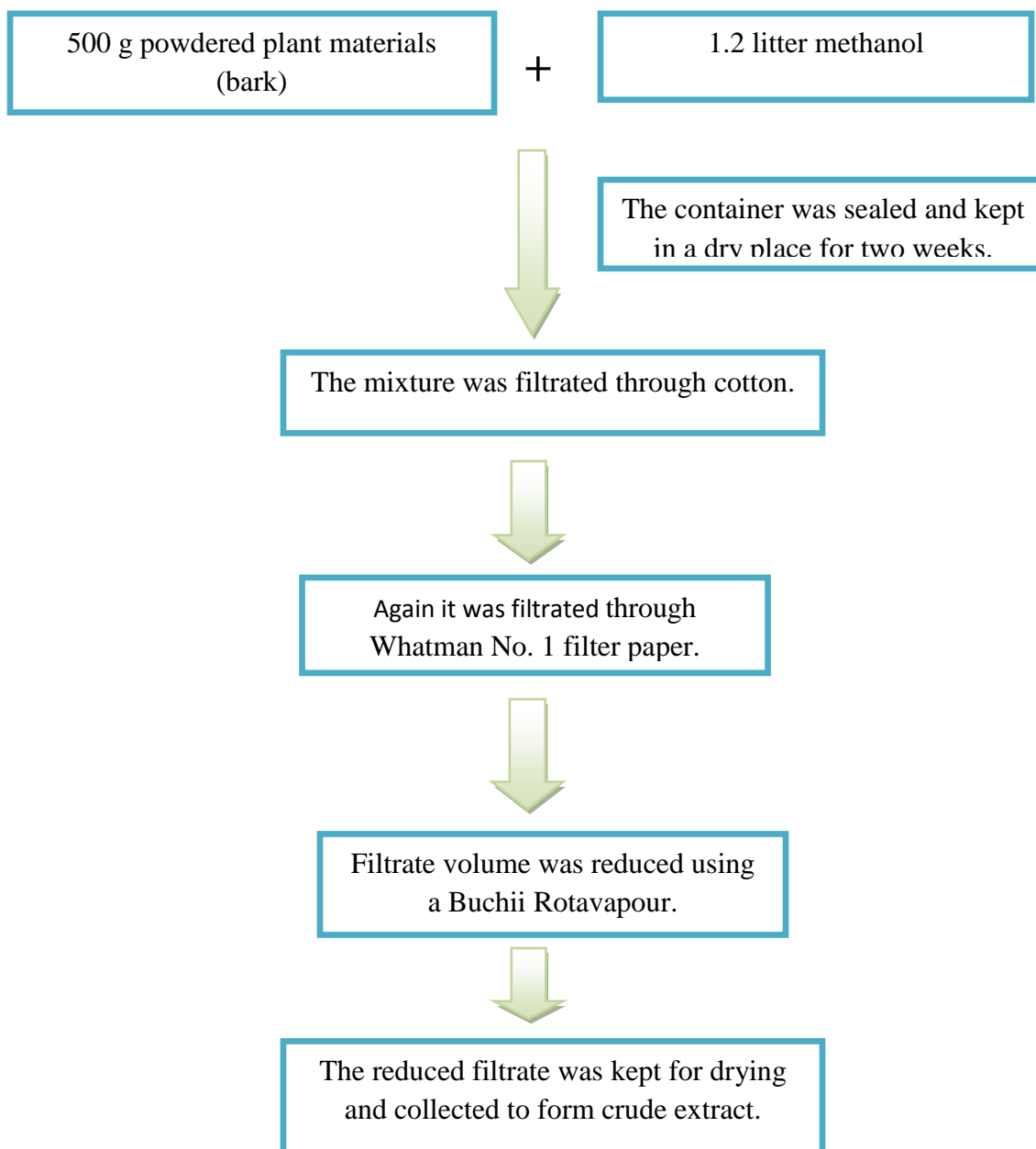


Figure 2.1: Schematic Diagram for the Preparation of Plant Material

2.2 Design of Biological Investigations

One of the successful sources of drugs is Natural products. Based on “rational” drug design, many new chemotherapeutic agents are synthetically derived. The natural products have advantage above synthetic or semi-synthetic drug and that leads optimally to materials containing new structural features with significant biological activities. The drugs which are used now-a-days come from natural sources. Practically every pharmacological group of drugs may include a natural product prototype. As sources of plants for the use of analysis, preclusion, and treatment of diseases is very promising (Setzer W.N., 2000).

Natural products are mainly metabolites, which can be derived naturally and/or can be the by-products from microorganisms, plants, or animals. Structural diversity is the major advantages of natural products for random screening. It is achievable to isolate a number of homologues and obtain structure- activity relationship, wherever bioactive natural products take place as a part of a family of related molecules. Lead compounds can be optimized either by traditional medicinal chemistry or by application of combinatorial approaches which are found from screening of natural products (Wikipedia 2007). Overall, natural products library, seems more likely to make available the chemical diversity to yield a hit than a library or similar number of compounds made by combinatorial synthesis, is used in case of screening assays where is present no information about low molecular weight leads. Since, for biological activities only a small portion of the worlds’ biodiversity has been examined and it can be assumed that natural products will continue to offer novel leads for novel therapeutic agents.

