2026 Volume: 02 Issue: 03 www.ijrpa.com ISSN 2456-9995 Research Article

International Journal Research Publication Analysis

Page: 01-26

PHYTOCHEMICAL STUDY OF METHANOLIC EXTRACT OF *SYZYGIUM SAMARANGENSE* STEM BARK

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Article Received: 14 February 2026
Article Revised: 05 March 2026
Published on: 25 March 2026

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DOI: https://doi-doi.org/101555/ijrpa.3781

ABSTRACT

Herbal medicine has been widely used for centuries in traditional systems such as Ayurveda, Unani, and Siddha, and continues to play a significant role in modern healthcare. A large proportion of the global population relies on plant-based remedies due to their effectiveness, cultural acceptance, and relatively fewer side effects. The present study focuses on the pharmacognostic, phytochemical, and in vitro antidiabetic evaluation of the methanolic extract of Syzygium samarangense stem bark. The plant Syzygium samarangense, belonging to the family Myrtaceae, is known for its diverse medicinal properties, including antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities. In this study, the stem bark was collected, authenticated, shade-dried, and subjected to various pharmacognostic evaluations such as organoleptic, microscopic, and physicochemical analysis. Parameters like moisture content, ash values, extractive values, foaming index, swelling index, and fluorescence analysis were determined to establish the quality and purity of the crude drug. Phytochemical screening of the methanolic extract revealed the presence of important bioactive constituents such as alkaloids, flavonoids, phenolic compounds, glycosides, tannins, saponins, and terpenoids, which are responsible for various pharmacological activities. Quantitative estimation of total phenolic, flavonoid, tannin, and glycoside content was also carried out. Additionally, GC-MS analysis was performed to identify the chemical constituents present in the extract. The in vitro antidiabetic activity was evaluated using α-amylase and α-glucosidase

inhibition assays. The results indicated that the extract possesses significant inhibitory activity, suggesting its potential role in controlling postprandial hyperglycemia.

INTRODUCTION

Traditional medical systems such as Ayurveda, Unani, and Siddha have long used herbal treatments to treat a variety of health issues. In these systems, plant-based medications were the primary form of healthcare, passed down through generations. The continued use of these cures indicates their long history and the trust built up over years of practical experience, with many herbal medicines having well-established traditional value and use.

About 75–80% of people worldwide still rely on herbal medications for primary healthcare, mostly in underdeveloped nations, due to their greater cultural acceptability, more compatibility with human bodies, and less adverse effects. According to the World Health Organization (WHO), between 75 and 80 percent of people worldwide still rely heavily on herbal remedies for their medical needs. The cultural acceptance of herbal treatments, their compatibility with the human body, and their relatively lower incidence of adverse effects are the main reasons for their widespread use.

HERBS AS MEDICINE

Herbal medicine is defined by the World Health Organization (WHO) as the use of herbs, herbal materials, herbal preparations, and final herbal products that comprise plant parts or other plant elements or combinations. Plant parts like leaves, stems, flowers, roots, and seeds are the source of these herbs.

Herbal medicine is also known as herbalism or phytomedicine. It is the study of use of medicinal plants. It includes modern standards of testing of herbs and medicines derived from natural sources, few high-quality clinical trials and standards of purity. Practice of using herbs and herbal preparation to maintain health and to prevent alleviate, or cure disease or a plant or a plant part or an extract or mixture of these used in herbal medicine.

ANTI-DIABETIC

Diabetes mellitus is a collection of metabolic illnesses that affect a large number of people around the world. It is characterized mostly by chronic hyperglycaemia caused by insulin secretion or action abnormalities. It is predicted that the global diabetes population would reach 366 million by 2030. Despite the fact that diabetes cases are increasing on a daily basis, no other method of therapy has been successfully established thus far. Thus, the current study aims to provide insight into the pathophysiological and etiological

elements of diabetes mellitus, as well as the treatments available for this metabolic condition. A brief overview of diabetes mellitus and the experimental screening model, together with its pertinent mechanism and importance, are also included in the review. Alloxan and streptozotocin are mostly utilized to assess a drug's antidiabetic efficacy. A list of medicinal plants that have been evaluated for their ability to prevent diabetes in rats with alloxan-induced diabetes is included in this reviewIn order to reduce the complications associated with diabetes and related disorders, the information presented in this study will assist researchers in developing alternatives to insulin and oral hypoglycaemic medications for the treatment of diabetes mellitus.

FLAVANOID CONTENT

The most prevalent polyphenols found in plant-based meals are flavonoids. They are characterized by a 15-carbon skeleton, arranged as C6-C3-C6, with variable substitutions making up the distinct subclasses. Bioactive food components called flavonoids may improve health and aid in the prevention of chronic illness. In the field of vascular health, where a meta-analysis showed improvements in blood pressure and flow-mediated dilatation, there is the strongest evidence for preventing chronic diseases. There is no known flavonoid deficient illness.

PHENOLIC CONTENT

Phenolic content refers to the total amount of phenolic compounds present in a plant extract, food material, or biological sample. Phenolic compounds are a large and diverse group of secondary metabolites characterized by one or more hydroxyl (–OH) groups attached directly to an aromatic ring. These compounds include simple phenols, phenolic acids, flavonoids, tannins, lignans, and stilbenes. They play an important role in plant physiology by providing protection against ultraviolet radiation, pathogens, and herbivores, and they also contribute to pigmentation, growth regulation, and structural integrity of plant tissues.

