Phytochemical Investigation, Standardization & Biological Evaluation of the Leaves of Barringtonia acutangula & it's Formulation

Thesis submitted in partial fulfilment of the requirement for the degree

of Master of Pharmacy

Under the guidance of

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2024

CERTIFICATE

This is to certify that **Moulisha Biswas,** Final year Masters of Pharmaceutical Technology (M. Pharm) examination student of Department of Pharmaceutical Technology, Jadavpur University, Class Roll No. 002211402053, Registration No. 163693 of 2022-2023, Examination Roll No. M4PIP24003 has completed the Project work entitled **"*Phytochemical Investigation, Standardization & Biological Evaluation of the Leaves of Barringtonia acutangula & it's Formulation*"** under my supervision, in the Division of Pharmacology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata – 700032.

She has incorporated her findings into the thesis of the same title being submitted by her in partial fulfillment of the requirement for the award of **Degree of Master of Pharmaceutical Technology**. I am satisfied that she has carried out her thesis with proper care and confidence to my entire satisfaction.

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Certificate of Approval

This is to certify that **Moulisha Biswas,** Final year Masters of Pharmaceutical Technology (M. Pharm) examination student of Department of Pharmaceutical Technology, Jadavpur University, Class Roll No. 002211402053, Registration No. 163693 of 2022-2023, Examination Roll No. M4PIP24003 has completed the Project work entitled **"*Phytochemical Investigation, Standardization & Biological Evaluation of the Leaves of Barringtonia acutangula & it's Formulation*"** under the guidance of **Dr. Nilanjan Ghosh** during her Master's Curriculum. This work has not been reported earlier anywhere and can be approved for submission in partial fulfilment of the course work.

(Signature of External Examiner)

DECLARATION

I hereby declare that **"Phytochemical Investigation, Standardization & Biological Evaluation of the Leaves of *Barringtonia acutangula* & it's Formulation"** is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Signature of the student:

Full Name:

Date:

ACKNOWLEDGEMENT

To bring something into existence is truly a work of **God**, I bring out my M. PHARM. work report endeavors biggest gratitude to **God almighty**.

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Finally, I extent my thanks to **You** (who are reading this) for your considerable interest and patience to go through entire project work.

Dated: **Moulisha Biswas**

DEDICATED

TO

THE CREATOR

&

MY FAMILY

PREFACE

The thesis entitled "***Phytochemical Investigation, Standardization & Biological Evaluation of the Leaves of Barringtonia acutangula & it's Formulation****"* consisted the project done by the author in the Department of Pharmaceutical Technology, Jadavpur University, for the Master Degree in Pharmacology.

India has started the use of medicines from the very beginning of civilization. The use of medicinal plant is found in *Rig Veda*. Ayurvedic mainly deals with the properties of different drugs obtained from natural source. Some plants are not cited in any book or literature showing their medicinal use but people have been using them generation after generation. Such Folklore and Traditional plants are neither investigated in the light of treating the diseases nor properly documented. Since long back people are using natural products without any hesitation and they do not bother regarding its scientific validity. This lack of awareness, blind faith on natural drugs and lack of documentation is depriving India to progress with highest momentum in the field of herbal research and herbal medicine. India has a vast collection of plants, animals and marine organisms; only a scientific approach with better momentum is needed to explore their utility in human beings. However, recently the untapped wealth of herbal source has become a major thrust area for searching the new bioactive molecules.

The present study is done on the plant *Barringtonia acutangula Linn.*(Lecythidaceae) commonly known as freshwater mangrove and mango-pine in English, Hijal in Bengali, is the large evergreen tree grown in the coastal wetlands of the South-East Asian countries including India. This plant had traditionally used in India for numerous therapeutic uses. Small leaves of this plant had been consumed as food, in somewhere people used to eat fresh leaves as vegetables. Research on the plant has found numerous medicinal uses, such as antitumor in seed extract, anti-microbial, inhibition of *Helicobacter pylori*, anti-nociceptive activity, hepato-protective activity and to combat with metabolic disorder.

My present study has been aimed to evaluate standardization parameters along with the biologial properties of propanol extract of *Barringtonia acutangula* leaves (PEBA) and it's formulation in rodents.

My thesis consists of the above mentioned chapters in proper order with appropriate references in every chapter. The results of different phytochemical and pharmacological studies are summarized in the form of table as well as figure indicating statistical significance level and concentration drawn in the manner to justify the work scientifically.

Moulisha Biswas

BRIEF OUTLINE OF THE WORK

The work described in this thesis mainly segmented into **seven chapters**.

The **first chapter** mentioned the introduction related to the detailed literature review on the plant *Barringtonia acutangula*, regarding the Traditional medicinal system mainly on Ayurvedic formulation prepared from different Indian medicinal plants and information on some metabolic disorders and their treatment with the help of medicinal plants.

The **second chapter** deals with the Aim & Objectives and especially on the scientific utility of the project work i.e. the extraction, biological evaluation and determination of the standardization parameters of the extract as well as the Ayurvedic formulation prepared the leaves of *Barringtonia acutangula*.

The **third chapter** mentioned the extraction of leaves of *Barringtonia acutangula* and preparation of the Ayurvedic formulation from the leaves of *Barringtonia acutangula*.

The **fourth chapter** describes the determination of the standardization parameters of the propanol extract of *Barringtonia acutangula* leaf according to the protocol of Ayurvedic Pharmacopoeia of India and determination of standardization parameters of the Ayurvedic formulation reffered to the protocol of Ayurvedic Formulary of India from leaves of *Barringtonia acutangula*.

The **fifth chapter** covers the evaluation of lethal dose for the 50% population (acute toxicity) of propanol extract of the leaves of *Barringtonia acutangula* in mice.

The **sixth chapter** deals with the antioxidant profile of the extract in different antioxidant models like DPPH scavenging, superoxide, lipid peroxidation etc.

The **seventh** i.e**. the last chapter** covers the hypoglycemic potential of extract against Streptozotocin induced diabetic rats.

CONTENT

CHAPTER - I

INTRODUCTION

India has started the use of medicines from the very beginning of civilization. The use of medicinal plant is found in *Rig Veda*. Ayurvedic mainly deals with the properties of different drugs obtained from natural source. Ayurveda had been introduced on earth more than 5000 years earlier, the holistic approach[1,2,3].According to our ancient traditional knowledge, during the creation of World various diseases emerges; to protect the and cure from the sufferings Lord Brahma first transfer the knowledge to Draksha Prajapati, then Draksha Prajapati transfer the wisdom to Ashwini kumars, then Ashwini kumars transfer the knowledge to Indra and Indra further transfer the knowledge to Bharadwaj, then Bharadwaj transfer the knowledge to Atreya, from Atreya Punarvasu get the knowledge of Ayurveda. Further Punarvasu has 6 students – Agnivesa, Bhela, Jatukarma, Parasara, Hariti, Ksarapaini. All these students contribute in developing Ayurveda, but among them Agnivesa was the most intelligent and he contributed 'Agnivesa Samhita' in written form (first written text of Ayurveda) and further Charaka implemented his text and which further known as 'Charaka Samhita'.[4]

Ayurveda evolved around preventive and personalised medicine by balancing tridosha i.e. vayu, pitta, kapha and Triguna i.e. satyo, tamo and rajo. Ayurveda understood and performs, according to individual Prakriti, an environmental factor Amazingly, Ayurveda incorporate food (ahara) [4] and drug (ausadha) [4] in the therapeutics to maintain the balance of Dosas. As we humans are more familiar to food intake rather than drugs. But interestingly, we use spice in our food is actual the herb as a natural remedy for medicine. Ayurvedic principles are developed on the basis of clinical observation. But the world, indeed, the scientific rationality and these rationalities are rarely based on the ancient text. But if we use traditional knowledge of ayurvedic, which is embedded in our Samhita's, Vedas, Lokas Dincharya and etc. For developing personalised medicines for Ayurveda, we have to understood and examine the individual Prakruti and using ancient texts, we can target the specific herb or medicine. And by using modern techniques, we can determine its physiochemical factors by using chromatography and phytochemical test by which we can determine various secondary metabolites and the mechanism actions.

Some plants were not cited in any book or literature showing their medicinal use but people have been using them generation after generation. Such Folklore and Traditional plants are neither investigated in the light of treating the diseases nor properly documented. Since long back people are using natural products without any hesitation and they do not bother regarding its scientific validity. This lack of awareness, blind faith on natural drugs and lack of documentation is depriving India to progress with highest momentum in the field of herbal research and herbal medicine. India has a vast collection of plants, animals and marine organisms; only a scientific approach with better momentum is needed to explore their utility in human beings.

Pharmacological Activities of some bioactive molecule:

The secondary metabolites have proved their biological activities. Although applications of these secondary metabolites are successful as therapeutically important agents but extensive exploratory activities in this particular field have been underway during recent years. Some important results are summarized below;

A. Antimalarial Activity: Triterpenoid analogue Arjunolic acid recently isolated from stems and leaves of *Humiria balsamifera* showed antimalarial activity against Chloroquine Resistant strain[5].

B. Anticancer Activity: The anticancer activity was tested against some human cancer cell lines.Betulinic acid a very well known triterpene present in many plants inhibits the proliferation of vincristine resistant derivative of K562 that displayed several multidrug resistant characteristics[6].

C. Anti diabetic Activity: Numerous medicinal plants proved hypoglycemic activity. Corosolic acid presents in leaves of *Lagerstroema speciosa* showed anti diabetic activity in type-II diabeties (NIDDM)[7].

D. Hepatoprotective Activity: It had been observed that Sarmentolin a triterpene analogue isolated from *Sedum sarmentosum* possess hepatoprotective activity[8].

E. Anti-inflammatory Activity: Numerouscrudedrugsshowedpotent anti-inflammatory action when tested in carragenan induced paw odemain rat. Two polyhydroxylated triterpenes obtained from*Salviahierosolymitana* showed inhibition croton oil induced ear odema in mice[9].

F. Anti- Leishmanial Activity: Besides apigenin, friedelin triterpenic acid like Arjunolic acid, tormentic acid, hyptatic acid obtained from *Pourouma guianensis*leafproved activity against*Leishmaniasp*. It had been observed that Pristimerin a quinonemethide triterpene from the chloroform extract of *Maytenus senegalensis*bark possess potent anti- leishmanial activity[10].

G. Anti- HIV Activity: Leaves of *Rosawoodsii, Syzygiumclaviflorum* and the whole plant of *Phoradendrumjuniperinum* and *Hyptiscapitala*posses a number of of triterpenoids. It had been observed that vaticinone, cycloartanesobtained from *Vaticacinerea*leaf and stem inhibit the replication of HIV-1. It had been reported that protostane triterpenoids isolated from trunk, bark and stem of *Garcinia speciosa* possess anti- HIV- 1 activity[10].

H. Cardiovascular Activity: It had been showed that lupeol and lupeol linoleate possess cardio-protective action. Ursolic acid having ursen skeleton proved the inhibition of atherosclerosis[11].

