Phytochemical screening and Antimicrobial activity of the Methanolic extract of *Polyalthia suberosa* leaf

Sayma Sadia

20346064

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing a degree at Brac University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

_____

Sayma Sadia

20346064

Approval

The project titled "Phytochemical screening and Antimicrobial activity of Methanolic extracts of *Polyalthia suberosa* leaf" submitted by Sayma Sadia (ID: 20346064) of Summer 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

Supervised By:

_____

Dr. Farhana Alam Ripa

Associate Professor

School of Pharmacy, BRAC University

Approved By

Dean:

_____

A. F. M. Yusuf Haider, PhD

Acting Dean, School of Pharmacy

Professor, Department of Mathematics and Natural Science

BRAC University

Ethics Statement

This study does not involve any kind of animal and human trial.

Abstract:

Polyalthia suberosa is a medicinal plant known for its traditional use in treating inflammation, pain, and infections. Traditionally, it has been used in South and Southeast Asian medicine to treat fever, wounds, and gastrointestinal disorders, highlighting its therapeutic potential. The present study was conducted to find out the the phytochemical constituents and antimicrobial effect of the methanolic extract of leaves of *P.suberosa* (MPSL). The phytochemical screening was performed following the standard method. Antimicrobial activity was tested against 5 pathogenic bacteria (*Bacillus cereus, Bacillus subtilis, Escheichia coli, Klebsiella peunomoniae, Pseudomonas aeruginosa)* by the Disk diffusion method. Alkaloids, tannins, flavonoids, saponins, glycosides, phenolic compounds, steroids and resin were some of the chemical constituents identified during phytochemical screening. The plant showed moderate antimicrobial activity against tested microorganisms. Although the experimental extract showed antimicrobial activity against the tested pathogenic microbes. Therefore, the MPSL extract can be used as a new source for the development of novel antimicrobial drugs. However, further investigation is required to find out the responsible phytoconstituents for the aforementioned activity.

Keywords: *Polyalthia suberosa*; phytochemical screening; antimicrobial activity; Disk diffusion method.

Dedication

I would like to dedicate this project paper to my parents.

Acknowledgements

First and foremost, I thank Almighty Allah for giving me the strength and determination to complete this job.

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WHO World Health Organization

BNH Bangladesh National Herbarium

THP Traditional Health Practitioners

LE Leaves Extract

ICDDRB International Centre for Diarrhoeal Disease Research, Bangladesh

Chapter 1: Introduction

Since antiquity, mankind have depended on plants having therapeutic powers as a conventional means to address ailments. Many historical documents emphasize nature's ability to restore. The acknowledgment of medicinal plants arises from humanity's persistent struggle against disease, prompting the investigation of therapeutic substances in diverse plant components, including leaves, flowers, bark, stems, roots, seeds, and fruits. Contemporary science progressively integrates phytochemicals into pharmacotherapy, leading to the creation of novel drugs sourced from these natural materials. This transition has created new opportunities for research and enhanced the proficiency of both pharmacists and physicians in medication development (Srivastava, 2018). For ages, medicinal plants have been integral to healing practices throughout various cultures globally. They remain pivotal in both conventional and contemporary medicine as vital resources for pharmacological development. The World Health Organization (WHO) indicates that around 80% of the global population, particularly in rural areas of underdeveloped countries, depend on herbal treatments for their fundamental health requirements (Mamedov, 2012). Moreover, more than 50 significant pharmaceutical medications sourced from tropical plants are presently accessible in global marketplaces (Hosseinzadeh et al., 2015). The WHO highly endorses the use of plant-based medicinal agents for their safety, efficacy, accessibility, and affordability in both traditional and modern medical practices. In Bangladesh, more than 1,000 plant species are recognized for their medicinal properties, with around 455 to 747 species recorded for therapeutic applications against several health issues (Alamgir and Fatema, 2014). The south-western section of the country, comprising Khulna, Satkhira, Bagerhat, and Patuakhali, encompasses the Sundarbans, the biggest mangrove forest globally, which harbors a diverse array of medicinal plants. The Bangladesh National Herbarium (BNH) has identified and documented 33 medicinal plants within the Khulna division for

their application in traditional medicine. Included is *P.suberosa*, sometimes referred to as the "Ham Jam" tree, which is one of the most prevalent species in the Sundarbans (Rahman, 2021).