GC-MS

Gas chromatography–mass spectrometry (GC–MS) is an analytical method that combines the properties of gas-chromatography and mass spectrometry to identify distinct chemicals within a test sample. Applications of GC–MS include drug detection, fire investigation, environmental analysis, explosives investigation, food and flavour analysis, and identification of unknown materials, including that of material samples collected from planet Mars during probe expeditions as early as the 1970s. GC–MS can also be used in airport security to detect drugs

in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously considered to have disintegrated beyond identification. Like liquid chromatography–mass spectrometry, it allows analysis and detection even of minute amounts of a chemical.

1. PLANT PROFILE

Syzygium samarangense, which belongs to Myrtaceae family, is widely cultivated in Asia and the Pacific region. This plant is generally known as Jambu Semarang, java apple, java rose apple, mountain apple, Samarang rose apple, wax Jambu, wax apple, jambul, Malay apple, chompu-khieo, or makopa.[13] The bark, fruit, and flower have pharmacological properties such as antibacterial, anticancer, antidiabetic, anti-inflammation, anti-mutation, antinociceptive activity, antioxidation activities, antiulcerogenic effect, and wound healing activity.

Fig.no 2.1 *Syzygium samarangense.*

Botanical Source: ***Syzygium samarangense***

Family: Myrtaceae

Taxonomic position

Kingdom	**Plantae**
Sub Kingdom	Tracheobionta
Super Division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Order	Myrtales
Family	Myrtaceae
Genus	Syzygium
Species	samarangense
Scientific name	*Syzygium Samarangense*

Common names

Kannada: Pannerale Telugu: Neredu Marathi: Jaman Malayalam: Chambekka

Description

- **Tree:** Grows to 12 meters tall, with a short, crooked trunk that often branches low to the ground.
- **Leaves**: Evergreen, aromatic, and oval-shaped. When crushed, they have a distinct smell.
- **Bark**: Brownish - gray in colour.
- **Flower**: White to yellowish-white, with four petals and numerous stamens.
- **Fruit**: 4–6 cm Bell Shape, colours ranging from white, pale green, or green to Red, Purple, or crimson. The skin is thin, and the flesh is white, spongy, and juicy.
- **Seeds:** Contains one to two seeds, which are small and rounded.
- **Stem:** Woody, erect, and forms the main supporting axis of the plant. It is moderately thick and cylindrical, with a smooth to slightly rough outer surface.

USES

- Compounds like **vescalagin** from the fruit have shown potential to **improve insulin resistance** and glycemic metabolism in experimental models.
- Extracts also protected pancreatic β-cells and improved insulin and glucose markers in diabetic rat studies.
- Plant parts used for diarrhoea and dysentery due to their astringent properties.
- Flowers and fruit are traditionally used to reduce fever.
- Bark decoctions are applied to wounds and skin itches.

Fig. no 2.2 Flower of *Syzygium samarangense*

Fig no.2.3 Fruit of *Syzygium samarangense*

Fig. No 2.4 Stem of *S. samarangense*

Fig. No 2.5 Whole tree of *S. samarangense*

2. AIM AND OBJECTIVES

AIM:-

To carry out Pharmacognostic and phytochemical, invitro anti diabetic activity of methanolic extract of *Syzygium samarangense* stem bark

OBJECTIVE:-

- To identify, collect & authenticate the plant *Syzygium samarangense*.
- To carry out pharmacognostic studies.
- To extract the drug using methanol by maceration.
- Phytochemical study on of methanolic extract of *Syzygium samarangense* stem bark.
- Pharmacological study on methanolic extract of *Syzygium samarangense* stem bark.

3. MATERIALS AND METHODS

I. PLANT COLLECTION AND DRYING

The stem barks of the plant *Syzygium samarangense* were collected from Kasaragod. The plant material was taxonomically identified by the botanist, Dr. Biju P, Associate professor, Department of Botany, Government College Kasaragod. The bark was dried under shade for about 7 days and then powdered with mechanical grinder and stored in an air tight container.

II. PHARMACOGNOSTICAL STUDIES

1. Organoleptic evaluation.

Organoleptic evaluation can be done by means of organs of sense. This refers to the evaluation of drug by colour, odour, size, shape, taste and special features including touch, texture etc. For this purpose, authentic specimen of the material under study and sample of Pharmacopoeia quality should be available to serve as a reference. However, the judgment based on the sensory characteristics like odour, taste etc.

Colour:

The untreated samples were examined under diffused sunlight or an artificial light source with wave length similar to day light.

Size: Size was measured using graduated ruler in millimeters.

Odour and taste:

Samples were crushed by gentle pressure and can be examined by repeated inhalation of air over the material.

Texture and fracture:

The texture was examined by taking small quantity of material and rubbed in between the thumb and fore finger. Bent and rupture caused to the sample provided information of the brittleness and appearance of the fractured plane as fibrous, smooth, rough, granular etc.

2. Microscopic Evaluation

- **Histology of Bark**

Fresh barks were collected. Transverse sections were prepared by cutting with razor blade at right angle to the longitudinal axis of the material. The sections were transferred to a watch glass containing water with the help of a brush. Thick and oblique ones were rejected. The selected sections were then cleared by warming with few drops of 5% w/v of potassium hydroxide solution. The sections were washed using tap water and stained with phloroglucinol: concentrated HCl (1:1). The sections were then mounted using glycerin with cover slip on a clean glass micro slide and observed under microscope.