I. Cholesterol Lowering Activity: It had been reported that cholesterol lowering and anti-atherosclerosis activity of Prostane and Fusidane (29- desmethylprostane) derivatives isolated from

Fusidiumcoccinenum. Some pentacyclic triterpenic acids inhibit human ACAT-1 and ACAT- 2 activity and may be useful for treatment of hyper cholestoromia and atherosclerosis.
Different disease, their pathogenesis and present therapies are shortly discussed as the present work carried out on that respective disorder[11].

Oxidation & Antioxidants

Antioxidants are largely incorporated in food materialsformaintenance of health and to combat with diseases like cancer and for other sickness. Preliminaryobservationadvisedthat antioxidant can providewellbeing. The uses of herbal antioxidants in medicine are used forprotectingthebioactive constituents[12].

The oxidative challenge in biology and the role of antioxidants

Organisms contain a complex network of antioxidant metabolites and enzymes that work altogether to prevent oxidative damage to cellular materialslike DNA, proteins and lipids. In general, antioxidant systems can prevent the reactive species from being formed, or remove them before they can damage vital materials of the cell[12,13].

The use of oxygen for generating metabolic energy produces reactive oxygen species. Peroxide is also produced from the oxidation of reduced flavoproteins. In plants, algae, and cyanobacteria, reactive oxygen species are produced during photosynthesis, mainly under the conditions of high light energy. The carotenoids are found to prevent the production of reactive oxygen species[14].

The oxidation reactions are important for organisms, but they can also create damage; that's why, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E, carotenoids, polyphenolic flavonoids.[15].

Diabetes mellitus

Diabetes mellitus, ismainly referred to as **diabetes**—is a metabolic diseases in which a person has increased blood sugar level because the body cannot produce enough insulin, or because cells do not respond to the insulin produced by the pancreas. This increased level of blood sugar produces the other symptoms like polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger)[16].

Types of diabetes:

- **Type 1 diabetes:**This type creates from the body's failure to produce own insulin, and the person needs to inject insulin from outside. (It is also known as *insulin-dependent* diabetes mellitus, *IDDM* and *juvenile* diabetes.)
- **Type2 diabetes:**It creates from the insulin resistance in whichthe cells fail to utilize insulin properly.
- **Gestational diabetes:** This condition may occurto pregnant women, who never had diabetes before, but used to have an increased blood glucose level during pregnancy.

Type II diabetes can be controlled by changing life style, food habit andwith medicines. Diabetes without proper treatments can cause numerous complications like hypoglycemia, diabetic ketoacidosis, coma. Serious long-term complications such as cardiovascular disease, chronic renal failure and retinaldamage are related to diabetes. Treatment of diabetes is very important, as well as blood pressure control and lifestyle[16].

Anti-diabetic drugs

Anti-diabetic drugs can be defined as the medicinal agents which can treat diabetes mellitus by lowering blood glucose levels.Insulin is called oral hypoglycemic agents or oral antihyperglycemic agents. There areseveral classes of anti-diabetic medications and their selection depends on the types of thediabetes, age as well as other factors.

Diabetes mellitus type I: Insulin should be administered in Type I, which is to be injected s.c.

Diabetes mellitus type II: Treatments including (I) agents which can increase the amount of insulin secreted by pancreas (II) agents which elevates the sensitivity of target organs to insulin and (III) agents which can decrease the rate at which glucose is absorbed from the gastrointestinal tract.

Several groups of drugs, mostly given by mouth, are effective in Type II diabetes, also in combinations.

Classification of Oral Hypoglycemic Agents

Sulfonylureas

First-generation agents	Second-generation agents
Tolbutamide	Glipizide
Acetohexamide	Glyburide
Tolazamide	Glimepiride
Chlorpropamide	

Meglitinide: Repaglinide, Nateglinide

Biguanide: Metformin, Phenformin, Buformin

Thiazolidinediones: Rosiglitazone, Miglitol

Alpha-glucosidase inhibitors: Acarbose, Pioglitazone

Some plants and their parts used are listed below as diabetic therapy: [16]

Names of the Plants	Parts Used
Aegle marmelos	Leaf
Areca catechu	Nut
Centella asiatica	Bark and fruit
Ficus glomerata	Fruit and bark
Glycine max	Dry seed powder
Morus indica	Leaf
Ocimum americanum	Whole plant

The present study is done on the plant *Barringtoniaacutangula Linn.*(Lecythidaceae) commonly known as freshwater mangrove and mango-pine in India, Hijal in West Bengal, is large evergreen tree grown in the coastal wetlands of the South-East Asian countries including India. This plant had been found for numerous therapeutic uses.Thefresh leaves of the plant have been incorporated in food as vegetables. Research on the selected plantproved potential actions like antitumor effects in seeds, anti-microbialproperty,inhibitoryeffects on *Helicobacter pylori*, anti-nociceptive property, hepato-protective activity and to combat with metabolic disorder[17].

Literature Review on the plant *Barringtonia acutangula*


Barringtonia acutangula is a large tree briefly deciduous; it grows upto 13 meters tall. The plant is cultivated for local uses, primarily as a medicine and also as vegetables. It is found in Asia -, Indian subcontinent, Myanmar, Malaysia, Vietnam, Indonesia, Philippines, Thailand, Australia[18].

Description of the plant:

Leaf: Simple, alternate, stipulate, 7.5—12.5 cm long, obovate to oblong-oval, bright green, not shining, petioles 0.6—1.2 cm long.

Flowers: Regular, bisexual, with petals cream colored and stamens dark bright crimson, petals 4, small, 0.6 cm long, imbricate, distinct; simple.

Fruit: Fibrous, 3.1—3.7 cm long, oblong-ovoid, narrowed at base, truncate at both ends, capped with small, persistent calyx segments.

Part(s) used for therapeutic uses: Fruits, Roots, and leaves

Distribution: Sub Himalayan tracts Assam,Bengal,Bihar, Orissa, Central, and South India and also found in Southern Asia, Philippines, Australia.

Flowering: From May to September.

Vernacular names of the Plant

1. **English**: freshwater mangroveand mango-pine
2. **Sanskrit**: Hijjala, Samudraphala (as it occurs along the streams in the coastal area),
3. **Assamese**: Hindole
4. **Bengali**: Hijjala
5. **Hindi**: Hijjala, Samudraphala

Scientific Classification[19,20,21].

- **Kingdom**: Plantae
- **Subkingdom**: Tracheobionta
- **Family**: Lecythidaceae
- **Genus**: Barringtonia
- **Species**: *Barringtonia acutangula*

Description –>

Colour – Brown

Odour – Characteristic

Taste – Bitter

Chemistry:

The plant contains potent opoid painkillers. It contains gallic acid, , bartogenic acid, Saponins, barringtoside A, barringtoside B, barringtoside Cand stigmasterol.

Uses

1. **Food:** Fresh leaves of the plant used as vegetables in food.
2. **Medicine:** The plant was found numerous therapeutic purposes such as the antitumor effects of the seeds, antibiotic, inhibitory effects on *Helicobacter pylori*, antinociceptive property and antifungal potential[28,29].

The common edible parts are the fresh leaves, fruits, and seeds, whereas the bark and roots are also orally administered or applied topically as medicine, to treat malaria, cough reliever, cure from stomach ache, to combat with skin diseases, and for wound-healing[30,31].

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CHAPTER – II

AIM, OBJECTIVES & SCIENTIFIC UTILITY OF THE WORK

Herbal drugs implemented significantly towards the development of modern therapy. Many of the modern clinicians are now more inclined towards the use of ancient medicine. For instance, plant-drugs constitute approximately 25% of all the prescribed medicines even today in the most advanced countries like the USA. Ninety percent of world populations rely on ancient system for primary health maintenance.

Plants have been a potential source of therapy because of their potential bioactive molecules. Therefore, my present study is conceptualized on the basis of finding of bioactive molecule along with the different pharmacological action and safety potential of the selected plant along with its formulation.

But, no scientific investigation along with its standardization parameters and pharmacological evaluation of the propanol extract and the formulation from the leaves is still reported on *Barringtonia acutangula* leaf.

Therefore, my present study was objected to investigate the standardization parameters along with the pharmacological properties and toxicity profile of propanol extract of *Barringtonia acutangula* in different animal models and development of a classical Ayurvedic formulation from the leaves of the selected plant.

CHAPTER – III

Extraction of the Propanol Extract of the Leaves of *Barringtonia acutangula*

&

Preparation of the Ayurvedic Formulation from the Leaves of *Barringtonia acutangula*

Medicinal activity of the crude drugs present in plants such as Steroids, diterpene, triterpene, flavones, alkaloids etc as well as in the form of ether, ester and glycosides. Plant contains secondary metabolites that show many pharmacological activities. These secondary metabolites are nothing but some chemical group having a particular structure. The tests are available to identify the particular phytochemicals group in the extract. These are based on colour reaction or precipitation response to a particular chemical reagent.

Extraction:

This is the process contains separating out the active constituents from a plant or plant part with the help of solvent is called extraction.

- Marc: - Plant residue left after extraction.
- Miscella:- Mixture of active constituents and solvent.
- Rinsing:- Dissolution of extractive substances out of the disintegrated cells of the parts of the plant.
- Lixiviation:- Extraction with only water as a solvent from the plant part.

Basic principle of extraction:-

Process of extraction of crude drug plants can be classified into following two major steps:-

1. Establishment of concentration equilibrium between the solution and solid residue.
2. Exhaustive extraction of the drug material.

The main parameter is the establishment of concentration equilibrium between the solution and solid residue, which comes to a halt when distribution of the extractive substances between the miscella and the drug residue has become zero[1,2].

Factors influencing extraction of herbal drugs:-

- Amount and nature of plant material:-When amount of the plant part increased then percentage of extractive value will be increased.
- Nature and volume of the solvent:- If volume of the menstrum is increased then percentage of extractive value will be increased.
- Temperature: - Though increase in temperature increases the rate of extraction yet is avoided as this causes denaturation of thermo labile constituents.
- Pressure: - Application of pressure on the plant material facilitates extraction. (Mainly used to extract oils from seeds)

- Dissolution of disintegrated plant cells into the solvent:- This increases the rate of extraction as due to the disintegration of cells the solvent easily penetrates the plant material and dissolves out the phytoconstituents.

- Dissolution of intact cells of plant material:- If the intact cells are subjected to solvent treatment then rate of extraction is very poor as the solvent cannot penetrate the cells and dissolve out the extractive substances.
- Imbibition of drugs:- The plant cells dialate after administering solvent to them which facilitates diffusion of solvent into the cells and hence accelerates extraction.
- pH of the solvent:- "Alkali likes alkali , Acid likes acid" based on which the extraction occurs depending upon the polarity.
- Moisture present in the drug:- If there is some moisture present either in the drug or in the solvent, then it will interfere with the extraction process[1,2].