Figure 1 Plant P.suberosa (Florida plants,2020)

1.1 The Description of the *P.suberosa*

P.suberosa, referred to as the corky debbar tree, is native to the tropical and subtropical areas of South and Southeast Asia, encompassing Bangladesh, India, Sri Lanka, China, and the Philippines (Asian Plant, n.d.). Historically, it has been employed in traditional medicine among diverse nations for its medicinal attributes. It possesses a historical significance in South and Southeast Asia, where it was conventionally employed in indigenous medicine for the treatment of fever, pain, and digestive disorders. The species was initially characterized by Scottish botanist William Roxburgh and then classed by George Henry Kendrick Thwaites in the 19th century. The enduring therapeutic application in Ayurvedic and indigenous healing traditions underscores its ethnobotanical significance, which has influenced contemporary pharmacological research. This perennial shrub or diminutive tree generally attains heights ranging from 6 to 10 meters. The plant features a thick, corky bark, thought to be an adaptation to fire-prone habitats (Asian Plant, n.d.). The leaves are slender, oblong-lanceolate, and long-acuminate, displaying a glossy aspect. The inflorescences are

extra-axillary, positioned opposite the leaves or somewhat beneath them, featuring greenish-yellow petals and many stamens (Asian Plant, n.d.). *P. suberosa* flourishes in open woodlands at lower altitudes. It exhibits tolerance to diverse soil types, necessitating well-draining soils for optimal growth (CABI, n.d.). The plant exhibits shade tolerance and can endure cultivation, browsing pressure, and fire, demonstrating its resilience in many ecological situations (CABI, n.d.). Historically, several components of *P. suberosa* have been utilized in traditional medicine for diverse diseases. In Bangladesh, the bark serves as a febrifuge, analgesic, and laxative (Asian Plant, n.d.). The roots are employed as an abortifacient and to alleviate abdominal pain resulting from muscular cramps (Asian Plant, n.d.). Latex has been utilized as a rudimentary filling for dental cavities in tropical areas (Asian Plant, n.d.). The plant comprises numerous bioactive chemicals. The leaves and stems produce triterpenes, including suberosol, which exhibit anti-HIV replication action (StuartXchange, n.d.). Alkaloids such as oxostephanine and lanuginosine, extracted from the stem bark, demonstrate antibacterial activity (StuartXchange, n.d.). The mature fruits of *P. suberosa* are consumable and possess vital elements. The proximate analysis of the fruit indicates a protein content of 1.96%, along with substantial levels of iron, sodium, potassium, calcium, copper, manganese, and zinc (StuartXchange, n.d.). *P. suberosa* is categorized as invasive in Cuba; nevertheless, there is insufficient evidence of detrimental environmental impacts in other regions (CABI, n.d.). Its extensive native range and flexibility indicate that, when properly managed, it can positively contribute to diverse ecosystems.

1.2 Taxonomy of *P.suberosa*

Kingdom: Plantae

Subkingdom: Tracheobionta (Vascular plants)

Division: Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Order: Magnoliales

Family: Annonaceae (Custard apple family)

Genus: Polyalthia

Species: Polyalthia suberosa (Roxb.) Thwaites

1.3 Global and Traditional habitat of *P.suberosa*

P.suberosa is a small evergreen species native to multiple Asian countries and has subsequently been introduced to various other regions around the world. This species is found in a natural distribution throughout tropical and subtropical regions, usually residing in open woodlands and thickets at lower elevations. The species is recorded in its native range across India, Bangladesh, Sri Lanka, Myanmar, Thailand, Vietnam, Laos, Cambodia, and the Philippines. This species exhibits a clear preference for well-drained soils and is often found in moist, tropical ecosystems. In India, this phenomenon is predominantly noted in evergreen forests at elevations reaching around 1,000 meters. *P.suberosa* has established itself outside its native range, becoming naturalized in various areas, including regions of the United States like Florida, Hawaii, and Cuba. Due to its aesthetic appeal and versatility, it is cultivated