- **Powder Analysis**

For powder analysis the plant was collected and washed thoroughly with water to remove the unwanted matter. This was further dried in the shade. After complete drying, the plant was

powdered and passed through sieve no. 60. A small quantity of the powder was treated with phloroglucinol and conc. HCl (1:1) solution for the detection of various microscopic characters proving the authenticity of the drug. Another sample was mounted in water to see whether it contained calcium oxalate crystals and yet another sample in iodine solution to detect the presence of starch grains.

MACERATION

Fresh and healthy plant material of *Syzygium samarangense* (commonly known as wax apple) was collected, washed thoroughly with distilled water to remove adhering impurities, and shade-dried at room temperature until a constant weight was obtained. The dried material was then coarsely powdered using a mechanical grinder and stored in an airtight container. A known quantity of the powdered plant material was transferred into a clean, dry, stoppered container and soaked in a suitable solvent (methanol) in an appropriate drug-to-solvent ratio. The mixture was kept at room temperature for 3–7 days with occasional shaking to ensure proper penetration of the solvent and efficient extraction of phytoconstituents. After the maceration period, the extract was filtered using muslin cloth followed by Whatman filter paper to remove the marc. The residual plant material was pressed to recover the remaining solvent, and the combined filtrate was concentrated under reduced pressure or evaporated on a water bath to obtain the crude extract, which was then stored in a desiccator or airtight container for further phytochemical and pharmacological studies.

2. Determination of foreign matter

About 100-500 g dried bark of the plant to be examined was weighed and spread out in a thin layer. The foreign matter was detected. It was separated, weighed and the percentage of foreign matter was calculated.

3. Determination of moisture content

Five grams of the powdered bark were placed in a tared evaporating dish. Drying was carried out at 105℃ for 5 hours. The drying was continued at 1 hour interval until difference between two successive weighing corresponded to not more than 0.25%. Constant weight was reached when two consecutive weighing, after drying for 30 minutes and cooling for 30 minutes in desiccators, showed not more than 0.01g difference.

4. Determination of Ash value

The ash value is an important parameter for the evaluation of crude drugs, due to the variation

of values within fairly wide limits. The ash value of any organic material is composed of inorganic materials like metallic salts and silica. The following three different methods were adopted,

a. Total ash
b. Acid insoluble ash
c. Water soluble ash
d. Sulphated ash

Ashing involves an oxidation of the components of the products and a high ash value involves the contamination, substitution, adulteration or carelessness in the preparation of crude drugs for marketing.

a. Total ash

Two grams of powdered bark material were accurately weighed out in a crucible previously ignited for 30 minutes. The material was spread in an even layer and ignited at a temperature not more than 450°C until it was white indicating the absence of carbon. Cooled in desiccator and weighed. Calculated the content of total ash per gram of air-dried material.

$$\%\, Total\ ash = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

b. Acid insoluble ash

To the crucible containing the total ash, 25 ml of 2N hydrochloric acid was added, covered with a watch-glass and boiled gently for 5 minutes. The watch-glasses were rinsed with 5 ml each of hot water and added into the crucible. Collected the insoluble matter on an ashless filter-paper and washed with hot water until the filtrate was neutral. Filter-paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a desiccator for 30 minutes; then weighed. Calculated the content of acid-insoluble ash per gram of air-dried material. The results are given in

$$\%\, Acid\ insoluble\ ash = \frac{weight\ of\ acid\ insoluble\ ash}{weight\ of\ sample} \times 100$$

c. Water soluble ash

To the crucibles containing the total ash, 25 ml each of water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered-glass crucible. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C. The weight of this residue in mg was subtracted from the weight of the total ash.

$$weight\ of\ water\ soluble\ ash = weight\ of\ total\ ash - weight\ of\ water\ insoluble\ ash$$

d. Sulphated ash

One gram of powdered drug was accurately weighed and taken in a tared silica crucible which was previously ignited and weighed. The drug was ignited gently at first until the substance was thoroughly charred. It was then cooled, the residue was moistened with 1 ml of sulphuric acid, heated gently until the white fumes were no longer evolved and ignited at 800oC ± 25oC until all black particles have disappeared. The crucible was allowed to cool and weighed. **Determination of Extractive Values**.

This method determines the number of active constituents in a given amount of plant material when extracted with solvent. The extractive value is used as a means of evaluating crude drug which are not readily estimated by other means.

$$\%Extractive\ value = \frac{weight\ of\ extract\ obtained}{weight\ of\ sample} \times 100$$

- **Ether soluble extractive value**

Five grams of the coarsely powdered air-dried drug was macerated with100 ml of ether in a closed flask for twenty-four hours, shaking frequently for six hours and allowing to stand aside Dept of Pharmacognosy, MDCP PAGE 2 1 for eighteen hours. It was filtered rapidly, taking precautions against loss of solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flatbottomed shallow dish and dried at 105˚C to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air- dried drug.