EXPERIMENTAL

Collection and Authentication of the selected Plant material

Barringtonia acutangula leaves were collected in January 2023 from Nadia district of West Bengal, India. The plant material was authenticated by the Taxonomist, BSI, India, Central National Herbarium, Howrah district of West Bengal, India. The voucher specimen [CNH/Tech.II/2023/188] was kept in the laboratory.

Preparation of extract

The leaves were shade-dried and then powdered, passing through sieve no #20, and were stored in air tight container.

The dried plant material was successively treated with pet ether (60-80°C), acetone and propanol by percolation technique. The extract was evaporated to dryness to get solvent free extract (5.98%, 15.96% and 21.45% respectively)[3,4].

Extraction of Phytoconstituents from the leaves of *Barringtonia acutangula*

The dried leaves of *Barringtonia acutangula*(1.2 kg) wastreated with pet ether (60-80°C), acetone and propanol by percolation method (3×5 lt). The extract was dried to get extract (5.98%, 15.96% and 21.45% respectively).

Significance of the preparation of Ayurvedic formulation

In some cases, Ayurvedic formulations are applied along with other natural agents like honey or milk, to obtain specific actions, depending on the disease condition. In Sanskrit, these herbal products are called Anupan such Ayurvedic products can minimize the side effects[5,6].

Preparation of formulation[5,6]

The formulation was prepared as per the following scheme:

Hijal shade dried leaf 250 gram were crushed

↓

Then 1.5 litre distilled water was incorporated and was kept it for 48 hours.

↓

Then that mixture was heated and reduced

↓

Miscella was collected

↓

Reduced it to 250 ml

↓

Added sodium benzoate 0.5% at 40◦C and add 0 .01% sodium methyl paraben and add 0.0375% sodium propyl paraben [Percentage should be calculated against 250 ml of decoction, after cooling added 18.75 ml Daburhoney mixed it carefully].

↓

Preserved in amber coloured glass bottle

Storage Condition

The formulation was preserved in the amber coloured glass bottle at room temperature.

All the standardization parameters were performed both for the plant extract as well as for the classical Ayurvedic formulation which was prepared from the leaves of *Barringtonia acutangula*.

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CHAPTER – IV

Standardization of the Propanol Extract of the Leaves of *Barringtonia acutangula* & Standardization of the Ayurvedic Formulation from the Leaves of *Barringtonia acutangula*

Significance and Utility of Standardization Parameters:

Standardization parameters play a crucial role in various fields, including pharmaceuticals, nutraceuticals, ayurvedic medicine, and natural product development. The significance and utility of standardization parameters are multifaceted and extend across different aspects of product quality, efficacy, safety, regulatory compliance, and consumer trust. Here are some key points highlighting the importance of standardization parameters[1]:

1. **Ensuring Product Quality**[1]: Standardization parameters define the essential quality attributes that a product must meet consistently across different batches. By establishing specifications for factors such as potency, purity, composition, and stability, standardization ensures product quality, efficacy. This consistency is essential for building consumer trust and confidence in the product.
2. **Enhancing Efficacy**[1]: Standardization parameters help ensure that the product contains the desired levels of active compounds or ingredients that are responsible for its therapeutic effects. By setting standards for potency and bioavailability, standardization ensures that the product delivers the intended health benefits reliably and consistently. This is especially critical in herbal medicine and natural product development, where variations in plant composition can impact efficacy.
3. **Facilitating Reproducibility**[1]: Standardization parameters provide a framework for reproducibility in product manufacturing. By defining specific criteria for raw materials, manufacturing processes, and quality control measures, standardization enables manufacturers to produce consistent and reliable products that meet predetermined quality standards. Reproducibility is essential for ensuring that the product performs as intended and yields consistent results.
4. **Regulatory Compliance** [1]: Standardization parameters are often required by regulatory authorities to ensure that products meet safety, quality, and efficacy standards. Regulatory bodies may mandate specific quality control measures, testing methods, and acceptance criteria for products to be approved for sale in the market. Adhering to standardized parameters helps manufacturers demonstrate compliance with regulatory requirements and ensures product safety for consumers.
5. **Consumer Safety**[1]: Standardization parameters play a crucial role in safeguarding consumer health and safety. By setting limits for contaminants, heavy metals, microbial load, and other harmful substances, standardization helps prevent the presence of potentially

hazardous materials in the product. Ensuring that products meet established safety standards protects consumers from adverse effects and promotes public health.

6. **Promoting Transparency**[1]: Standardization parameters contribute to transparency in product labelling and marketing. Transparent labelingisbased on standardized parameters helps build consumer trust and fosters a positive relationship between manufacturers and consumers.
7. **Supporting Research and Development**[1]: Standardization parameters provide the safety of new products during the research and development phase. By establishing reference standards and acceptance criteria, standardization facilitates comparative studies, quality assessment, and formulation optimization. This framework accelerates the product development process and ensures that new products meet predefined quality standards.

A. Organoleptic evaluation

The sensory features of the extract as well as the finished liquid formulation product thus prepared were noted.

B. Physico-chemical and chromatographic parameters

Different physico-chemical parameters of this product like specific gravity, miscibility/solubility, pH, viscosity, solubility, preliminary phytochemical analysis, total solids content, ash values, extractive values were determined according to the protocols prescribed in the API. Thin layer chromatography of extract and formulation wereperformed on the pre-coated plates of silica gel $60F_{254}$ by using mobile phase ethyl acetate: methanol = 5: 5.

C. Phytochemical tests of propanol extract and the formulation of the plant

Phytochemical evaluation helps to investigate the groups of phytochemicals of extract and formulation which is very much helpful in searching for bioactive molecules.

Different type of phytoconstituents are-:

1. Steroid.
2. Terpenoid.
3. Phenolic compound.
4. Alkaloid.
5. Glycoside.

6. Tannins.
7. Fixed oil.
8. Saponins.

Primary metabolites of the plants [2]-:

1. Carbohydrate.
2. Fat.
3. Protein.

I. Test for Carbohydrate[3] –

a. **Molisch's reagent:** Sample was taken and the solution and some drops of Molisch's reagent was added to it. Then added few drops of concentrated H_2SO_4 and purple to violet color ring form in case of positive response.

II. Test for Protein[3] **–**

Ninhydrin Test – To 1 ml of sample,Ninhydrinreagenthad been added. Violet colour was formed in positive response.

III. Test for Fat[3] **–** A filter paper was taken and drop a few drops of extract to it. The filter paper became translucent in positive response.

IV. Identification of Steroids[3]–

Libermann-Burchad'sreagent -

To the extract2 ml of acetic anhydride and 2 ml of concentrated H_2SO_4were added. Reddish brown ring formation at the junction for steroids was observed.

V. Test for Terpenoid[3]–

Libermann-Burchad'sreagent -

2 ml extract was taken after that,acetic anhydride and 10ml H_2SO_4were added under cooling and then ethanol was added. Formation of bluish green colourindicatedforTerpenoid.

VI. Identification of Phenol[3]–

a. **Ammonia Test –**

Added 3 drops of sample on filter paper and was exposed to Ammonia vapour. Yellow colour appeared in positive response.

b. **Shinoda test –** Added extract to the test tube to the 2ml dil. HCl. Red/magenta colour was appeared in positive response.

VII. Test for Alkaloids[3]–

a. Mayer's reagent–

Propanol extracthad been taken in the test tube then Mayer's reagent was added to it. Cream precipitation was formed in positive response.

b. Wagner's reagent –

2-4 ml propanol extract was taken to the test tube and Wagner's Reagent was added. Reddish brown colour appeared in positive response.

c. Hager's test –

2-4 ml sample had beentaken in test tube and Hager'sreagentwasadded. Yellow precipitation was formed in positive response.

d. Murexide test -

2-4 ml propanol extract had been taken in test tube and added 1 ml $KClO_3$ and 1 drop of HCl and were added and heated it. Then it was exposed to NH_3. Purple colourwas formed form in positive response.

VIII. Test for Glycosides[3]–

a. Baljet's test –

Added extract to the test tube then added sodium picrate to it. Yellow to orange colour appeared in positive response.

IX. Identification of Tannins[3]–

a. Ferric chloride reagent –

3 ml propanol extract was taken and few drops of ferric chloride reagent were added. Dark blue colour appeared for hydrolysable tannin in positive response.Greencolour appeared for condensed tannin in positive response.

X. Test for Fixed oil[3] **-**

Spot test –

A filter paper was taken and added drop a few drops of extract to it. The filter papers became translucent in positive response.

XI. Test for Saponins[3]–

Froth Test –

2 ml sample had been taken in a test tube; 7 ml distilled water has been added and was shaken for 12 seconds thensample was allowed to stand for 10 seconds. Appearance of stable foam indicated for saponins.

XII. Detection of Flavonoid[3] – To the 2 ml propanol extract ethanol had been added. Then the sample was poured on a filter paper and was exposed to ammonia vapor. Yellow spot was observed in positive response.

XIII. Detection ofReducing Sugar[3] **–**

a. Fehling reagent -

A few ml samples had been taken in test tube and Fehling's reagent had been administered in it.

b. Benedict's test –

Propanol extracthad been taken in test tube and 3-5 drops Benedict's reagent had been incorporated.

Presence of Phytochemicals in different extracts[4]

Type of Extracts	Constituents Present
Propanol extract of *Barringtonia acutangula*	Terpenoid, Saponin, Glycoside, Flavonoid, Tannin
Ayurvedic formulation from the leaves of *Barringtonia acutangula*	Terpenoid, Saponin, Glycoside, Flavonoid, Tannin

D. Identification of the Retention Factor (Rf values) of compounds with the help of Thin Layer Chromatography Technique

Basic principle of TLC technique is based on adsorption chromatography[5].

R_f value[5]:

It can be defined as it is a physical parameter and definite for a definite compound with respect to the definite solvent system. R_f value doesn't have any unit because it is a ratio.

R_f = distance which was travelled by the solute/ distance which was travelled by solvent.

Classifications of TLC plate

1. Preparative plate (Stationary phase is silica gel G)
2. Pre-coated plate. (stationary phase is silica gel $60F_{254}$)

Factors influencing TLC -:

1. Mobile phase.
2. Stationary phase.
3. Nature of the sample.
4. Temperature.
5. Humidity.
6. Spot application.
7. Sample volume.
8. Impurities present in the mobile phase.

Application of TLC-:

1. Detection of R_f values of organic compound.
2. Determination of R_f values of different marker constituents which can be compared with the test sample.
3. Detection of R_f value of unknown phytoconstituent.
4. Detection of R_f values of impurity present in the sample.
5. Detection of active constituent present in the extract.
6. Detection of R_f value of synthetic re agents, synthetic compound and synthetic intermediates.

After completion of TLC of *Barringtonia acutangula* leaf extract the R_f value obtained under UV light 365 nm are follows and as it doesn't have any monograph for standardize parameter, I repeat this experiment to set a standardized parameter.

R_f = distance which was travelled by the solute/ distance which was travelled by solvent.