across various tropical climates. A notable morphological feature is its corky bark, which provides protection against mechanical injury and enhances the species' resilience in various environmental conditions. This characteristic enhances its capacity to thrive in diverse ecological environments, ranging from untouched lowland forests to human-modified habitats, highlighting its ecological flexibility. This species in Bangladesh exhibits remarkable adaptability across a range of ecological zones, thriving in diverse habitats. The tree is primarily located in forests and elevated areas throughout the country, encompassing regions such as the Sundarbans, Khulna, Satkhira, Sylhet, Gazipur, and Tangail (Forest Emission Factor Database, n.d.; Plant Specimen Database Program, n.d.). This species has also been documented in roadside scrub jungles and sal forests, demonstrating its ability to thrive in disturbed and secondary habitats (Plant Specimen Database Program, n.d.). *P. suberosa* thrives in well-drained, loamy soils and is frequently found in moist, tropical climates. It demonstrates remarkable adaptability to seasonal changes, thriving in both flood-prone lowlands and elevated terrains, highlighting its ecological flexibility (CABI Compendium, n.d.). This species contributes to local ecosystems through its role in enhancing biodiversity and stabilizing soil. The occurrence in diverse habitats underscores its ecological importance and ability to thrive in varying environmental conditions.

Figure 2 Distribution Map of P.suberosa (World flora,2017)

1.4 Medicinal applications around the world

The leaves of *P.suberosa* has a wide array of therapeutic uses acknowledged throughout several cultures, especially in South Asia. Historically, the leaves are utilized for their anti-inflammatory, antibacterial, analgesic, and antioxidant attributes, rendering them significant in traditional medicine for addressing diverse health issues. The leaves are frequently employed to mitigate discomfort, diminish inflammation, and address gastrointestinal disorders such as diarrhea and dysentery (Ali et al., 2018). Moreover, they are utilized topically to address wounds, lacerations, and dermal infections owing to their antibacterial properties (Rahman et al., 2020). In conventional Ayurvedic and Unani treatment, P. suberosa leaves are utilized to alleviate fever and respiratory disorders, typically administered as decoctions or poultices. The leaves are thought to possess antipyretic properties, aiding in the alleviation of fever and discomfort linked to illnesses (Hossain et al., 2017). Moreover, studies indicate that the phytochemicals present in the leaves, including alkaloids, flavonoids, and terpenoids, enhance these therapeutic advantages, validating their application in contemporary herbal therapy (Jalil et al., 2021). Research has underscored the plant's potential in contemporary pharmacology, with P. suberosa extracts exhibiting antibacterial, antioxidant, and anti-inflammatory properties, thereby corroborating numerous traditional applications (Ahmed et al., 2019). The plant's many therapeutic properties have generated increasing interest in its integration into modern healthcare practices, especially in areas where it is native. *P.suberosa* leaves possess significant therapeutic properties, integrating traditional wisdom with contemporary scientific inquiry to endorse their extensive application in global herbal medicine.

1.5 Traditional applications in Bangladesh

In Bangladesh, the leaves of *P.suberosa* are extensively utilized in traditional medicine for their diverse medicinal characteristics. Local cultures employ the leaves to address many maladies, particularly highlighting their anti-inflammatory, analgesic, and antibacterial properties. The leaves are commonly processed into decoctions or pastes, which are either consumed or applied externally to mitigate symptoms of fever, discomfort, and dermal infections. The leaves are frequently utilized to alleviate fever and address respiratory ailments, with numerous practitioners advocating for a tea derived from the leaves to help diminish body temperature and relieve discomfort (Hossain et al., 2017). *P.suberosa* leaves are utilized to treat gastrointestinal disorders, including diarrhea and indigestion. The leaves possess modest antibacterial properties, rendering them effective against intestinal infections (Ali et al., 2018). In rural regions, leaves are occasionally mashed and utilized as poultices for the treatment of wounds and injuries, aiding in infection prevention and facilitating healing due to its antibacterial properties (Rahman et al., 2020). Contemporary research corroborates these traditional use by identifying bioactive components in the leaves, including alkaloids, flavonoids, and terpenoids, that enhance their therapeutic efficacy (Jalil et al., 2021). The scientific validation of the plant's therapeutic characteristics underscores its importance in both traditional and modern herbal therapy. The extensive utilization of *P. suberosa* in Bangladesh highlights its significant contribution to the local healthcare system, especially in regions with restricted access to contemporary medical treatment.

1.6 Aim of the study

The study is to identify the phytochemical ingredients and antibacterial properties of *P.suberosa*.

1.7 Objectives of the study

The objectives of the study are

1. To find out the chemical components of *P.suberosa* leaf.

2. To find out the antimicrobial activities of this plant.

3. To carry out any novel components to develop a drug for any chronic disease.

Chapter 2: Methodology

2.1 Collection and Identification of *P.suberosa* leaf

Fresh leaf samples of *P.suberosa* were gathered from the Savar Botanical Garden, located in Savar, Dhaka, Bangladesh. This botanical garden is noted for its extensive variety of plant species, featuring numerous rare and medicinally significant plants. The specified portions of the plant were gathered and submitted to the National Herbarium of Bangladesh (NHB), in Mirpur, Dhaka, for verification. Following identification, the leaf's underwent a comprehensive rinsing with running tap water to eliminate surface debris, soil particles, and other contaminants, thereby ensuring the samples were appropriate for the next laboratory procedures.