- **Ethanol soluble extractive value**

Macerated 5 grams of coarsely powdered air-dried bark of *Syzygium samarangense* with 100ml ethanol in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and then allowed to stand undisturbed for another 18 hours. Filtered rapidly, by taking precaution against loss of alcohol. Then 25 ml of the filtrate was evaporated to dryness in a

tared flat bottomed shallow dish, dried at 105ºC and weighed. Calculated the percentage w/w ethanol soluble extractive with reference to the air-dried material.

- **Water soluble extractive value**

Macerated 5 grams of coarsely powdered air-dried barks of *Syzygium samarangense* with 100ml water in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and then allowed to stand for another 18 hours. Filtered rapidly, then 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish and dried at 105ºC and weighed. Calculated the percentage w/w of water-soluble extractive with reference to air dried material.

5. Loss on Drying

Determining the amount of volatile matter (i.e., water drying off from the drug) in the drug is a measure of loss on drying for substances appearing to contain water as the only volatile constituent, the procedure given below was followed. Accurately weighed drug samples were placed (without preliminary drying) in a tarred evaporating dish. They were dried at 105˚C for 5 hours, and weighed; percentage loss on drying was calculated with reference to initial weight. The percentage of loss on drying was calculated using the following formula:

$$\% \text{ Loss on drying} = \frac{Initial\ weight - Final\ weight\ Initial\ weight}{Initial\ weight} \times 100$$

6. Determination of Foaming index

Many medicinal plant materials contain saponins that can cause persistent foam when aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of foaming index. Weighed accurately about 1g of coarsely powdered drug and transferred to 500ml conical flask containing 100ml of boiling water maintained at moderate boiling at 80-90ºC for about 30 mins then made it cold, filtered into a volumetric flask and added sufficient water through the filter to make the volume up to 100ml. Cleaned 10 stopper test tubes were taken and marked with 1-10. The successive portions of 1,2ml up to 10ml drug was taken in separate tubes and adjusted remaining the volume with liquid up to 10ml in each. After closing the tube with stoppers, shake them for 15sec and allow to stand for 15min then measure the height.

$$\textbf{Foaming index} = \frac{1000}{a}$$

Where, a = Volume (ml) of decoction

7. Swelling index

The swelling index is the volume in ml occupied by the swelling of 1gm of plant material under specified conditions. A specified quantity of the plant material previously reduced to the required fineness was accurately weighed and transferred into a 25ml glass stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated portion about 125mm, marked in 0.2ml divisions from 0 to 25ml in an upward direction. 25ml of water was added and the mixture was shaken thoroughly every 10min for 1hour, allowed to stand for 3 hours at room temperature. The volume in ml occupied by the plant material was measured including any sticky mucilage. The mean value of the individual determination, related to 1gm of plant material was calculated.

8. Fluorescence analysis

Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluorescence in day light. If the substances themselves are not fluorescence, they may often be converted into fluorescent derivatives by applying different reagents. Hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation.

Organic molecules absorbed light usually over a specific range of wavelength; many of them re emit such radiations. So, if the powder is treated with different chemical reagent and seen in the UV cabinet, different colours will be produced. Therefore, it can be used for the identification of the drug. The fluorescence characteristic of the drug powder with different chemical reagent was studied by observing under UV light. The fluorescence analysis is a tool for the determination of constituents in the plant that gives that gives a definite idea of chemical nature.

III. PHYTOCHEMICAL STUDIES

Phytochemical Screening:

i. Chemical tests for alkaloids

A small portion of the dried alcoholic extract was shaken (acidified) with dilute hydrochloric acid and filtered. The acidified filtrate was tested with the following reagents, to detect the presence of alkaloids.

- **Mayer's Test**

The acidified extract (two ml) was treated with 1 ml of Mayer's reagent (potassium mercuric iodide), shaken, and noted for the presence of a creamy precipitate.

- **Hager's Test**

The acidified extract (two ml) was treated with 1 ml of Hager's reagent (saturated picric acid solution) and observed for the presence of yellow precipitate.

- **Wagner's Test**

The acidified extract (two ml) was treated with a few ml of Wagner's reagent (solution of iodine in potassium iodide) and observed for the presence of a reddish- brown precipitate.

- **Dragendorff's Test**

The acidified extract (two ml) was treated with a few ml of Dragendorff's reagent (Potassium bismuth iodide) and observed for the presence of orange-red precipitate.

ii. Chemical test for glycosides

A small portion of the extract was hydrolyzed with dilute hydrochloric acid for a few hours in a water bath and the hydrolysate was later subjected to the following tests to detect the presence of glycosides.

- **Legal's Test**

The residue (dry extract) left after evaporation was dissolved in a few millilitres of pyridine. Two millilitres of freshly prepared sodium nitroprusside solution was added to it and then made alkaline with sodium hydroxide solution. It was observed for the formation of pink red colour.

- **Baljet's Test**

The few ml of the extract was treated with 1ml sodium picrate solution and a yellow to orange colour reveals the presence of cardiac glycosides.

- **Liebermann Burchard's Test**

The five ml of the hydrolysate taken in a test tube was evaporated, the residue taken in dry chloroform (one ml) and then it was mixed with two ml of specially distilled acetic anhydride

followed by a few drops of concentrated sulphuric acid through the sides of the test tube. It was then observed for the development of a deep red colour in the lower portion and green colour in the upper portion which changed to blue and violet.