2.3 Experimental Design

2.3.1 Investigation of chemical compounds by phytochemical screening

The methanolic bark extract of *Albizia richardiana* was subjected to analysis chemical screening for the identification of the presence of bioactive substances like, carbohydrate, tannin, glycosides, alkaloids, and saponins etc. by using standard procedures.

2.3.2 Brine Shrimp lethality Bioassay

For the screening of pharmacological activities in plant extracts, Brine shrimp lethality bioassay (Mayer *et al.*, 1986) has been suggested. Rapid, inexpensive and simplicity (eg.no aseptic techniques are required) are the advantages of this method. For statistical validation, this method easily utilizes a large number of organisms and requires no special equipment and a relatively small amount of sample. Additionally, it does not require animal serum.

2.3.3 Evaluation of hypoglycemic activity

For the evaluation of hypoglycemic activity, the most acceptable method is glucose tolerance test (GTT). In this medical test, glucose is given and blood samples are taken subsequently to find out how quickly the glucose is cleared from blood. The test is usually used for the purpose of determining diabetes, insulin resistance, and occasionally reactive hypoglycemia or rare disorder of carbohydrate metabolism. Many variation and development of the GTT have been done over the years for various purposes, with different standard doses of glucose, different routes of administration, different intervals and durations of sampling with the measurement of various substances in addition to blood glucose.

Chapter 3

Investigation of Chemical Compounds by Phytochemical Screening

Chapter 3

Investigation of Chemical Compounds by Phytochemical Screening

3.1 Introduction

For the preliminary analysis and identification of nature and quantity of bioactive chemical substances present in a given plant material, phytochemical screening can act as an important and essential means of analysis.

For the present investigation, the methanolic bark extract of *Albizia richardiana* was subjected for phytochemical analysis to identify the presence of bioactive substances like, carbohydrate, tannin, glycosides, alkaloids, and saponins etc. by using standard procedures.

3.2 Methods of the experiment

A large variety of qualitative tests are available for the phytochemical screening of medicinal and other plants. There are some common standard tests described here which are simple, rapid and available for such phytochemical screening.

3.2.1 Test for Carbohydrates

- ✓ **Molisch's test:** At first, a test tube was taken and 2 mL of plant's aqueous extract was poured into it, then 2 drops of freshly prepared 10% of alcoholic solution of alpha naphthol was added in the test tube and mixed thoroughly. After that, 2 mL of conc. sulphuric acid was allowed to flow down the side of the inclined test tube so that the acid forms a layer beneath the aqueous solution. If carbohydrate is present, a red or reddish violet ring is formed at the junction of the two layers, which is followed by formation of a dark purple solution on standing or shaking. Then, it was shaken followed by standing for about two minutes. After that, the solution was diluted with 5 mL of water. A dull violet precipitate formed immediately will indicate the presence of carbohydrate in the plant material.

- ✓ **Fehling's test:** For doing the Fehling's test, 1 mL of a solution, which was the mixture of Fehling's solutions A and B (equal in volume), was added to the 2 mL volume of the methanolic extract of the plant material. After that the mixture was boiled for a few minutes. Formation of a red or brick-red precipitate will indicate the presence of reducing sugar in plant extract.

3.2.2 Test for Combined Reducing Sugar

About 1 mL of plant's aqueous extract and 2 mL of dilute hydrochloric acid were boiled together for 5 minutes. Then the mixture was cooled followed by neutralization by using sodium hydroxide solution. At the end, Fehling's test was carried out. A combined reducing sugar is present (due to the release of the reducing sugars on hydrolysis), if a brick-red precipitate is formed.

3.2.3 Tests for Glycosides

For doing this test, a small quantity of an alcoholic extract of the fresh and dried plant material was dissolved in about 1 mL of water. After that a few drops of aqueous sodium hydroxide solution were added to it. If the plant material contains glycosides, a yellow color may develop.

3.2.4 Test for Glucosides

A small quantity of plant's alcoholic extract was allowed to dissolve using both water and alcohol. After that, the solution was divided into two portions and was treated in the following ways:

- ✓ First portion was boiled up with solution containing equal volumes of Fehling's solutions A and B and note was taken whether any brick-red precipitate was formed or not.
- ✓ Other portion was boiled for about 5 minutes with a few drops of dilute sulphuric acid. After that, the mixture was neutralized by using sodium hydroxide solution. Then a

solution containing equal quantity of Fehling's solutions A and B, was added to it followed by boiling the mixture again.

The second experiment was carried out with the hydrolysed extract. Formation of a brick-red precipitate and formation of no such precipitate in first experiment may suggest the presence of glucosides in the extract.

3.2.5 Tests for Saponins

- ✓ About 0.1 g of the powdered plant material was allowed to be dissolved in about 10 mL of water. Then the mixture was allowed to be boiled for about 3-5 minutes. Then the mixture was filtered. After cooling, about 5 mL of the filtrate was diluted with water and then shaken vigorously. Formations of persistent frothing, which remain stable on heating, indicate the presence of saponins.
- ✓ Alternatively, this test can be performed by shaking 0.5 g of plant's alcoholic extract with water. A similar result can be obtained.