2.2 Process of Extraction

Many steps are included in the process of extraction; those can be divided into two parts.

2.2.1 Processing plant part

Leaf's were washed carefully by water and remove the dirt and dust particles. Afterwards, the washed parts were spread out over clean papers for natural drying in the room environment for two weeks.

Figure 3 Steps of preparation and drying

2.2.2 Size Reduction

The dried leaf's are currently being cut into smaller fragments in order to decrease their size. The leaf's are fragmented into smaller fragments. Subsequently, these smaller fragments are individually granulated into fine powders and weighed. The unrefined powder was stored in a clean, airtight glass container that was labeled with the necessary information.

2.2.3 Maceration of extraction

Subsequently, the extraction procedure was macerated using methanol as the organic solvent. 1000 gm of stem powder was weighed and thoroughly soaked in 1.5L of methanol in a glass container at ambient temperature (22-25 °C) for 14 days. The plant part was ensured that it was thoroughly soaked in the solvent by conducting regular agitating.

2.2.4 Filtration

Subsequently, the solutions saturated with methanol underwent an initial filtration through a clean cotton cloth, followed by a secondary filtration using filter papers to yield a clear solution containing the crude extract.

2.2.5 Evaporation process

The separated crude extracts were transferred to the rotary evaporator to obtain the concentrated filtrate by removing the methanol. The crude solution was heated to 45-50 degrees Celsius for 40 minutes at 800 mbar vacuum pressure to separate the methanol solution from the solvent for collection.

2.2.6 Extraction

The extraction process is described in the (figure 4).