- **Bontrager's Test**

A little of the residue obtained from the hydrolysate was mixed with water and shaken with an equal volume of chloroform. The chloroform layer was separated to which dilute ammonia solution was added and shaken well and noted whether any pink colour was present in the ammoniacal layer.

- **Modified Bontrager's Test**

The residue obtained was treated with ferric chloride and dilute HCl, for the oxidative hydrolysis of C-glycoside. Then it was extracted with chloroform. The chloroform layer was separated, and dilute ammonia solution was added and shaken. The ammoniacal layer was observed for pink in colour.

iii. Chemical test for phenolic compounds and tannins

- **Ferric chloride Test**

A small quantity of the extract diluted with water was treated with dilute ferric chloride solution (5%) and observed for the presence of blue colour.

- **Gelatin Test**

The extract dissolved in water was filtered. To the filtrate, 2% solution of gelatin containing 10% sodium chloride was added. Noted for the presence of milky white precipitate.

- **Lead acetate Test**

The extract dissolved in water was treated with a 10% lead acetate solution. Noted for the presence of bulky white precipitate.

- **Decolorization Test**

The extract dissolved in water was treated with a dilute potassium permanganate solution. Noted for the decolorization of potassium permanganate.

iv. Chemical test for flavanones and flavonoids

Aqueous sodium hydroxide Test Aqueous sodium hydroxide solution was added to the few ml of the extract and the presence of yellow coloration of the solution was noted.

The filter paper was wetted with a small quantity of alcoholic solution of the extract.

That filter paper was exposed to ammonia vapours and noted the yellow colour.

v. Chemical test for carbohydrates

A small quantity of ethanolic extract was mixed with water or alcohol and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates.

Molisch's Test

The filtrate (two ml) was treated with a few drops of Molisch's reagent and two ml of concentrated sulphuric acid was added through the sides of the test tube without shaking. Observed for the presence of a violet ring at the junction of two solutions.

- **Benedict's Test**

The filtrate (a few drops) was treated with two ml of Benedict's reagent. Then the mixture was heated in a boiling water bath for two min and the presence of red precipitate was noted.

- **Fehling's Test**

The filtrate (one ml) was treated with 1 ml each of Fehling's solutions A and B and boiled in a water bath for half an hour, then observed for the presence of red residue at the bottom of the test tube.

vi. Chemical test for proteins and amino acids

- **Millon's Test**

The extract (two ml) was treated with a few drops of Millon's reagent (1gm of mercury+ 9ml of fuming nitric acid) and observed for the presence of white precipitate, which on warming turned into a red-coloured solution.

- **Biuret Test**

The extract (two ml) was treated with one drop of 2% copper sulphate solution. To this 1ml of 95% ethanol was added followed by an excess of potassium hydroxide solution and observed for the presence of violet-coloured solution.

- **Ninhydrin Test**

The extract (a few ml) was treated with two drops of ninhydrin solution and heated in a water bath and then the presence of violet colour was noted.

vii. Chemical test for terpenoids

- **Salkowski's Test**

The extract (a few ml) was dissolved in chloroform. An equal volume of concentrated sulphuric acid was added to it and noted for the appearance of red colour in the chloroform layer and greenish-yellow fluorescence in the acid layer.

viii. Chemical test for sterols

A small amount of the alcoholic extract was refluxed with a solution of alcoholic potassium hydroxide until saponification was observed. The mixture was diluted and extracted with solvent ether. The ethereal extract was evaporated, and the residue was subjected to Liebermann Burchard's and Salkowski's tests.

- **Liebermann-Burchard Test**

The residue was taken with dry chloroform (one ml) and then it was mixed with two ml of specially distilled acetic anhydride followed by a few drops of concentrated sulphuric acid through the sides of the test tube and observed for the development of a deep red colour in the lower portion and green colour in the upper portion which changes to blue and violet.

- **Salkowski's Test**

The residue was dissolved in chloroform and an equal volume of concentrated sulphuric acid was added to it and observed for the red colour in the lower layer.

ix. Chemical test for saponins Foam or Froth

Test A small quantity of extract was diluted with 20 ml of distilled water in a graduated cylinder. The suspension was shaken for 15 minutes and waited to see if any froth was formed.

x. Chemical test for gums or mucilage

To 10 ml aqueous extract of the plant, 25 ml of absolute alcohol was added with constant stirring. Filtered and the precipitate formed was dried in air and examined for swelling properties.

- **GAS CHROMATOGRAPHY-MASS SPECTROSCOPY(GC-MS) ANALYSIS**

Perkin Elmer was used for the GC-MS analysis of methanol, ethyl acetate, and chloroform extract. Helium was used, as carrier gas at a constant flow rate of 1ml/min to separate the components of a fused silica column packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30m x 0.25 mm ID) that was utilized in the Clarus 680 GC analysis. 260ºC was the injector temperature set for the chromatographic run. The oven temperature for the 1 µL of extract sample that was injected into the device was set to 60ºC for two minutes, 300ºC at a rate of 10ºC per minute, and 300ºC for six minutes. Condition for the mass detector were a 240ºC transfer line, a 240ºC ion source, an ionization mode electron impact at 70 eV, a 0.2 second scan time, and a scan interval. The pieces spanning 40-600Da. The component spectrums were compared to the database of known component spectrum kept in the GC-MS

NIST (2008) library.