Procedure[5] -

$60F_{254}$ Pre-coated TLC plates were used for the whole procedures. The spot of the extract was applied with capillary tube on the percolated plate and had been dried. After that, plate had been incorporated inside the TLC solvent camber and the mobile phase had been allowed to move upwards. Then the plate had beentaken out from the chamber and dried and kept inside the iodine chamber or UV Cabinet to visualize the spots given by the compounds.

Sample preparation: Propanol extract of *Barringtonia acutangula* leaf was taken with capillary tube and was applied over $60F_{254}$ precoated plate.

Mobile phase:

Ethyl acetate: Methanol (1:1)

E. Total Ash[6]:

Total ash can be defined as the amount of inorganic substances formed after the incineration of the plant part. Total ash usually consists of physiological and non – physiological ash.

Material, Apparatus and Instrument Required -

Crucible with Lid, Tong, Muffle Furnace, Hot Air Oven, Plant material, Desiccator, Acetone

Procedure[6] –

A crucible was taken and rinsed with Acetone and was dried for 15 minutes. After drying the crucible was kept into the desiccators for cooling. After cooling take the weight of empty crucible. Added 5 gm of crushed, shaded dried leaf in crucible and placed into muffle furnace keeping at 460˚C to 550˚C. Incinerate the leaf until it became carbon free then it had been cooled and weighed. The total ash had been collected and was calculated percentage of total ash.

F. Acid Insoluble Ash[6]:

The term acid insoluble ash denotes the inorganic residues that remain after the crude drug is incinerated to detect its mineral content. Acid insoluble ash is mainly measure the amount inorganic substances present in a crude drug.

Material, Apparatus and Instrument Required -

Crucible with lid, Total Ash, Ashless Filter Paper, Funnel, ConicalFlask, Measuring Cylinder, Muffle furnace, dil. HCl.

Procedure[6] –

Take a crucible and rinse with Acetone and was dried for 15 minutes. After drying the crucible was putinto the desicator for cooling. Total Ash was boiled for sometimes with 25 ml dilute HCL. Residue had been collected in a crucible or on an ashless filter paper and incinerated in Muffle Furnace until the mater became complete white ash. Then percentage of acid-insoluble ash was calculated.

G. Evaluation of Moisture Content[7]:

Material, Apparatus and Instrument Required -:

Shaded dried and crushed leaf, LOD bottle with Lid, Hot Air Oven, Tong, Weight Machine, Desiccator.

Procedure:

A Petri dish was taken and weighed; to it the shaded dried and crushed leaf was taken. Noted the weight of Petri dish with shaded dried and crushed leaf and placed it to the Hot Air Oven at 105°C. The drying was continued and weighing at half an hour interval until a constant weight came. When the constant weight was reached after two consecutive weighing at 30 minutes interval, then it was cooled in thedesiccators and weighed.

H. pH Value[8]:

The pH of any liquid can be defined as the negative logarithm of the hydrogen ion concentration expressed in g, per litre. The pH value of a liquid can be determined by potentiometer.

Material, Apparatus and Instrument Required –

pH paper, pH meter, Extracts of leaf, Beaker.

Procedure –

(For pH paper)

Took about 20-30 ml extract and insert a pH paper to it and note the colour and compare with the standard given.

(For pH Meter)

Calibrate pH meter with pH 7,4,10 buffer. Took 30-40 ml of extract in a beaker and insert the electrode on it. Note the reading.

I. Specific Gravity (Wt./ml)[9]:

Material, Apparatus and Instrument Required –

Pycnometer, DM water, Extract.

Specific gravity[9] – The Specific gravity of a liquid is the weight of a given volume of the liquid at 25ºC compared with the weight of an equal volume of water at the same temperature, denoted as Wt. Per ml. Specific gravity of the liquid can be obtained by dividing the weight of the liquid in the pycnometer by the weight of water in it.

Procedure –

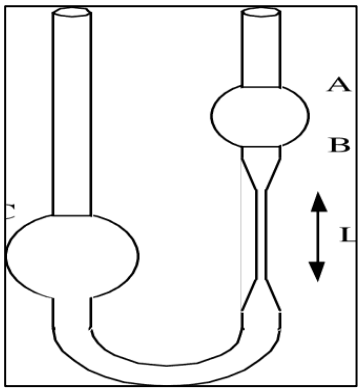
Apycnometer had been taken, dried and weighed. Both water and sample were incorporated accordingly and weighed. Subtracted the weight of empty pycnometer from the filled pycnometer was done. Specific gravity was measured.

I. Viscosity[10]:

Material, Apparatus and Instrument Required –

Ostwald viscometer, DM Water, Extract, Stopwatch

Definition: Viscosity is a physical property of a liquid, which denotes the resistance to flow of a fluid.. The unit of viscosity is the poise and the centi poise which is 1/100th of the poise.



Ostwald viscometer

Procedure –

The liquid was filled in an Ostwald viscometer. Time taken for the fluid to pass the two specified marks was measured. The kinematic viscosity is calculated from the following formula: Kinematic viscosity = kt Where k = the constant of the viscometer determined by observation on liquids of known kinematic viscosity. T = time in seconds for fluid to pass through the two specified marks.

J. Solubility Test[11]:

Material, Apparatus and Instrument Required –

Test tube, test tube Rack, Extract, Measuring cylinder, Hexane, Pet ether, Chloroform, Acetone, Methanol, Ethyl acetate, DM Water

Procedure –

5ml each solution to each 7 test tubes separately and add 2-3 ml of the sample (*Barringtonia acutangula* propanol extract) to each test tubes separately and vortex it. Note the observation.

K. Microbial Analysis[12,13]

This parameter was studied for detecting microorganisms which may cause disease or spoilage of the formulation.

Procedure –

Sterilize all the instrument, glassware &distilled water. Prepare peptone water & inoculate sample for growth & incubate for 24 hours.

Microbial determination

After preparation of the product and repeated after 3 months i.e., on the 91th day of its preparation[12,13].

L. Heavy metal content determination

The formulation was tested for arsenic (As), lead (Pb), cadmium(Cd), and mercury (Hg) with the help of atomic absorption spectrophotometer (AAS) as per the protocol described[14].

Analytical Results of the propanol extract of leaves of *Barringtonia acutangula*

Organoleptic evaluation

Description: Dry solid

Colour: Dark green to brownish.

Odour: Characteristic.

Taste:*Kashay*

Physico-chemical parameters

Miscibility/solubility

n-Hexane: Immiscible

Chloroform: Immiscible

Acetone: Miscible

Methanol: Miscible

Ethanol: Miscible

Water: Miscible

pH: 5.5

Total ash value:1.5% w/w

Acid insoluble ashvalue:0.05% w/w

Moisture content: 88.83 %

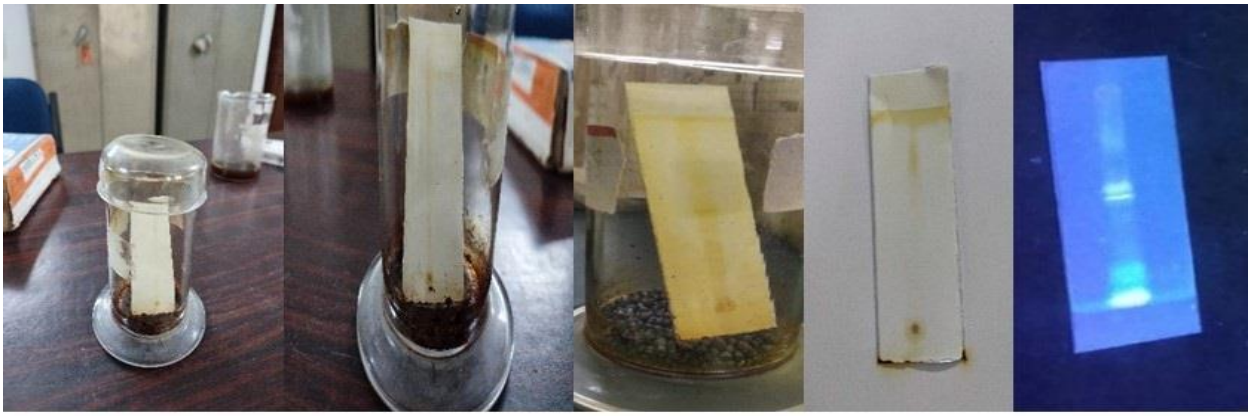
Preliminary phytochemial screening

The extract was contained alkaloids, steroids, terpenoids, condensed tannins, flavonoids, saponins, carbohydrates and reducing sugars.

TLC Rf values

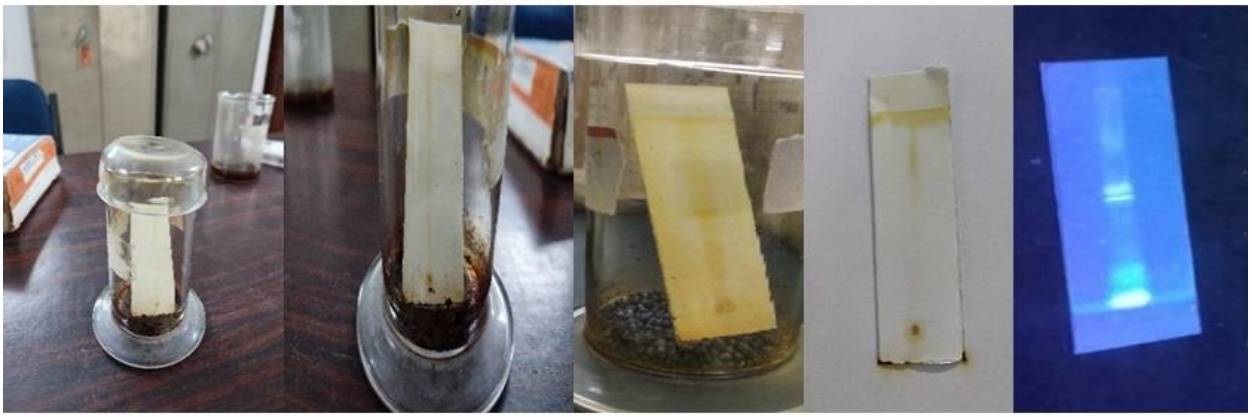
Visualisation:

Underultra-violet light (254 nm) and ultra-violet light (366 nm) the different $R_f$values were found to be 0.96, 0.87, 0.85, 0.81, 0.65,0.56, 0.48 and 0.41



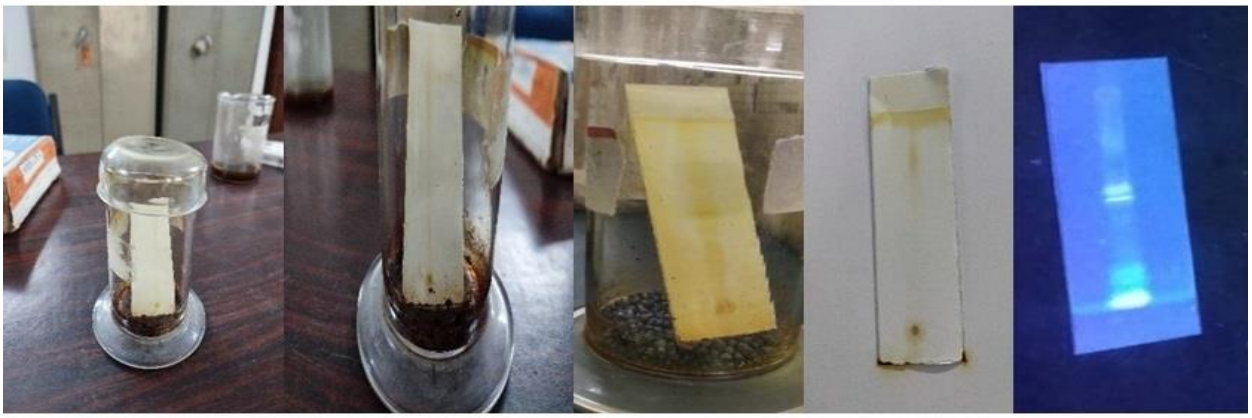
Iodine Chamber 1 | Iodine Chamber 2 | After H_2SO_4 Spray | Naked Eye | In UV Chamber at 366 nm

Set 1 TLC Plate



Iodine Chamber 1 | Iodine Chamber 2 | After H_2SO_4 Spray | Naked Eye | In UV Chamber at 366 nm

Set 2 TLC Plate



Iodine Chamber 1 | Iodine Chamber 2 | After H_2SO_4 Spray | Naked Eye | In UV Chamber at 366 nm

Set 3 TLC Plate

Microbial contamination

The observations have been presented in the Table 1 below:

Table 1: Observed microbial loads of propanol extract of *B.acutangula* leaf.