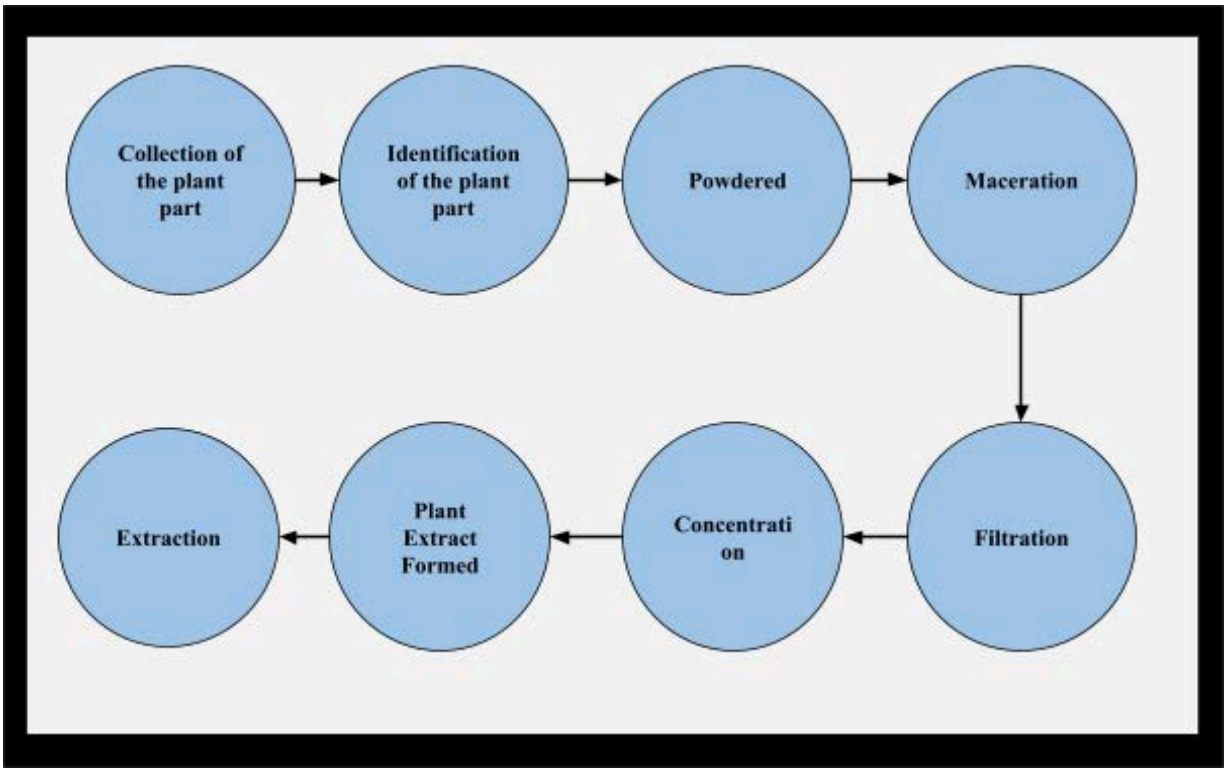

Figure 4 Procedure of Extraction

2.3 Phytochemical Screening

The presence or absence of phytochemical compounds were determined by performing certain qualitative tests.

2.3.1 Alkaloids – Mayer's and Wagner's Tests

Alkaloids precipitate upon reaction with reagents containing heavy metal salts. A segment of the methanolic extract was individually subjected to treatment with Mayer's reagent (potassium mercuric iodide) and Wagner's reagent (iodine in potassium iodide). The emergence of a cream-colored (Mayer's) and reddish-brown (Wagner's) precipitate validated the presence of alkaloids at a high intensity (+++).

2.3.2 Flavonoids – Alkaline Reagent Test

Flavonoids interact with alkaline substances to generate a temporary yellow hue, which vanishes with acidification. The extract was subjected to a few drops of sodium hydroxide solution, subsequently followed by dilute hydrochloric acid. A golden hue that became colorless following acidification signified a moderate presence (++) of flavonoids.

2.3.3 Tannins – Ferric Chloride Test

Ferric chloride interacts with tannins to produce colorful complexes as a result of phenolic group present. A little quantity of 5% ferric chloride solution was included into the extract. A deep green hue indicated a significant presence (+++) of tannins.

2.3.4 Saponins – Froth Test

Saponins possess surfactant characteristics and produce stable foam when agitated with water. The extract was agitated thoroughly with distilled water and allowed to settle. The development of enduring foam signified a moderate presence (++) of saponins.

2.3.5 Steroids – Salkowski's Test

Steroidal chemicals interact with concentrated sulfuric acid in chloroform, resulting in distinctive color changes. The extract was combined with chloroform, subsequently followed by the meticulous incorporation of strong sulfuric acid. A subtle reddish-brown hue at the interface signified a moderate presence (+) of steroids.

2.3.6 Terpenoids – Salkowski's Test

Terpenoids have analogous reactions to steroids under Salkowski's circumstances, producing distinctive colors. The identical approach utilized for steroids was implemented. A faint brownish-red hue at the interface signified a moderate presence (+) of terpenoids.

2.3.7 Glycosides – Keller-Killani Test

Cardiac glycosides with deoxy sugars undergo a reaction with glacial acetic acid and ferric chloride, subsequently treated with strong sulfuric acid, resulting in a reddish-brown ring at the interface. The extract was combined with glacial acetic acid and ferric chloride, thereafter incorporating strong sulfuric acid gradually. The emergence of a reddish-brown ring validated the moderate presence (++) of glycosides.

2.3.8 Phenolic Compounds – Ferric Chloride Test

Phenolic compounds react with ferric chloride to form blue-black or green complexes due to their hydroxyl groups. A few drops of ferric chloride solution were added to the extract. A dark green coloration indicated a moderate presence (++) of phenolic compounds.

2.3.9 Carbohydrates – Molisch's Test

Carbohydrates undergo dehydration in the presence of concentrated sulfuric acid to form furfural derivatives, which react with α-naphthol to produce a violet-colored ring. The extract was mixed with Molisch's reagent and concentrated sulfuric acid was added carefully along the side of the test tube. The absence of a violet ring indicated a negative result (–), suggesting the absence of free carbohydrates.

2.3.10 Proteins – Millon's Test

Millon's test was conducted to identify proteins in the extract of *P.suberosa*. Upon the introduction of Millon's reagent and subsequent gentle heating, a red hue was noted, signifying the presence of tyrosine-containing proteins in the plant. No color change was detected, suggesting the absence of proteins in the extract.

2.3.11 Resin Test

The extract was solubilized in alcohol and subsequently subjected to water treatment. The emergence of turbidity or precipitate validated the existence of resinous chemicals in the sample.