QUANTIFICATION OF SECONDARY METABOLITE

a. ESTIMATION OF TOTAL PHENOLIC CONTENT (FOLIN CIO-CALTEAU METHOD)[13]

Preparation of reagents

a. Folin Cio-Calteau reagent (2N): it was diluted to 1:10 ml with water.

b. Sodium carbonate solution: 20% of sodium carbonate (anhydrous) made with 100 ml distilled water.

Preparation of standard graph of Gallic acid

10 mg of gallic acid was weighed and made up to 10 ml with methanol in a 10ml standard flask. From the above solution (1mg/ml), 1ml was pipetted out and made up to 10 ml with methanol to get 100µg/ml gallic acid standard solution (stock solution). From the stock solution 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 ml were pipetted out and made up to 10 ml with water to get 2, 4, 6, 8, 10, 12, 14 µg/ml solutions respectively. To the above solutions, 5 ml of Folin Cio-Calteau reagent and 4 ml of 20% sodium carbonate solution were added after 5 minutes. It was stirred and incubated at room temperature for 30 minutes. After 30 minutes, absorbances of the solutions were measured at 765 nm using Shimadzu UV Visible spectrophotometer. The absorbance values were plotted against concentration and a standard graph was obtained.

b. ESTIMATION OF TOTAL FLAVANOID CONTENT[13]

- **Method:** Aluminium chloride colorimetric method**.**
- **Materials required:** Standard Quercetin, Aluminum chloride solution (10%), sodium nitrite solution (5%), sodium hydroxide solution (1 M), methanol, distilled water, extracts**.**

- **Procedure:**

Concentration of 2,4,6,8, and 10 µg/ml were generated from the prepared stock solution, and extracts were also prepared. 4 ml of water and 0.3 ml of 5% sodium nitrite were added to each of them.0.3 ml of a 10% aluminium chloride solution was added after 5 minutes.2ml of 1 M sodium hydroxide was added after 6 minutes, and 10ml of distilled water were added in total. After mixing the solutions, a UV Visible spectrometer was used to test the absorbance at 510nm against the blank, which did not have any aluminium chloride added. The standard graph of quercetin was used to compute the percentage of total flavonoid content.

c. ESTIMATION OF TOTAL TANNIN CONTENT [13]

The Folin-Ciocalteu method was used to measure the tannins. A volumetric flask (10ml) was filled with 7.5ml, of distilled water, 0.5ml of Folin-Ciocalteu phenol reagent ,1ml of 35% sodium carbonate solution, and approximately 0.1ml of the sample extract. The flask was then diluted to 10ml with distilled water. The mixture was well shaken and allow to sit at room temperature for half an hour. The same procedure as previously described was used to generate a series of reference standard solutions of tannic acid (2,4,6,8 and 10 µg/ml). An UV /visible spectrophotometer, was used to measure the absorbance of the test and standard solution at 700nm in relation to the blank. Three separate analyses were performed to estimate the tannic content. The amount of tannin in the sample was stated as milligrams of tannic acid equivalents per gram of dried material.

d. ESTIMATION OF TOTAL GLYCOSIDE CONTENT

8ml of plant extract was transferred to a 100ml volumetric flask and 60ml of H_2O and 8ml of 12.5% lead acetate were added, mixed and filtered.50ml of the filtrate was transferred into another 100ml flask and 8ml of 47% Na_2HPO_4 were added to precipitate excess Pb^{2+} ion. This was mixed and completed to volume with water. The mixture was filtered twice through same filter paper to remove excess lead phosphate. 10ml of purified filtrate was transferred into clean Erlyn – Meyer flask and treated with 10ml Baljet reagent. A blank titration was carried out using 10ml distilled water and 10ml Baljet reagent. This was allowed to stand for one hour for complete colour development. The colour intensity was measured colorimetrically at 495nm.

Calculation % of total glycoside = (A x 100) /75 g%

IV. PHARMACOLOGICAL STUDY

❖ INVITRO ANTIDIABETIC ACTIVITY[14,15]

i. Alpha amylase inhibition assay

α amylase inhibitors decrease the high glucose levels that can occur after a meal by slowing the speed the speed with which alpha amylase can convert starch to simple sugars. This is of importance in diabetic people where low insulin levels prevent the fast clearing of extracellular glucose from the blood. Hence diabetics tend to have low alpha amylase levels in order to keep their glucose levels under control. These oligosaccharides are converted into glucose by α glucosidase enzyme. This conversion into glucose is inhibited by α glucosidase inhibitors.

Preparation of test and standard samples

In α amylase inhibition assay, acarbose 50 mg was used as the standard sample solutions of concentration (50, 100, 150 and 200 µg/ml) were used for the assay. Test sample solution was prepared from various extracts of the drug. The concentrations of sample solution were 200, 400 and 600 µg/ml.