Sl. no.	Parameters	Permissible limits as per API[#]	Results/g*
1	Microbial plate count	10^5/g	82205
2	Yeast and mould	10^3/g	824
3	*Staphylococcus aureus*	Nil	Nil
4	*Salmonella sp*.	Nil	Nil
5	*Pseudomonas aeuroginosa*	Nil	Nil
6	*Escherichia coli*	Nil	Nil

*Repeat study after 3 months (90 days) did not show any appreciable deviation from the above initial results. [#]API: Ayurvedic Pharmacopoeia of India.

Heavy metal contents

The observation has been depicted in the Table 2 below:

Table 2: Permissible limits and observed heavy metal contents of propanol extract of *B.acutangula* leaf.

Sl. no.	Heavy metals	Permissible limits (ppm) as per API[#]	Results (ppm)
1	Arsenic	3	0.06
2	Lead	10	2.36
3	Cadmium	0.3	< 0.05*
4	Mercury	1	< 0.05*

*Below the limit of 0.05 ppm. [#]API: Ayurvedic Pharmacopoeia of India.

Analytical Results of Ayurvedic Formulation from the leaves of *Barringtonia acutangula*

Organoleptic evaluation

Description: Free flowing syrupy liquid.

Colour: Dark brown to blackish.

Odour: Characteristic.

Taste:*Kashay* to *Madhur*.

Physico-chemical parameters

Miscibility/solubility

n-Hexane: Immiscible

Chloroform: Immiscible

Acetone: Miscible

Methanol: Miscible

Ethanol: Miscible

Water: Miscible

Specific gravity: 1.80 at 25°C

pH: 5.5

Viscosity: 2.1$KgM^{-1}S^{-1}$ at 25°C

Total ash value: 4.83 w/w

Acid insoluble ashvalue:0.05w/w

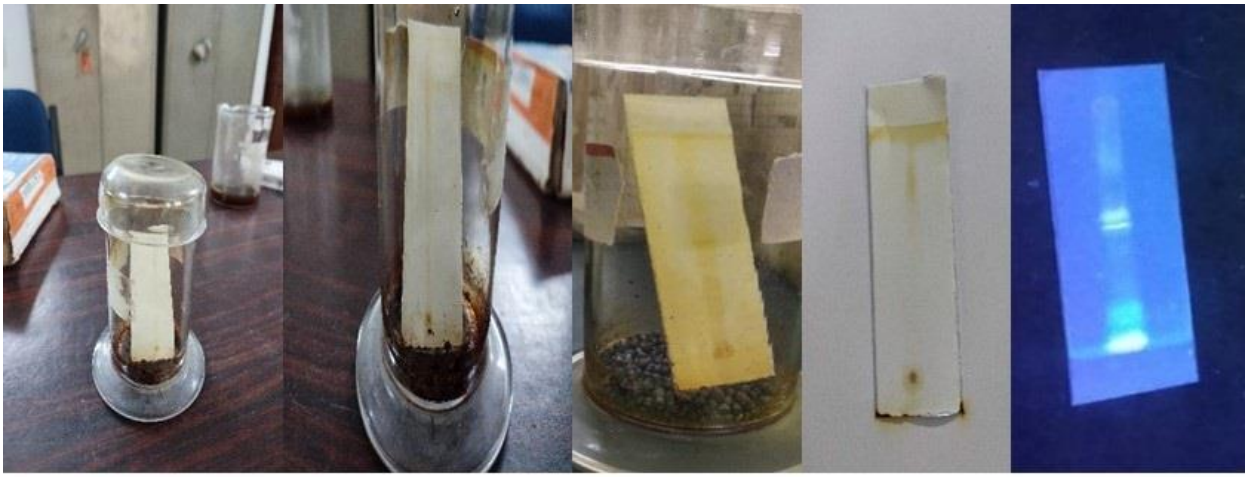
Total solids content: 85.93 %

Preliminary phytochemial screening

The prepared formulation was contained alkaloids, steroids, terpenoids, saponins, phenolic compounds, condensed tannins, carbohydrates and reducing sugars.

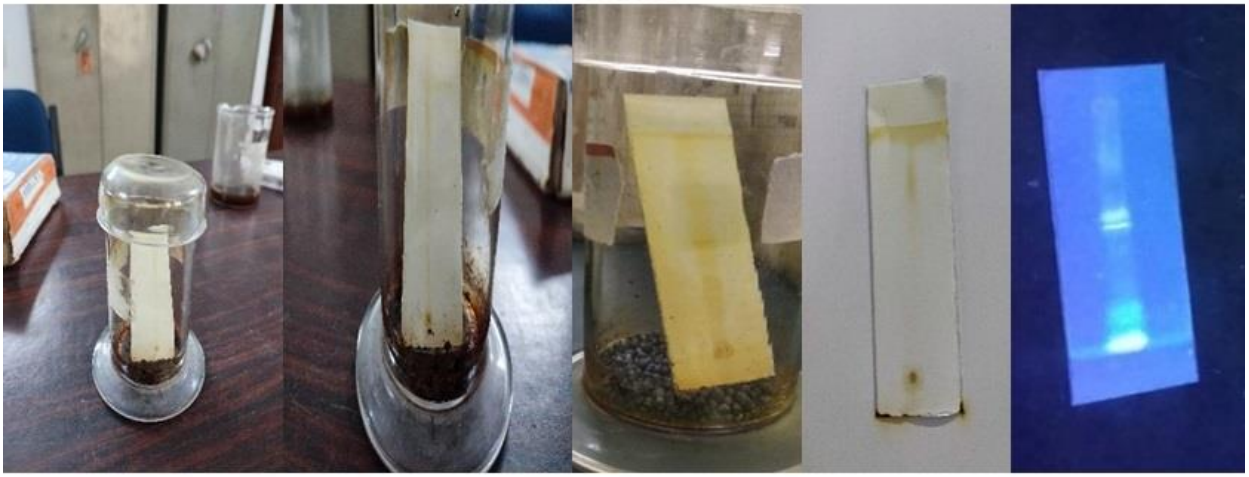
TLC Rf values

Observed at daylight: No visible spots
Observed at254 and 366 nm: Rf_1= 0.66, Rf_2= 0.21, Rf_3= 0.07
Observed at iodine chamber: Rf_1= 0.75, Rf_2= 0.68, Rf_3= 0.21



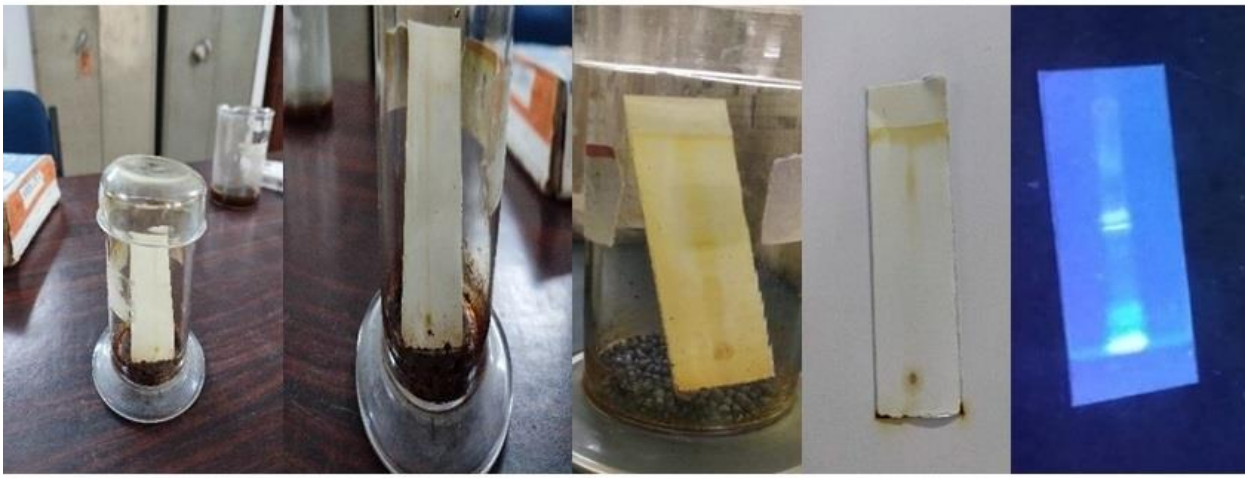
Iodine Chamber 1 | Iodine Chamber 2 | After H_2SO_4 Spray | Naked Eye | In UV Chamber at 366 nm

Set 1 TLC Plate



Iodine Chamber 1 | Iodine Chamber 2 | After H_2SO_4 Spray | Naked Eye | In UV Chamber at 366 nm

Set 2 TLC Plate



Iodine Chamber 1 | Iodine Chamber 2 | After H_2SO_4 Spray | Naked Eye | In UV Chamber at 366 nm

Set 3 TLC Plate

Microbial contamination

The observations have been presented in the Table 3 below:

Table 3: Permissible limits and observed microbial loads of formulation prepared from *B.acutangula* leaf.

Sl. no.	Parameters	Permissible limits as per API[#]	Results/g*
1	Microbial plate count	10^5/g	92205
2	Yeast and mould	10^3/g	889
3	*Staphylococcus aureus*	Nil	Nil
4	*Salmonella sp*.	Nil	Nil
5	*Pseudomonas aeuroginosa*	Nil	Nil
6	*Escherichia coli*	Nil	Nil

*Repeat study after 3 months (90 days) did not show any appreciable deviation from the above initial results. [#]API: Ayurvedic Pharmacopoeia of India.