3.2.6 Tests for Flavonoids

- ✓ A small quantity of plant's alcoholic extract was taken and a few drops of conc. hydrochloric acid were added into it. Formation of red color immediately after the addition may suggest the presence of flavonoids in the plant material.
- ✓ A test tube was taken and about 0.5 mL of an alcoholic extract of the sample was poured into it. Then about 5-10 drops of conc. hydrochloric acid with a small piece of magnesium or zinc ribbon was added to it. Then the mixture was boiled for about few minutes. The result will be based on the development of the following color change like-orange to red (flavanes), red to crimson (flavanols), crimson to magenta (flavanones) and occasionally crimson to green or blue color (flavonoids).

3.2.7 Test for Tannins

- ✓ Ferric chloride test: About 5-10 mL of distilled water and 0.5 g of either an alcoholic or aqueous extract of the plant material were taken. Then the extract was dissolved in water after which it was filtrated. After that, a few drops of 5% ferric chloride solution was added to the filtrate. Formation of blue, blue-black, green or blue-green colors precipitate may indicate the presence of tannins. On the addition of a few mL of dilute sulphuric acid, this color may disappear and may form a yellowish brown precipitate.

3.2.8 Test for Resins

A small quantity of chloroformic or ethanolic extract was dissolved in about 5 to 10 mL of acetic anhydride by using gentle heat. After that, the solution was cooled and then about 0.05 mL of sulphuric acid was added to it. A bright purplish red color, rapidly changing to violet, may form if resin is present in the plant extract.

3.2.9 Tests for Proteins

- ✓ Millon's test: About 5-6 drops of Millon's reagent was added to a solution containing a small quantity of methanolic extract of the plant material which dissolved in about 1 mL of distilled water. On heating, if a white precipitate turns into red colour, it may suggest the presence of protein in the sample.
- ✓ Biuret's test: About 5-8 drops of 10% sodium hydroxide solution and 1-2 drops of 3% copper sulphate solution were added to 1 mL of a hot aqueous extract of the plant material. A red or violet color may produce if protein is present.

3.2.10 Test for Alkaloids

Alkaloid extract does not always give satisfactory results while using organic solvents or even aqueous organic solvents directly, though alkaloids occur in plants both as free bases and as their salts. In this case, extraction of the alkaloidal constituents can be affected by using either acidified dilute alcohol or pretreatment of the plant material with dilute ammonia solution or calcium hydroxide with sufficient water. This is performed to moisten the powdered plant

material prior to extraction with organic solvents. Extraction of the material can be completed by either maceration or percolation or by the use of Soxhlet extractor.

- ✓ **General laboratory test:** About 0.5 g of the extract was added to 5 mL of 1% hydrochloric acid and then the mixture was put on a steam bath, after which it was filtered. Then about 1 mL of the filtrate was treated with a few drops of each of the following reagents, separately. Turbidity or formation of the respective colored precipitates may indicate the presence of alkaloids in the extract:
- ✓ **Mayer's reagent** (potassio-mercuric iodide solution): In presence of alkaloids, this reagent will produce white or creamy white precipitate.
- ✓ **Hager's reagent** (1% solution of picric acid): In presence of alkaloids this reagent will produce yellow crystalline precipitate.
- ✓ **Wagner's reagent** (saturated solution of I₂ in KI): In presence of alkaloids this reagent will produce brown or deep brown precipitate.
- ✓ **Dragendorff's reagent** (bismuth potassium iodide solution): In presence of alkaloids this reagent will produce orange or orange-red precipitate.
- ✓ **Tannic acid solution (10%)**: In presence of alkaloids this reagent will produce dirty white or black precipitate.

3.3 Results and discussion

Phytochemical screening test of the methanolic plant extract of *Albizia richardiana* suggests the presence of bioactive substances like- carbohydrates, saponin, glycosides, glucosides, alkaloids in the bark extract of plant. Results of phytochemical screening of *Albizia richardiana* is given in the following table-1.

Table-3.1: Result of phytochemical screening

Test	MEB
Carbohydrates	+
Saponins	+
Glucosides	+
Glycosides	+
Flavonoids	-
Steroids	-
Tannins	-
Resins	-
Alkaloids	+
Proteins	-

(+) = present, (-) = absent, MEB= Methanolic extract of bark

Chapter 4

Brine shrimp lethality bioassay

Chapter 4

Brine shrimp lethality bioassay

4.1 Introduction

For the evaluation of cytotoxicity, the bioactive compounds of natural and synthetic origins are assayed by Brine Shrimp Lethality bioassay. This is a rapid and comprehensive bioassay method. In the discovery of new bioactive natural products, the method utilizes in vivo lethality in a simple zoological organism (Brine nauplii) which is a convenient monitor for screening cytotoxic activity. Brine toxicity is closely associated to 9KB (human nasopharyngeal carcinoma) cytotoxicity ($p=0.036$ and $\kappa = 0.56$). However, the assay contains some advantages for being rapid (24 hours), inexpensive, and simple (e.g., no aseptic technique are required). For the purpose of statistical validation, a large number of organisms can also easily be utilized by this method. Apart from this the method requires relatively small quantity of sample (2-20 mg or less) with no special equipment. Additionally, no animal serum is needed for cytotoxic activity evaluation.