METHOD

1. Incubate 250 µl of plant extracts with varying concentration (12.5 - 100µg/ml) and 250 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing alpha amylase solution (0.5 µg/ml) at 25ºC for 10 minutes.
2. Add 250 µl of 1 % starch solution in 0.02 M sodium phosphate buffer pH 6.9 to each tube at timed intervals.
3. Incubate the reaction mixture at 25 ºC for 10 minutes.
4. The reaction will stop with 500 µl of di - nitrosalicylic acid colour reagent.
5. The test tubes are then incubated in a boiling water bath for 5 minutes, then cool to room temperature.
6. Dilute the reaction mixture by adding 5 ml distilled water and measure the absorbance at 540 nm.
7. Acarbose at various concentrations (12.5 - 100 µg/ml) was include as a standard.
8. Without test substance was set up in parallel as a control and each experiment was performed in triplicates.
9. The results were expressed as percentage inhibition, which was calculated using the formula,

Percentage inhibition = $[(A_{\circ} - A1) / A_{\circ}] \times 100$

Where, $A_{\circ}$ = Absorbance of the control

A1 = Absorbance of the sample/ standard

Calculation of IC50

Based on the graph, the amount of medication (µg/ml) needed to scavenge 50% hydrogen peroxide was determined. For the inhibitory concentration of the samples as well as the standard, the IC_{50} value was computed and tabulated.

ii. Alpha glucosidase inhibition assay

α glucosidase inhibitors work by slowing down alpha amylase's ability to convert such into

simple sugars, which reduces the possibility of high glucose levels following a meal. This is a crucial for diabetics since low insulin levels make it difficult for the blood to quickly remove extracellular glucose. The sample extract's α amylase inhibitors stop carbohydrates from breaking down into smaller forms called oligosaccharides. The enzyme α glucosidase then breaks down these oligosaccharides into glucose. Alpha glucosidase inhibitors block this conversion to glucose.

Preparation of test and standard samples

Acarbose 50 mg was utilized as the standard sample solution in the α glucosidase inhibition assay, with concentration of 50, 100, 150, and 200 µg/ml. A test sample solution was made using different medication extracts. The sample solution had concentrations of 200, 400, and 600 µg/ml.

Method

A modest adjustment was made to the approach outlined by Shai et al. (2011) to examine the sample's impact on a glucosidase activity. For thirty minutes, 400 µl of α glucosidase (0.067 U/ml) was pre-incubated with varying concentrations of the sample. The reaction, was then started by adding 200 µl of 3.0 Mm (pNPG), which was employed as substrate, diluted in 0.1 M sodium phosphate buffer (pH 6.9). After 30 minutes of incubation at 37ºC, the reaction mixture, was halted by adding 2 millilitres of 0.1 M sodium carbonate. By measuring the yellow-coloured para-nitro phenol produced from pNPG at 400nm, the α glucosidase activity was ascertained. The findings were presented as an inhibition percentage.

Percentage of inhibition = [(A0 – A1) / A0] x 100

Where, A_0 = Absorbance of control

A_1 = Absorbance of sample/ standard

Calculation of IC50

The graph was used to compute the drugs concentration (µg/ml) needed to scavenge 50% hydrogen peroxide. The inhibitory concentration of the standard and the samples was determined, and the IC_{50} value was tabulated.

5. RESULT AND DISCUSSION

1. PLANT COLLECTION AND AUTHENTICATION

The plant *Syzygium samarangense* (Myrtaceae) was collected from Uliyathaduka, Kasaragod, Kerala. The plant material was taxonomically identified by the botanist Dr. Biju P, Department of Botany, Government college Kasaragod and dried under shade.

1. PHARMACOGNOSTIC STUDIES

a) Determination of moisture content of the stem bark

As previously mentioned, the moisture content of the plant's stem bark portion was ascertained. It was established how much moisture the plant's stem bark portion lost on average. Table No 5.1 presents the result.

Table no 5.1: Results showing moisture content of the stem bark of *S. samarangense.*

Sl no	Weight of empty petridish (g)	Weight of drug +empty petridish (g)	Weight of petridish +powder after drying (g)	LOD (g)	% Loss on drying (% w/w)	Average (% w/w)
1	50.37	52.37	52.19	0.18	9.0	9.3
2	52.76	54.76	54.57	0.19	9.5	
3	55.23	57.23	57.04	0.188	9.4	

b) Determination of ash value of stem bark

The ash values of the stem bark of plant were determined according to the procedure in the earlier. The percentage content of acid insoluble ash was found to be less than that of water-soluble ash. The results were presented below.

- **Total ash value**

Table no 5.2: Results showing total ash value of stem bark of *S. samarangense.*

Sl. No	Weight of empty crucible (g)	Weight of crucible + sample (g)	Weight of crucible + ash (g)	Weight of ash (g)	Percentage yield (% w/w)	Average (% w/w)
1	43.41	45.41	43.67	0.26	13	11.66
2	43.60	45.60	43.81	0.21	10.5	
3	43.75	45.75	43.98	0.23	11.5	

- **Acid insoluble ash**

Table no 5.3: Results showing acid in soluble ash value of stem bark of *S. samarangense.*

Sl. No	Weight of empty crucible (g)	Weight of crucible + sample (g)	Weight of crucible + acid insoluble ash (g)	Weight of acid insoluble ash (g)	Percentage yield (% w/w)	Average (% w/w)
1	18.9	20.9	18.98	0.08	4.0	4.1
2	20.6	22.6	20.69	0.09	4.5	
3	19.5	21.5	19.57	0.07	3.8	

- **Water soluble ash**

Table no 5.4: Results showing water soluble ash value of stem bark of *S. samarangense.*

Sl. No	Weight of empty crucible (g)	Weight of crucible + sample (g)	Weight of crucible + water insoluble ash (g)	Weight of water-soluble ash (g)	Percentage yield (% w/w)	Average (% w/w)
1	29.72	31.72	29.78	0.14	7.0	6.6
2	29.45	31.45	29.52	0.13	6.5	
3	29.72	31.72	29.78	0.13	6.5	