Heavy metal contents

The observations have been depicted in the Table 4 below:

Table 4: Permissible limits and observed heavy metal contents of formulation prepared from *B.acutangula* leaf.

Sl. no.	Heavy metals	Permissible limits (ppm) as per API[#]	Results (ppm)
1	Arsenic	3	0.06
2	Lead	10	2.36
3	Cadmium	0.3	< 0.05*
4	Mercury	1	< 0.05*

*Below the limit of quantization i.e., 0.05 ppm. [#]API: Ayurvedic Pharmacopoeia of India.

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CHAPTER-V

Acute Toxicity Study (LD_{50} Determination)

Toxicology is a branch of Pharmacology which describes undesired effect of medicinal agents on living organisms. Toxicity study is focused on the complications caused by the therapeutic use of a medicine. Attention must be taken towards solvent which may cause toxic effects. Animal care during toxicity tests is very important[1].

Ancient uses of natural agents can be divided into three categories as follows:

a) Previously known drugs which have been consumed for many years.

b) Not much known inside country but for which international experience is required.

c) This type represents a new compound for safety and efficacy.

First group consists mainly foodstuff products which have been consumed for a large period of time as traditional herbal remedies.

For the second category, it is necessary that needs to prove its reference in scientific literature confirming that the product is safe.

In case of third category, here it has been faced that a product not beforetestedtowards toxicological profile and should have to be investigated[3].

LD_{50} Value

TheLD_{50}Valuecan be defined as the dose of medicinal agent, which mainlykills one-half of population of the same species and strain. The median effective dose or ED_{50} is the dose (mg/kg) can be defined as which produces a desired efficacy in 50 percent of the test population[4]. In my present study, lethal dose (LD_{50}) of the propanol extract of *Barringtonia acutangula*in oral route was tested. Determination of the acute toxicity study is primary for the toxicological investigations of unexplored substances.

Acute toxicity was investigated on the plant extract by Up and Down procedure (UDP) as laid down by the guidelines provided by **OECD** (Organization for Economic Co-operation and Development)[5]. OECD guidelines for Testing of Medicines were reviewed for scientific progress. The idea of Up and Down testing protocol was first described by Bruce who implemented this method to investigate the acute toxicity of chemicals[6].

This protocol is very easy to apply the substances which can cause death within 24 to 48 hours.. A limit test can be done to investigate the medicinal agents which are mainly show low toxicity[7]. During this test all animals must be observed carefully for up to 48 hours before determining that the proper dose to be administered for the next animal[8].

DESIGN OF THE EXPERIMENT

MATERIALS & METHODS

Animals

Swiss albino mice (20-25 g) of male or female animals were taken for this investigation. The animals were divided into different groups and were placed in polyacrylic cages (37×21×11 cm) and were maintained under standard laboratory conditions (temperature 25±3° C with dark and light cycle 14/10 h) for seven days before staring of experiment. They were allowed to take standard dry pellet diet from Hindustan Lever, Kolkata, India and water *ad libitum*.

Preparation of Doses

In animals, volume must not be exceeded 1 ml/100 mg of animal's weight. In mice, volume must not be exceeded 1ml/100 g of animal's weight.

Procedure

The acute toxicity of propanol extract was determined according to the guideline No. 420 of OECD. Male Swiss albino mice were weighed 20-25 g were administered for this experiment. The extract was administered to different groups of animals at different doses in increasing order by oral route. All animals were observed for 14 days for their general behavior as well as mortality.

Observations

Each and every animal was observed during the 30 minutes after dose administration, during the 24 hours then activity was observed for 48 hours. Observations should include changes in skin, eyes and also respiratory, circulatory, autonomic and central nervous systems and behavioral pattern. Attentions must be directed to observe tremors, convulsions, salivations, diarrhea, lethargy, sleep and coma. 50 percent death had been observed at the dose of the 1000 mg/kg.

RESULT & CONCLUSION

After completion of acute toxicity studies it had been observed that the LD_{50} value of propanol extract had been found to be 1000 mg/kg b.w by oral route.

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CHAPTER VI

Evaluation of *In Vitro* Antioxidant Activity

Antioxidant protects and is added to food substances to inhibit the free radical inmedicinal substances[1,2]. Reactive Oxygen Species causes destructive and damage towards the components of cellular materials like proteins, lipid sand DNA and causes diseases like inflammation, genotoxicity, diabetes, cancer[3,4].

Antioxidants supplements can be administered to protect the human body toreduce oxidative stress[5].

Plants produced antioxidants have been proved to combat with oxidative stress in human.

REAGENTS AND PROCEDURES

Chemical

Ammonium thiocyanate, ferric chloride, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), Vitamin C, nitroblue tetrazolium (NBT), thiobarbituric acid (TBA), phenazine methosulfate (PMS),tricholoacetic acid (TCA), and potassium ferric cyanide were purchased from Sigma Chemical Co Ltd.. (Mumbai, India). All chemicals were of analytical grade.

Inhibition of free radical scavenging potential measured by 1, 1-diphenyl-2-picryl-hydrazil

The free radical scavenging potential of propanol extract was measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH) by using the reported protocol[6]. 0.1 mM solution of DPPH in propanol was prepared and 1 ml the solution was added to 3 ml of different concentration of propanol extract solution in propanol (10, 20, 40, 80, 160 & 320 µg/ml). The mixture had been shaken vigorously and was allowed to stand at room temperature for 30 min. Absorbance had been measured at 517 nm using Ultra Violet spectrophotometer. The absorbance indicated higher free radical scavenging potential. Ability to scavenge the DPPH radical had been calculated by using th following formula:

DPPH scavenging activity (%) = $[(A_0 – A_1) / A_0) \times 100]$

Where, A_0 is the absorbance of the control and A_1 is the absorbance of presence of propanol extract sample & standard.

Inhibition of Super oxide anion radical

Measurement of super oxide anion scavenging activity of propanol extract was based on the protocol described[7]. 1 mlnitroblue tetrazolium (NBT) in 100 mM phosphate buffer, pH 7.4 and different concentrations (10, 20, 40, 80, 160, 320 µg/ml) of the extract in distilled water were mixed. The reaction initialized with the addition of 100 µl phenazine methosulfate in 100 mM phosphate buffer, pH 7.4 to the sample. The sample was incubated at 28 °C for 10 min and

absorbance was read at 560 nm against blank. A decreased in the absorbance of sample indicated increased super oxide anion scavenging activity. Ascorbic acid had been used as standard drug.

Inhibition of Nitric oxide radical

Nitric oxide formed from sodium nitroprusside in water at physiological pH may react with oxygen to produce nitrite ions can beread by Griess reaction[8]. 5 ml of reaction mixture of sodium nitroprusside (10 mM) in phosphate-buffered saline (PBS) and different concentrations (10, 20, 40, 80, 160, 320 µg/ml) of propanol extract was incubated at 28° C for 160 min. After the incubation, 0.5 ml of the reaction mixture was removed and 0.5 ml of Gries reagent (1% sulphonilamide in 2% ortho phosphoric acid and 0.1 % naphthylethylene diamine dihydrochloride) were added. The absorbance was read at 548 nm. The percentage inhibition of nitrite oxide formed had been read by comparing the absorbance values of control and different concentrations of propanol extract.

Determination of inhibition of lipid peroxidation[9]

Animal liver had been collected, homogenized with 40 mM Tris-HCl buffer (pH 7.0) and centrifuged at 3000 g for 10 min to get clear supernatant. To 100 µl of each of 0.15 M KCl, 15 mM $FeSO_4$ and 6 mM Vitamin C were added and was incubated at 37 °C for 1 hr. After incubation 1.0 ml TCA (10%) was added to the reaction mixture and the sample was centrifuged at 3000 g for 20 min at 4° C. 3 ml supernatant was taken and 1.0 ml TBA was added to this fraction followed by heating on water bath at 60° C for 15 min. After cooling the reaction complex had been mixed with organic solvent and absorbance was had been read at 536 nm. Percentage inhibition of lipid peroxidation had been measured by comparing the absorbance values of control and different concentrations of propanol extract.

Evaluation of phenolic contents

Phenolic contents in *Barringtonia acutangula* leaf were measured with Folin-Ciocalteu reagent. 2 mg of the propanol extract solution had placed in a 100 ml Erlenmeyer flask diluted with distilled water (45 ml). 2 mlFolin-Ciocalteu reagent was added and the content of the flask was mixed properly. After 5 min, 2 ml of Na_2CO_3 (2%) was added, then the mixture was allowed to stand for 1 h with occasional shaking. The absorbance had been read at 750 nm in a spectrophotometer[9]. The amount of phenolic components in leaves of *Barringtonia acutangula* had been estimated in the standard pyrocatechol graph:

Absorbance = 0.001 × pyrocatechol (µg) + 0.0036

RESULTS & DISCUSSIONS

Different antioxidant models are used to estimate antioxidant potential towards mechanism of action of antioxidants. Mostly used models for the evaluation of antioxidant property are free

radical scavenging, superoxide anion radical scavenging and nitric oxide radical inhibition activities and evaluation of phenol compounds.

Antioxidant potential of propanol extract

Antioxidant potential of rat liver peroxidation due to the presence of bioactive moleculeslike flavonoids and biflavones was recorded. Therefore, the present experiment concludes the antioxidant potential of propanol extract can be caused to chelation of metal ions due to the presence of bioactive molecules.

Inhibitory potential of scavenging of DPPH radical

DPPH radical model is largely utilized and comparatively quick method for the detection of antioxidant potential. The mechanism of antioxidant on DPPH radical scavenging is primarily due to the hydrogen-donating ability. The absorption maximum of DPPH radical was 520 nm. The decreased in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecules which results in the scavenging of the radicals by hydrogen donation. It was observed that as a change in color from purple to yellow. So, DPPH is generally used as a substrate to detect the antioxidant potential of antioxidant[9]. It was reported that oxidative stress, which occurs due to free radical generation and causes chronic conditions such as arteriosclerosis[10].

Results of the study proved that propanol extract was effective as free radical scavenger. Table 1 illustrated sharp decrease in concentration of DPPH radicals due to the scavenging ability of propanol extract.

Inhibition of superoxide anion radical

Superoxide anion radical (O_2^-) generally produced from activated phagocytes like macrophages, monocytes, eosinophils and neutrophills and the production of O_2^- is a crucial factor for killing bacteria by phagocytes. In this protocol, superoxide anion, derived from dissolved oxygen from the reaction of PMS-NADH which reduces NBT[11]. Decline in absorbance at 566 nm with propanol extract indicated the consumption of free radical in the reaction mixtures; Table 2 proved the percent of inhibition of superoxide radical production by 20, 40, 60, 80, 100 µg/ml propanol extract compared with Ascorbic acid. All the concentrations of propanol extract showed a potential actionons uperoxide radical scavenging activity when compared to standard.