4.2 Materials

1. *Artemia salina* leach (brine shrimp eggs)
2. Sea salt (NaCl)
3. Small tank with perforated dividing dam to hatch the shrimp
4. Lamp to attract shrimps
5. Micropipette (100-1000 μ l)
6. Glass vials
7. Magnifying glass
8. Test samples of experimental plants

4.3 Experimental Procedure

In brine shrimp bioassay method, brine shrimp eggs were hatched in simulated sea water. Then different calculated amount of methanolic extract of bark of *Albizia richardiana* were dissolved in dimethylsulphoxide (DMSO) solvent and preferred concentration of the test sample were prepared. Then, the nauplii were counted by visual inspection after which the nauplii were transferred in vials containing 5 mL of simulated sea water. By using micropipette, samples of different concentrations were added to the premarked vials. The vials were then left for 24 hours and maintained in required conditions. Counting of the Survivors took place after 24 hours.

4.3.1 Preparation of seawater

For hatching of brine shrimp, simulated seawater was needed. For that purpose, 38g of sea salt (pure NaCl) was dissolved in 1 liter of distilled water. At first, 38g of sea salt (pure NaCl) was weighed by rough balance and dissolved in one liter of distilled water, in a small tank and after which it was filtered to obtain a clear solution.

4.3.2 Hatching of brine shrimps

Artemia saline leacheds (Brine Shrimp eggs) were collected from nearby pet shops was used as test organism. The quantity of simulated seawater was prepared according to the size of the tank, as the simulated seawater was poured down in a small tank. For this test, 2 liter of simulated seawater were prepared and then Shrimp eggs were added to the one side of the perforated divided tank and after which the side was covered.



Figure 4.1: Hatching of Brine Shrimps

The tank was kept under constant aeration for 48 hrs for hatching the shrimp and to be matured as nauplii (larvae). Then 10 living Shrimps were added to each of the test tubes, which containing 5 ml of Brine solution. All through the hatching time constant oxygen supply had to carry out, by using pump. The hatched shrimps were attracted to the lamp through the perforated dam and they were taken for experiment. 10 living shrimps were added to each of the test tubes, which containing 5 mL of seawater. This work is done by getting the help from a Pasteur pipette.

4.3.3 Preparation of Test Solutions

About 5 mg of the plant's methanolic extract was taken and then dissolved in DMSO. The concentration was adjusted to 320µg/ml by adding 3.9 ml DMSO and considering as the parent solution. A sequence of decreasing concentrated solutions was prepared by sequential dilution with std. DMSO. Then 50µl from each of these test solutions were withdrawn and added to pre-marked glass vials/test tubes containing 4.5ml of simulated seawater and 10 shrimp nauplii. So, the final concentrations were 320µg/ml, 160µg/ml, 80µg/ml, 40µg/ml, 20µg/ml, 10µg/ml, 5µg/ml, 2.5µg/ml, and 1.25µg/ml.

Table 4.1: Test samples with concentration values after serial dilution

Test Tube No.	Concentration (µg/mL)
1	320.0
2	160 .0
3	80 .0
4	40 .0
5	20 .0
6	10.0
7	5.0
8	2.5
9	1.25

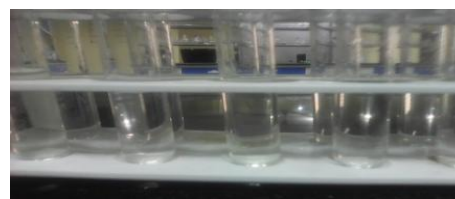
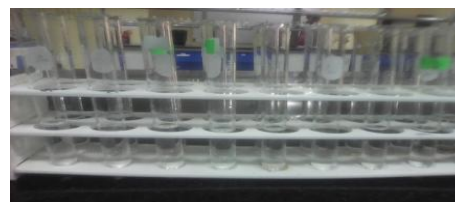


Figure 4.2: preparation of test solutions

4.3.4 Preparation of control group

In cytotoxicity study, test methods should be validated by using control groups. This ensures that only the tested agents participated and effects of the other potential factors are abolished. Two types of control groups are normally used,

i) Positive control

ii) Negative control

4.3.4.1 Preparation of the positive control group

In cytotoxicity study, positive control group gets much importance. It is not only accepted as a cytotoxic agent as the tested result is also evaluated with the result obtains from the positive control group. In this cytotoxicity study, vincristine sulphate was used as the positive control group. Precise quantity of the vincristine sulphate was dissolved in std. DMSO for getting an initial concentration of 20µg/mL. Serial dilutions are made using std. DMSO for getting the concentrations 10 µg/mL, 5µg/mL, 2.5µg/mL, 1.25µg/mL, 0.625µg/mL, 0.3125µg/mL, 0.15625µg/mL, 0.078125µg/mL, and 0.0390µg/mL. After that positive control groups were obtained by adding the positive control solutions to the premarked vials containing ten living brine shrimp nauplii in 5 mL simulated sea water.

4.3.4.2 Preparation of the negative control group

In the preparation of negative control group, 100µL of DMSO was added to each of three premarked glass vials/test tubes, which contain 5 mL of simulated sea water and 10 shrimp nauplii. The rate of mortality of brine shrimps in these vials/test tubes should not be very rapid. If it is, then the test will be considered as invalid as the nauplii may died due to some other reasons but not only for the cytotoxicity of the compounds.

4.3.5 Counting of nauplii

The vials/test tubes were inspected and the numbers of survivors were counted subsequent to 24 hours, by using a magnifying glass. The percent (%) of mortality was calculated for each dilution, individually. Linear regression was used by using a simple IBM-PC program to analyze statistically the concentration-mortality data. The median lethal concentration (LC₅₀) value indicates the either effectiveness or the concentration-mortality relationship of plant product.