- **Sulphated ash**

Table no 5.5: Results showing sulphated ash value of stem bark of *S. samarangense.*

Sl. No	Weight of empty crucible (g)	Weight of crucible + sample	Weight of crucible + sulphated ash (g)	Weight of sulphated ash (g)	Percentage yield (% w/w)	Average (% w/w)
1	31.41	33.41	31.50	0.09	3.5	3.6
2	30.28	32.28	30.35	0.07	3.7	
3	31.50	33.50	31.58	0.08	3.6	

c) Determination of extractive values of stem bark

The extractive value of the plants stem bark section, both water and alcohol soluble, were calculated using the previously described method. The water-soluble extractive value of *Syzygium samarangense* was found to be higher than alcohol soluble extractive value.

The outcomes are displayed below:

- **Water soluble extractive value**

2. **Table no 5.6: Results of water -soluble extractive value of stem bark of *S. samarangense.***

Sl no.	Weight of dry powder (g)	Weight of empty dish (g)	Weight of dish+ extract (g)	Percentage yield (% w/w)	Average (%w/w)
1.	5	153.24	153.42	3.6	3.53
2.	5	155.12	155.31	3.8	
3.	5	160.35	160.51	3.2	

Fig no 5.1: Water soluble extractive value.

- **Alcohol soluble extractive value**

3. **Table no 5.7: Results showing alcohol soluble extractive value of stem bark of *S. samarangense.***

Sl no.	Weight of dry powder (g)	Weight of empty dish (g)	Weight of dish+ extract (g)	Percentage yield (% w/w)	Average (%w/w)
1.	5	139.41	139.48	1.4	1.73
2.	5	140.21	140.30	1.8	
3.	5	141.11	141.21	2	

Fig no 5.2: Alcohol soluble extractive value.

a) Organoleptic characters

Fig no 5.3: Stem bark of *S. samarangense.*

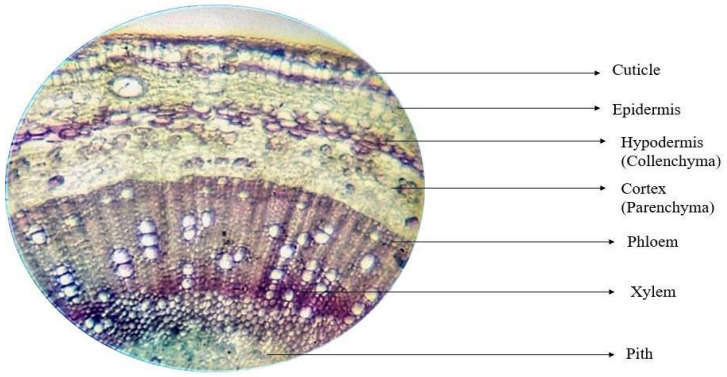
Organoleptic evaluation was carried by means of organs of sense. This included evaluation of drug by color; odour; size; shape; taste and special features including touch; texture etc.

Table no 5.8: Results after organoleptic evaluation of the stem bark of *S. samarangense.*

Macroscopic features	
Color	brownish-grey
Odour	No odor
Taste	No significant taste
Size	Thin to moderately thick, about 2–6 mm thick in mature trees.
Shape	Cylindrical

b) Microscopic evaluation

- **Transversesection**



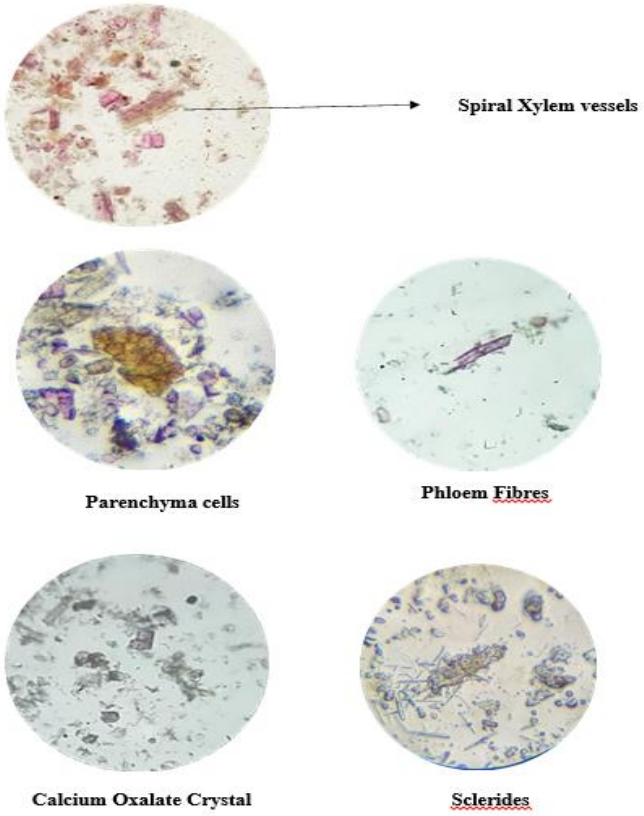


Fig no 5.4: Characteristic images of powder analysis.

- **Powder analysis**

The powder when observed under microscope exhibit the following characters.

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