Inhibition of nitric acid (NO^-) radical

Nitric oxide can cause ischemic renal injury to cause more damage. High concentration of nitric oxide (NO) has harmful effects, so it is necessary that the production of NO is to strictly monitored[12]. When NO is generated by macrophages, the nitric oxide radical may be converted into

peroxynitrites, which happen diverse chemical reactions such as nitration of tyrosine residue of protein, triggering lipid peroxidation, [13].

Table 3 described percentage inhibition of nitric oxide generation. Propanol extract showed potential inhibition of nitric oxide generation *in vitro* conditions.

Inhibition of lipid peroxidation

Propanol extract retarded the peroxidation of linoleic acid (LH). The retardation of LH peroxidation by propanol extract had been found to be due to termination of chain reaction[14]. Table 4 depicted percentage inhibition of lipid peroxidation of the different concentrations of propanol extract compared with standard drug Ascorbic acid.

Effects of phenolic components

Phenolic constituents are very important natural secondary metabolites due to their scavenging ability of free radicals[15]. Numerous experiments have provedthe therapeutic efficacy of phenolic components. It had been also seen that phenolic components coupled with antioxidant potential and showed an important role for stabilizing lipid peroxidation. 1ml propanol extract contained 120.7 µg of pyrocatechol. It can be said that up to 1 g of polyphenolic compounds consumed daily basis can shoe inhibitory action on mutagenesis in humans[16].

CONCLUSION

Based on the observation of the above study, it can be said that, propanol extract had shown potential *in vitro* antioxidants potential against different free radical scavenging model.

Table 1:DPPH scavenging potential of propanol extract of *Barringtonia acutangula* leaves

Treatment	Concentration (µg/ml)	% DPPH scavenging action	IC_{50} Value (µg/ml)
PEBA	10, 20, 40, 80, 160 & 320	25.53, 33.23, 42.61, 54.25, 72.82, 83.36	47.96
Vitamin C	2, 4, 6, 8, 10 & 15	25.70, 32.10, 42.50, 69.20, 92.20 & 97.23	7.06

Table 2:Super oxide inhibition action of the propanol extract of *Barringtonia acutangula* leaves

Treatment	Concentration (µg/ml)	% Super oxide scavenging action	IC_{50} Value (µg/ml)
PEBA	10, 20, 40, 80, 160 & 320	36.18, 45.92, 54.15, 66.13, 75.45, 85.35	20.42
Vitamin C	2, 4, 6, 8, 10 & 15	25.62, 35.25, 54.35, 69.23, 78.29 & 89.64	5.67

Table 3:Nitric oxide scavenging potential of propanol extract of *Barringtonia acutangula* leaves

Treatment	Concentration (µg/ml)	% of Nitric oxide scavenging action	IC_{50} Value (µg/ml)
PEBA	10, 20, 40, 80, 160 & 320	22.69, 36.18, 48.65, 54.19, 70.18, 81.13	40.31
Vitamin C	2, 4, 6, 8, 10 & 15	28.40, 45.82, 64.47, 76.87, 86.50 & 95.32	4.42

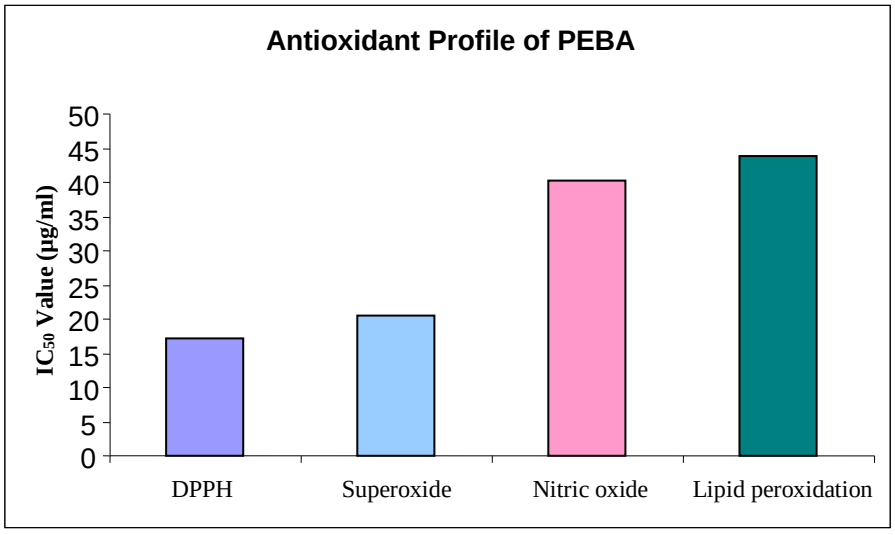
Table4: Lipid peroxidation scavenging potential of propanol extract of *Barringtonia acutangula* leaves

Treatment	Concentration (µg/ml)	% inhibition lipid peroxidation	IC_{50} Value (µg/ml)
PEBA	10, 20, 40, 80, 160 & 320	29.63, 36.27, 45.68, 59.25, 79.82, 89.36	43.81
Vitamin C	2, 4, 6, 8, 10 & 15	27.54, 38.58, 48.98, 60.43, 75.19 &82.34	6.12

Table5: Total Phenolic content present in propanol extract of *Barringtonia acutangula* leaves

Absorbance	Pyrocatechol Content (µg)/1000 vs of extract	Average (µg)
0.124	121.76	121.07±16.09
0.140	135.73	
0.108	105.72	

Figure 1:IC_{50} Values of the propanol extract of *Barringtonia acutangula* leaves against DPPH, Superoxide, Nitric oxide and Lipid peroxidation Models



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CHAPTER VII

Determination of Hypoglycemic action of Propanol Extract of *Barringtonia acutangula*

Diabetes mellitus is a metabolic disorder and characterized by hyperglycemia, glycosuria, and in some cases ketonemia. The minimum defining feature to detect diabetes mellitus is substantiated elevation of circulation glucose concentration[1]. In Etiology, the terminology diabetes is defined as to "pass through: a siphon". The word diabetes was derived from Greek word, "dia" means "through" and "betes" means "pass". Mallitus comes from the Greek word "sweet".

MATERIALS & METHODS

Drugs and chemicals

Streptozotocin (STZ), nitroblue tetrazolium (NBT), Glibenclamide;. All chemicals were of analytical grade.

Animals used

Wistar albino rats weighing 150-180 g were used throughout the study. The animals had been kept inside thepolypropelene cages and were kept under laboratory conditions (temperature 26±5° C, dark/light cycle 14/10 hours). Animals were allowed free access to food and water *ad libitum*. Animals had been acclimatized towards laboratory condition for 7 days before commencement of experiment.

Induction of Diabetes in Experimental Animals

Diabetes had been induced by intraperitoneal (i.p.) route of freshly prepared streptozotocin (STZ) (50 mg/kg. b.w) in ice cold citrate buffer (0.1 M, pH 4.5) to overnight-fasted rats[2]. After 2 days, fasting blood glucose levels had been measured and the animals showed blood glucose level ≥ 220 mg/dl had been used for experimental investigation[3].

Evaluation of fasting blood glucose (FBG) level

The animals had been divided into five groups (n = 6). Group I served as normal saline (5 ml/kg b.w.p.o.) control. Group II was diabetic (STZ) control. Group III and IV were obtained propanol extract of *Barringtonia acutangula*(100 and 200 mg/kg b.w., p.o. respectively) and group V were provided standard glibenclamide (0.5 mg/kg b.w., p.o.) daily for 15 days. Fasting blood glucose had been measured on 0^{th}, 5^{th}, 10^{th} and 15^{th} day with the help of glucometer (Accu-check®). 24 h of the last dose blood had been collected from overnight fasted animals of every group for evaluation of serum biochemical parameters like SGPT, SGOT, ALP, serum cholesterol.

Observation of body weight, liver and kidney weight

Body weights of animals of every group had been examined just before and 15

days after treatment. Liver and kidney weights of all animals were measured after post treatment sacrifice.

Evaluation of serum biochemical parameters

Collected blood had been used for evaluation of serum biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), serum cholesterol with the help of commercial kit.

OBSERVATIONS

Evaluation of fasting blood glucose (FBG) level

The fasting blood glucose reading of normal, diabetic treated animals were depicted in Table 1. Streptozotocin 50 mg/kg can form hyperglycemia as evidence seen significant increase in FBG parameter in Streptozotocin control group when comparing with normal control group. Administration of PEBA in STZ-induced diabetic animals at doses of 100 and 200 mg/kg b.w .showed potential and dose dependent decline in blood glucose levels while comparing with Streptozotocin control group. The FBG decreasing result by PEBA at the dose of 200 mg/kg b.w. had been observed to be more potent to that of the standard drug glibenclamide (0.5 mg/kg b.w.).

Body weight, liver and kidney weight

Body weight, liver and kidney weights of animals from Streptozotocin control group (after 15 days) had been slightly reduced when comparing with normal control group. PEBA at the dose 100 and 200 mg/kg b.w. improved body weight, liver and kidney weights to normal in a dose dependent manner comparison with STZ control (Table 2).

Estimation of serum biochemical parameters

Biochemical observations such as SGOT, SGPT,SALP and serum cholesterol in the Streptozotocin control group had been elevated while comparing to the normal control group. Treated with PEBA of dose of 100 and 200 mg/kg b.w. caused SGOT, SGPT, SALP and serum cholesterol levels to normal level in a dose dependent manner.

DISCUSSION

Streptozotocin is an antibiotic isolated from *Streptomyces sp*. Streptozotocin causes destruction of pancreatic β cell; therefore, it had been largely administered to produce diabetes mellitus in study animals[7]. After administration of Streptozotocinit

causes decomposition to generate isocyanate and methyl-diazohydroxide compound[8,9]. Streptozotocin destroy pancreatic β cells as well asdamagenephrons, hepatocytes and cardiomyocytes[10,11].

Hyperglycemia was studied after 2 days of Streptozotocin -injection. Treated with PEBA in Streptozotocin-administered animals, at both test doses started decreasing fasting blood glucose levels after 5 days and made them non-diabetic after 15 days. PEBA proved marked potential for controlling loss of body weight, liver and kidney weights of animals.

15 days treatment of PEBA controlled all the mentioned parameters to normal level in a dose dependent manner[12]. In my present study the increased serum cholesterol parameter in diabetic animals had been normalized by treating with PEBA. The results proved that propanol extract may inhibit the pathway of cholesterol synthesis in diabetic animals[13].

In my present study, administration of PEBA to Streptozotocin-induced diabetic animals explained sharp reduction in blood sugar level, normalization of serum biochemical parameters compared with Streptozotocin control rats.

Therefore, it can be said that propanol extract of *Barringtonia acutangula*(PEBA) leaf is potentially effective towards streptozotocin-induced diabetes in Wistar rats for its antioxidant potential.