After a certain exposure period, the concentration of the chemical which produces death in half of the test subjects is represented by this value.



Figure 4.3: counting of nauplii

4.4 Results and discussion of Brine Shrimp Lethality Bioassay

The degree of lethality was directly proportional to the concentration of the extract, ranging from the lowest concentration (1.25 $\mu\text{g/ml}$) to the highest concentration (320 $\mu\text{g/ml}$) (Table 4.2). The methanolic extract of the plant material had LC_{50} values of 22.03 $\mu\text{g/ml}$ while the LC_{50} of the reference anticancer drug, vincristine sulphate was 0.52 $\mu\text{g/ml}$ (Table 3). With increasing concentration of the sample the rate of mortality of the nauplii had increased (Figure-4.4 and Figure-4.5).

Table: 4.2- Effect of methanolic extract of *Albizia richardiana* on brine shrimp lethality test in *Artemia salina*

Sample conc. (µg/ml)	log conc.	No of nau. taken	No of nauplii dead			% of mortality	Vincristine sulphate				
			Exp. no 1	Exp. no 2	Avg. no		Std. conc. (µg/ml)	Log conc.	No of nau. taken	No of nau. dead	% of mortality
1.25	0.09	10	2	5	3.5	35	0.156	-0.806	10	3	30
2.50	0.39	10	5	3	4	40	0.312	-0.505	10	4	40
5.00	0.69	10	3	5	4	40	0.625	-0.204	10	5	50
10.00	1	10	5	5	5	50	1.25	0.096	10	6	60
20.00	1.30	10	7	3	5	50	2.5	0.397	10	8	80
40.00	1.60	10	7	3	5	50	5	0.698	10	9	90
80.00	1.90	10	5	5	5	50	10	1	10	10	100
160.00	2.20	10	5	7	6	60	20	1.310	10	10	100
320.00	2.50	10	9	5	7	70	40	1.602	10	10	100

Table: 4.3- Result of *Albizia richardiana* against on *Artemia salina*

Sample	LC ₅₀ (µg/ml)	Regression equation	R ²
Vincristine sulphate	0.52	Y = 32.61x+59.22	0.942
MEB	22.03	Y= 12.16x + 33.67	0.874

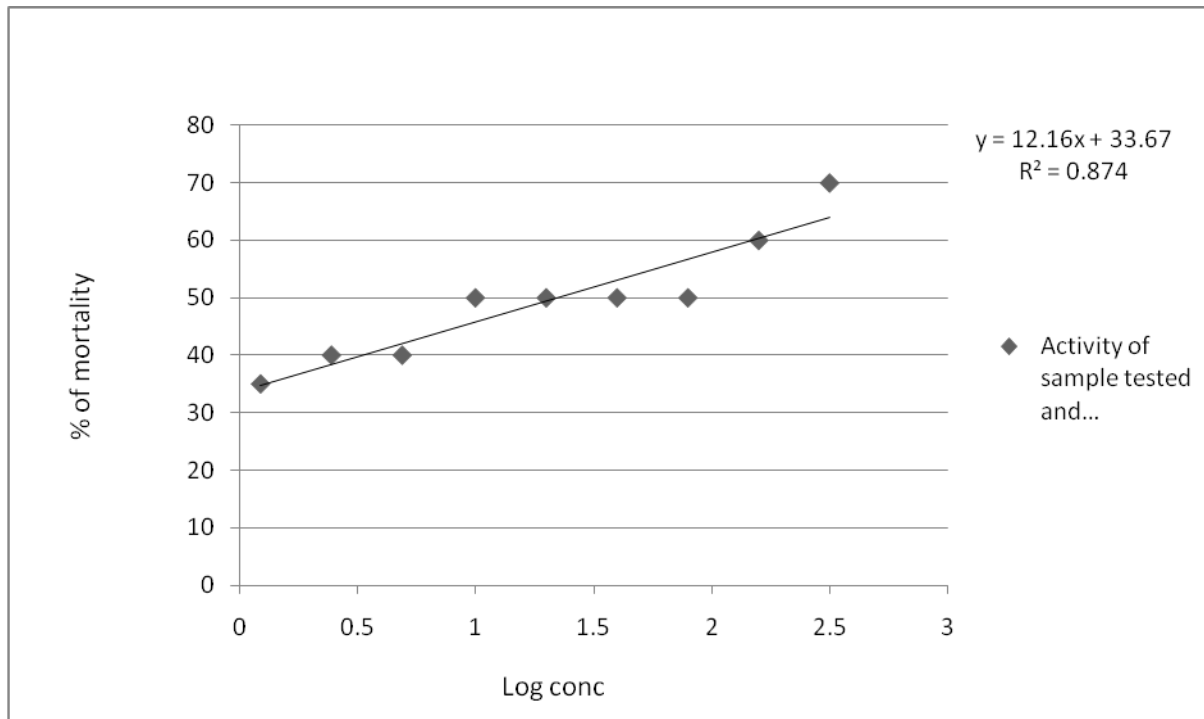


Fig 4.4: Activity of sample tested and determination of LC₅₀

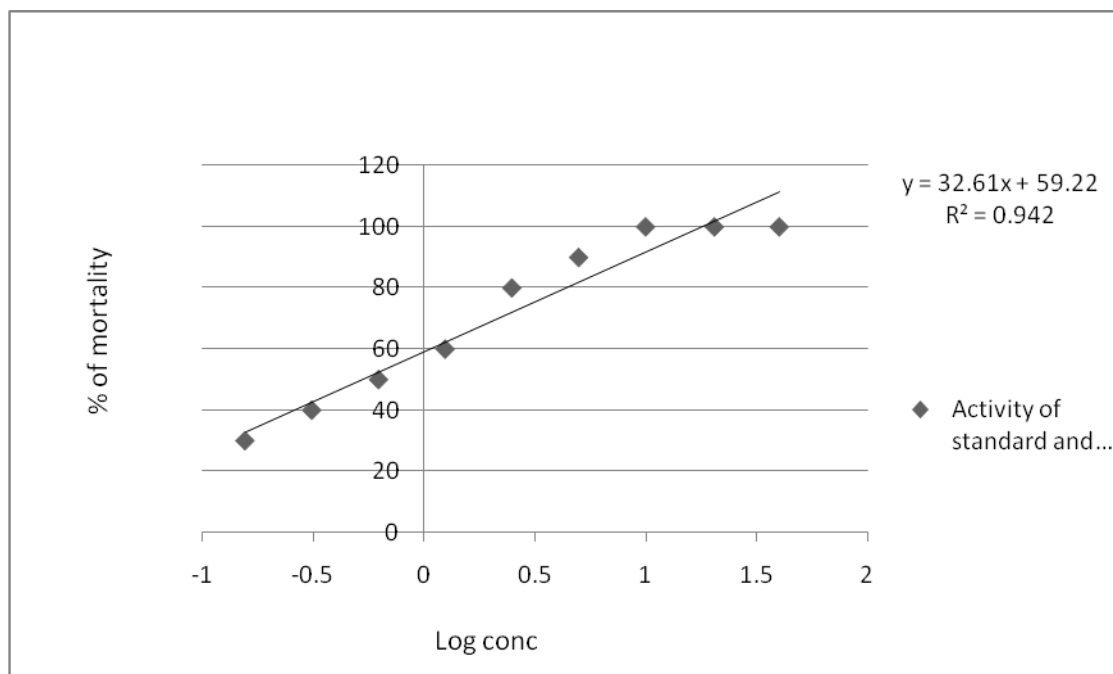


Fig 4.5: Activity of standard and determination of LC₅₀

Comparing the test result of the methanolic bark extract of *Albizia Richardiana* with standard, it may be suggested that the plant has no significant cytotoxic activity.

Chapter 5

Evaluation of Hypoglycemic Activity

Chapter 5

Evaluation of Hypoglycemic Activity

5.1 Introduction

Diabetes mellitus (Madhumeha) is a major metabolic disorder, affecting nearly 10% of the population and the rate of increasing of this percentage is also very rapid. This disease is also known as hereditary endocrine disorder. The classical symptoms of diabetes mellitus can be weight loss, polyurea, polydypsia and also polyphagia. They may also include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Though the herbal drugs are very compatible and easily metabolized in human systems with fewer side effects, controlling of diabetes without any side-effect is still a challenge to the medical system. For this purpose the demand of natural products which has anti diabetic activity with less side effects, has increased.

For the evaluation of hypoglycemic activity, the most acceptable method is glucose tolerance test (GTT). In this medical test, glucose is given and blood samples are taken subsequently to determine how quickly the glucose is cleared from blood. The test is usually used for the purpose of determining diabetes mellitus, insulin resistance, and occasionally reactive hypoglycemia or any rare disorders of carbohydrate metabolism. Many variations and development of the GTT have been done over the years for various purposes, with different standard doses of glucose, different routes of administration, different intervals and durations of sampling with the measurement of various substances in addition to blood glucose.

In this study, hypoglycemic effect of methanolic extract of bark of *Albizia richardiana* at 200 mg/kg and 400 mg/kg doses were examined and evaluated with related to that of control and standard group. In this test, the standard drug was Glibenclamide.

5.2 Experimental animals

For this experiment, Swiss-albino mice of either sex, aged 4-5 weeks, were used which were obtained from the Animal Resource Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR'B). Some necessary works had to be done, for instance- mice were housed in standard polypropylene cages, the room temperature was $24\pm 2^{\circ}\text{C}$, relative humidity 60-70%, in a 12hr light-dark cycle and their food was rodent food and their water was formulated by ICDDR'B. These animals were kept in the environment where the experiment took place before the test for at least 3-4 days, as they are very sensitive to environment changes.



Figure 5.1: Swiss albino mice



Figure 5.2: Polypropylene cages



Figure 5.3: Oral administration

5.3 Experimental design

For this experiment, twelve experimental animals were randomly selected and after that divided into four groups indicated as group-I, group-II, group-III and group IV. Each group contained 3 mice and received a particular treatment. The doses of the test samples and control materials were adjusted accordingly and each mouse was weighted properly before giving any treatment. Due to difficulty in observing the biological responses of three mice at a time getting same treatment, individual animal was identified of a group throughout the treatment. The animals can be individualized in the following way (figure 5.4) and marked as M1= Mice 1, M2= Mice 2 and M3= Mice 3.