Table: 1. Effect of propanol extract of *Barringtonia acutangula* on fasting blood glucose level (mg/dL)

Group	Dose	Day 0	Day 5	Day 10	Day 15
I (Normal)	5 ml/kg	78.29 ± 1.13	72.25 ± 5.26	72.56 ± 5.63	72.23 ± 3.92
II (STZ)	50 mg/kg	266.45 ±8.23*	279.83 ± 9.54*	275.66 ± 9.45*	289.65 ± 7.81*
III (STZ + PEBA)	100 mg/kg	261.45 ± 9.95	110.54 ±6.81**	91.03 ± 2.63**	87.66 ± 3.85**
IV (STZ + PEBA)	200 mg/kg	265.45 ± 8.51	89.59 ± 6.45**	82.27 ± 5.26**	73.66 ± 1.91**
V (STZ + Gliben.)	0.5 mg/kg	256.65 ± 4.35	92.63 ± 2.83**	79.54 ± 2.58**	69.29 ± 1.44**

Values were expressed as mean ± SEM (n = 6); *p<0.001 while comparing normal control and **p<0.001 compared with STZ control group.

Table: 2. Effect of propanol extract of *Barringtonia acutangula* on body weight,liver weight and kidney weight in animals

Group	Dose	Initial body wt (g)	Final body wt (g)	Liver wt (g)	Kidney wt (g)
I (Normal)	5 ml/kg	175.45 ± 3.83	182.67 ± 6.24	7.01 ± 1.92	2.08 ± 1.35
II (STZ)	50 mg/kg	166.33 ± 3.22	179.55 ± 5.54	2.95 ± 1.36*	0.94 ± 3.14
III (STZ + PEBA)	100 mg/kg	165.46 ± 5.26	169.72 ± 3.95	6.83 ± 2.38**	2.01 ± 4.56
IV (STZ + PEBA)	200 mg/kg	169.36 ± 6.28	172.66 ± 2.86	6.92 ± 2.62**	2.23 ± 7.22
V (STZ + Gliben.)	0.5 mg/kg	172.77 ± 3.54	178.34 ± 2.36	6.98 ± 2.15**	2.66 ± 4.63

Values are expressed as mean ± SEM (n = 6); *p< 0.001 compared with normal control and **p< 0.001 while comparingtostreptozotocingroup

Table: 3. Effect of propanol extract of *Barringtonia acutangula* on serum biochemical parameters

Group	Dose	SGOT (IU/L)	SGPT (IU/L)	SALP (IU/L)	Cholesterol (mg/dL)
I (Normal)	5 ml/kg	19.99 ± 6.91	23.81 ± 2.81	170.41 ± 13.24	149.66 ± 4.61
II (STZ)	50 mg/kg	44.54 ± 3.53*	41.54 ± 3.54*	256.45 ± 11.81*	204.61 ± 9.81*
III (STZ + PEBA)	100 mg/kg	27.92 ± 2.63**	29.63 ± 2.75**	218.29 ± 13.36**	191.83 ± 9.83**
IV (STZ + PEBA)	200 mg/kg	24.36 ± 2.36**	25.81 ± 2.37**	196.36 ± 15.61**	186.54 ± 8.27**
V (STZ + Gliben.)	0.5 mg/kg	22.54 ± 6.92**	26.83 ± 1.73**	169.27 ± 9.81**	159.72 ± 6.58**

Values were expressed as mean ± SEM (n = 6); *p< 0.001 compared with normal control and **p< 0.001 while comparingtostreptozotocin group.

Effect of propanol extract of *Barringtonia acutangula* on fasting blood glucose level [Fig. 1], liver and kidney weight [Fig. 2], SGOT, SGPT, ALP and Cholesterol Level [Fig. 3] in normal and diabetic rats

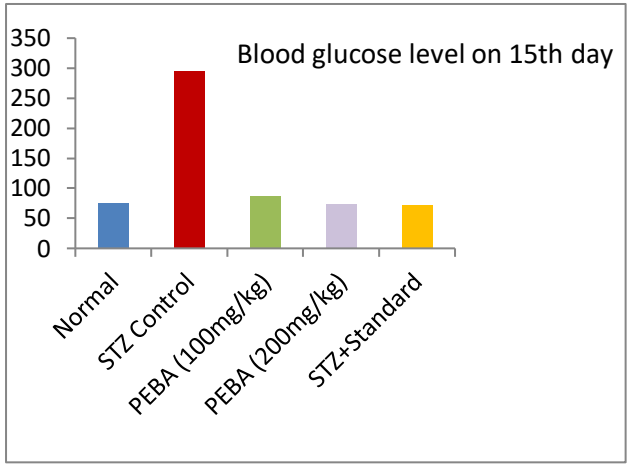

Figure: 1

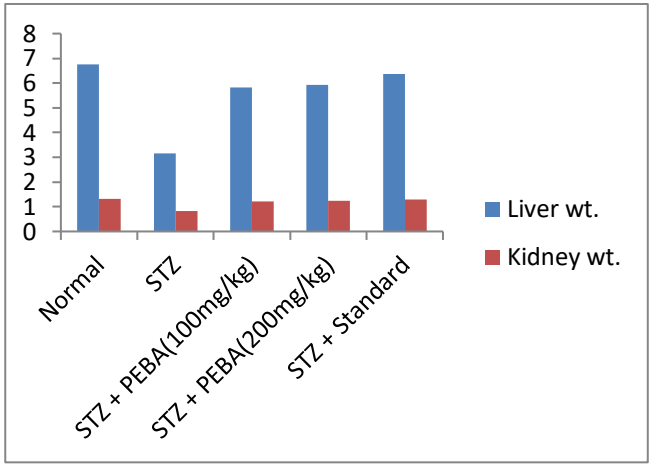

Figure: 2

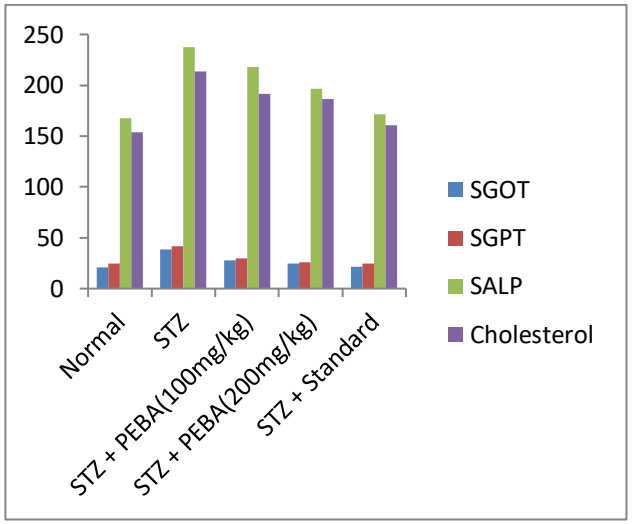

Figure: 3

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SUMMARY
&
CONCLUSION

The importance of plant products as a source of new therapeutic or other useful agent needs no introduction. Though the emphasis shifted to synthetic drugs and those of microbial origin since the middle of the last century, of late these has been a revival of interest in traditional medicines even in the developed countries. As a result the consumption of medicinal plants has almost doubled in Western Europe during the past decade. Many of the modern clinicians are now more inclined towards the use of traditional medicine. For instance, plant-drugs constitute approximately 25% of all the prescribed medicines even today in the most advanced countries like the USA.

The World Health Organization (WHO) has compiled a list of 20,000 medicinal plants used in different parts of the world. According to an estimate of the WHO, about the 80% of world's population rely on plant based medicines for their health care particularly the rural people in developing countries. By the mid-1980s most of the pharmaceutical manufacturers had abandoned exploring folk uses of plants in their search for new drugs. Now however, the pendulum is beginning to swing back towards an appreciation that plants used in traditional medicine can serve as a source of novel therapeutic agents.

Therefore, the present study is conceptualized on the basis of finding of the standardization of the extract and the formulation along with the different pharmacological activity and safety profile of the leaf of *Barringtonia acutangula* belonging to family Lecythidaceae. In India, the plant has been traditionally used for several medicinal purposes. The fruits of the plant are used as a tonic. Externally its leaf paste is used as a cover on sores and ulcers. The bark is antidysenteric, antipyretic, astringent, cardiotonic, lithotriptic, and tonic; a powder of the bark acts as diuretic in cirrhosis of liver and gives relief in symptomatic hypertension. A decoction of bark made with milk is given every morning on an empty stomach, or its powder with milk, as a cardiotonic. The powder of the bark is also given with honey in fractures and contusions with echymosis. Beside this, the extract of the bark as astringent is used for cleaning sores, ulcers, cancers etc. The extract of the bark is prescribed in scorpion-stings and lowering blood glucose. But, no pharmacological investigation along with its bioactive molecule is still reported on the propanol extract of *Barringtonia acutangula* leaf and its formulation.

Therefore, the present study is aimed to investigate the unexplored standardization parameters for the propanol extract as well as its classical formulation along with the pharmacological actions and toxicity profile of *Barringtonia acutangula* leaf in rodents.

The leaves of *Barringtonia acutangula* were collected during January 2023 from Nadia, West Bengal, India. The plant species was authenticated at Botanical Survey of India, Central National Herbarium, Howrah, West Bengal, India. The voucher specimen was maintained in our laboratory for future reference. The powdered plant material was defatted with petroleum ether and then the marc was extracted with propanol and the percentage of yield of the propanol extract was 21.45% w/w. Preliminary phytochemical studies of leaf extract revealed the presence of alkaloids, triterpenoids, tannins and flavonoids.

Repeated chromatographic purifications of the fractions and preparative thin layer chromatography led to provide the different retention factors for different bioactive compounds present in the extract as well as in the formulation. The LD_{50} values of the methanol extract of the leaves of the *Barringtonia acutangula* was found to be 1000 mg/kg in mice.

The propanol extract of *Barringtonia acutangula*(PEBA) leaves had shown profound antioxidant role on different antioxidant models like DPPH, Nitric oxide, Superoxide scavenging activity. Also the extract had very good reducible property and retarded the peroxidation of linoleic acid in lipid peroxidation model.

The anti-diabetic activity of the propanol extract of *Barringtonia acutangula*(PEBA) leaves had been seen in Streptozotocin induced diabetic rats. Administration of PEBA in STZ-induced diabetic rats produced dose dependently fall in blood glucose levels as compared with the STZ-control group. PEBA showed marked effect in controlling the loss of body weight, liver and kidney weights of diabetic rats. Elevation of serum biomarker enzymes such as SGOT, SGPT and ALP was observed in diabetic rats indicating impaired liver functions which may be due to hepatic damage. The decreased total protein content in STZ-induced animals also substantiated the hepatic damage by STZ. Treatment with PEBA restored all the above mentioned parameters towards the normal levels in a dose dependent manner. The elevated level of serum cholesterol in diabetic rats was also normalized after PEBA treatment.

After the method development & validation of the developed method we can conclude that this set of parameters can be taken as standard for the further researches. Overall, this research aims to bridge the gap between traditional Ayurvedic knowledge and modern scientific evidence, thereby offering new prospective on the potential use of *Barringtonia acutangula* leaf. Furthermore, research is needed to establish the classically claimed therapeutic uses of this leaf like in Diabetes & Metabolic disorders.