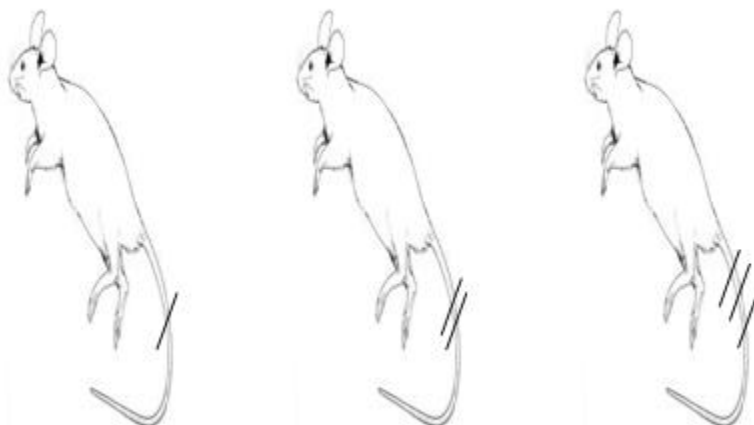


Figure 5.4: Numbering of mice

5.3.1 Preparation of test materials

In order to prepare the extract at doses of 400 mg/kg body wt and 200 mg/kg body wt of mice, at first the exact weight of the extracts were measured respectively. Then small amount of Tween-80 (a suspending agent) was added and triturated in unidirectional way. Normal saline was slowly added after proper mixing of extract and suspending agent. The final volume of the suspension required to make 3.0 ml suspension's volume was adjusted and stirred well by vortex mixture for the purpose of stabilizing the suspension.

In case of std. Glibenclamide, 10 mg tablet was dissolved into 3.0 ml normal saline (0.9%NaCl) for the dose of 10 mg/kg body weight.

5.3.2 Procedure

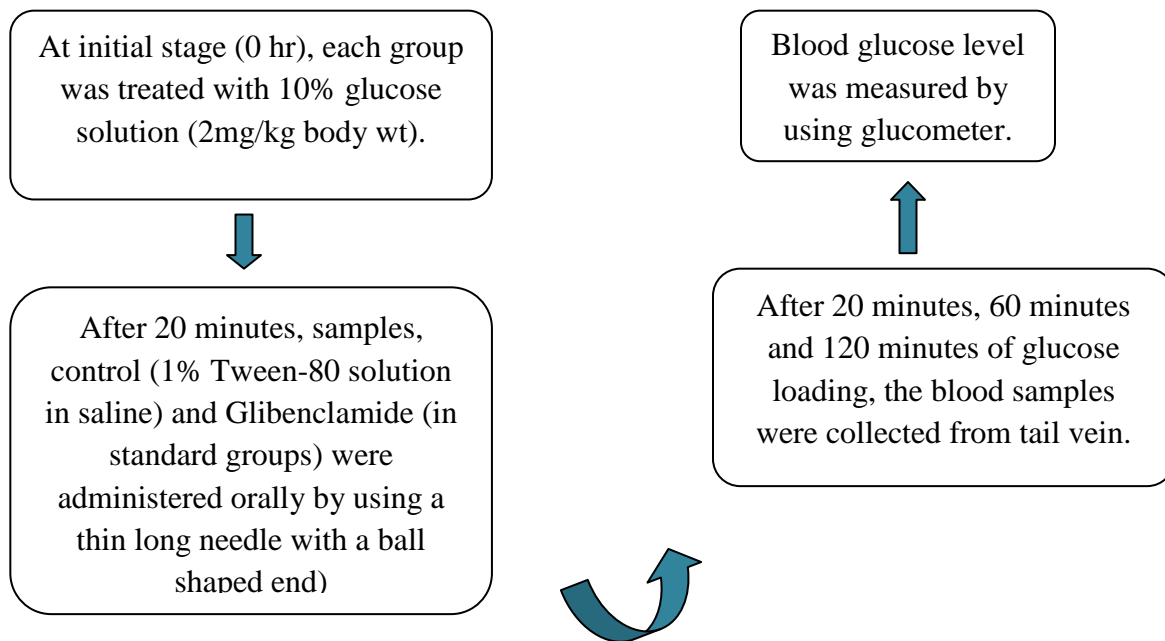


Figure 5.5: Schematic representation for determining blood glucose level of mice after administration of bark of *Albizia richardiana* extract



Figure 5.6: pricking of mice's tail

5.4 Results and Discussion

For the evaluation of hypoglycemic activity of methanolic bark extract of *Albizia richardiana*, 200 and 400 mg/kg dose of the extract were taken to observe whether they do have any effect on lowering of blood glucose level.

Table 5.1: Test materials used in the evaluation of hypoglycemic activity of crude extract of bark of *Albizia richardiana*

Code no.	Test Samples	Group	Identification	Dose (mg/kg)
CTL	1% Tween-80 & DMSO in normal saline	I	Control Group	0.1 ml/10 g of body wt
STD	Glibenclamide	II	Standard Group	10
ME 1	Methanolic bark extract of <i>Albizia richardiana</i>	III	Test Sample	200
ME 2	Methanolic bark extract of <i>Albizia richardiana</i>	III	Test Sample	400

Table 5.2: Plasma level of glucose (mmol/L) of mice at different time

Code no.	0 minute		20 minute		60 minute		120 minute	
	Data	Mean	Data	Mean	Data	Mean	Data	Mean
CTL	5.8	5.70	10.1	10.67	7.6	7.33	5.7	5.60
	5.8		10.9		7.2		5.8	
	5.5		11		7.2		5.3	
STD	4.1	4.17	3.6	3.73	3.3	3.53	3.6	3.30
	4.2		3.7		3.6		3.2	
	4.2		3.9		3.7		3.1	
ME 1	5.0	4.17	6.5	5.70	3.1	3.60	Low	2.90
	4.6		4.4		4.1		2.7	
	2.9		6.1		3.6		3.0	
ME 2	3.6	4.30	7.0	9.13	2.7	3.90	3.7	3.50
	4.8		12.3		5.2		4.1	
	4.4		8.1		3.8		2.7	

Table 5.3: % reduction of plasma glucose level by test materials

Code no.	% reduction		
	After 20 minutes	After 60 minutes	After 120 minutes
STD	10.55	15.35	20.86
ME 1	-36.69	13.67	30.46
ME 2	-112.32	9.30	18.61

From the above table it is clear that, ME 1 and ME 2 do not have any significant percentage of reduction values than STD after 20 minutes of administration, but do have greater effects after 60 and 120 minutes of administration.

Table 5.4: Hypoglycemic activity of crude extract of bark of *Albizia richardiana*

Code no.	Plasma level of glucose (Mean)			
	0 minute	20 minute	60 minute	120 minute
STD	4.17	3.73	3.53	3.30
ME 1	4.17	5.70	3.60	2.90
ME 2	4.30	9.13	3.90	3.50

5.4.1. Statistical data evaluation

For the comparison of the test samples with both control group and standard group, standard t-Test of the test samples was carried out along with calculation of statistical significance of them.

Table 5.5: Statistical evaluation of the data

Code No	t-Test value	Degree of Freedom	P value	Level of significance
STD	3.0412	6	0.0228	Statistically significant
ME 1	2.4401	6	0.0505	Not quite statistically significant
ME 2	1.1956	6	0.2770	Not statistically significant

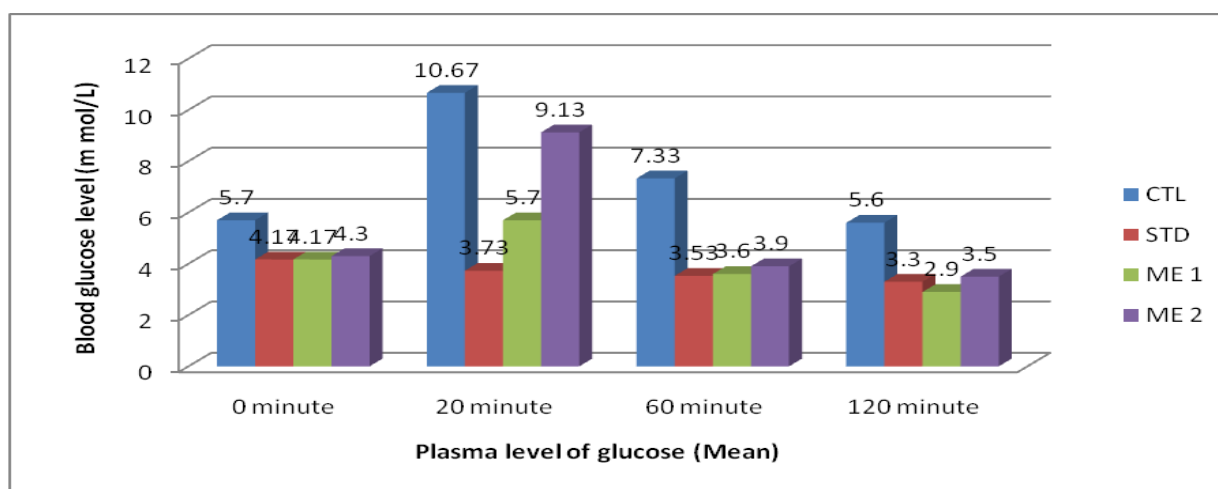


Figure 5.6: Plasma level of glucose of different groups of mice at different times of bark extract of *Albizia richardiana*

No statistically significant hypoglycemic activity of methanolic bark extract of *Albizia richardiana*, at both doses of 200 mg/kg and 400 mg/kg body wt was observed.

Chapter 6

Conclusion

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Conclusion

The methanolic extract of bark of *Albizia richardiana* (Fam. Fabaceae, subfam. Mimosoideae) was investigated for the evaluation of phytochemical profile as well as biological profile of this plant.

The result of the present study shows that the plant contains some of the important bioactive constituents like more instance- carbohydrate, glucosides, glycosides, saponin and alkaloids. It was also observed from the investigation that the plant extract not responded positively in brine shrimp lethality bioassay with LC_{50} value of about 22.03 $\mu\text{g/ml}$. However, no statistically significant hypoglycemic activity of methanolic bark extract of *Albizia richardiana*, at both 200 mg/kg and 400 mg/kg body wt doses was observed.

Thus, the present study has revealed potential bioactivity of *Albizia richardiana* (Fam. Fabaceae, subfam. Mimosoideae), encouraging further extensive studies to find out their unexplored efficacy and to rationalize it as a traditional medicine.

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