2.4 Disk Diffusion method for testing Antimicrobial Activity

The Disk diffusion assay is a standardized and widely recognized technique for assessing the antimicrobial effectiveness of diverse substances. This technique involves placing discs containing the test extract on agar plates that have been pre-inoculated with bacterial strains, with the resulting inhibition zones indicating antibacterial activity.

2.4.1 Microorganisms used for the test

Both gram-positive and gram-negative bacteria were used to perform the antimicrobial test. Names of the gram-positive and gram-negative bacteria used during the test.

Gram Positive	**Gram Negative**
Bacillus Cereus BTCC19	*Escherichia coli (ATCC25922)*
Bacillus Subtilis BTCC17	*Klebsiella Peunomoniae CRL(ICDDR,B)*
	Pseudomonas aeruginosa (ATCC27853)

Table 1 Name of the Gram-positive and Gram-negative bacteria used in this experiment

2.4.2 Sterilization procedure

All necessary apparatus for the experiment, including beakers, cotton, conical flasks, and forceps, were sterilized using an autoclave and maintained in an aseptic environment prior to utilization. All procedures were conducted within the laminar air flow hood, and the UV lamp was activated for one hour prior to the commencement of the experiment to ensure a

controlled atmosphere. Sterilized, single-use petri dishes were employed to conduct the experiment.

2.4.3 Sample Preparation

A single stock solution was produced for the test, from which sample solutions with concentrations of 1000 µg/ml were derived.

2.4.4 Experimental Procedure

7 grams of nutrient broth were measured and dissolved in 250 milliliters of distilled water to make the culture medium for bacterial cultivation. Subsequently, one conical flask containing 50 ml of the produced broth was inoculated with the five specified bacterial strains. The conical flasks were maintained in the incubator in a shaking position for 24 hours at a temperature of 37 °C. Following a 24-hour period, the conical flasks were removed from the incubator and placed in a controlled environment for subsequent usage. The agar medium was subsequently made by dissolving 3.3 g of M.H. agar in 100 ml of distilled water. The prepared agar was thereafter placed on Petri dishes and allowed to cool at room temperature. Once the agar medium solidified in the petri dishes, the prepared bacterial strains from the conical flasks were meticulously dispersed on them using cotton swabs. Kanamycin discs were employed as the standard in all petri plates and positioned meticulously. Similarly, the produced sample discs and blanks were placed in petri plates and incubated at 37 °C for 24 hours to create an ideal environment for bacterial growth. Following a 24-hour period, the petri dishes were retrieved from the incubator, and the inhibition zones of both the standard and sample discs were assessed.

Chapter 3: Result

3.1 Phytochemical screening of methanolic extracts of *P.suberosa*

A preliminary phytochemical screening of the methanolic extract of *P.suberosa* leaves was conducted to ascertain the presence of several bioactive secondary metabolites employing established qualitative techniques. The tests identified the presence of various essential phytoconstituents, including alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, glycosides, phenols, resins, and carbohydrates (Table 2). Alkaloids were validated using Mayer's and Wagner's assays, signifying their possible medicinal significance. Flavonoids were identified by the alkaline reagent test, whilst tannins were verified through the ferric chloride test. The detection of saponins was evidenced by sustained foaming in the froth test. Terpenoids and steroids were detected via the Salkowski reaction, yielding distinctive reddish-brown and greenish hues, respectively. Glycosides were identified using the Keller-Killiani test, and phenolic substances were further validated by the ferric chloride reaction. Carbohydrates were detected by Molisch's test, however proteins were lacking as evidenced by a negative Millon's test. Resins were detected utilizing the acetone-water assay. The findings indicate that *P.suberosa* possesses a diverse array of pharmacologically active chemicals, corroborating its traditional therapeutic applications and underscoring its potential for more pharmacological research.

Phytochemicals	Test Name	Result
Alkaloids	Mayer’s and Wagner’s test	+
Flavonoids	Alkaline Reagent Test	+
Tannins	Ferric Chloride Test	+
Saponins	Froth Test	+
Steroids	Salkowski’s Test	+
Terpenoids	Salkowski’s Test	+
Glycosides	Keller-Killani Test	+
Phenolic Compounds	Ferric Chloride Test	+
Carbohydrates	Molisch’s Test	+
Proteins	Millon's Test	-
Resin	Acetone water Test	+

Note: This table shows the bioactive compounds that are present in the *P.Suberosa* leaf. Positive or **(+)** indicates the presence of the compound and Negative or **(-)** indicates the absence of the compound.

Table 2 Phytochemical screening results of Methanolic Extract of P.suberosa

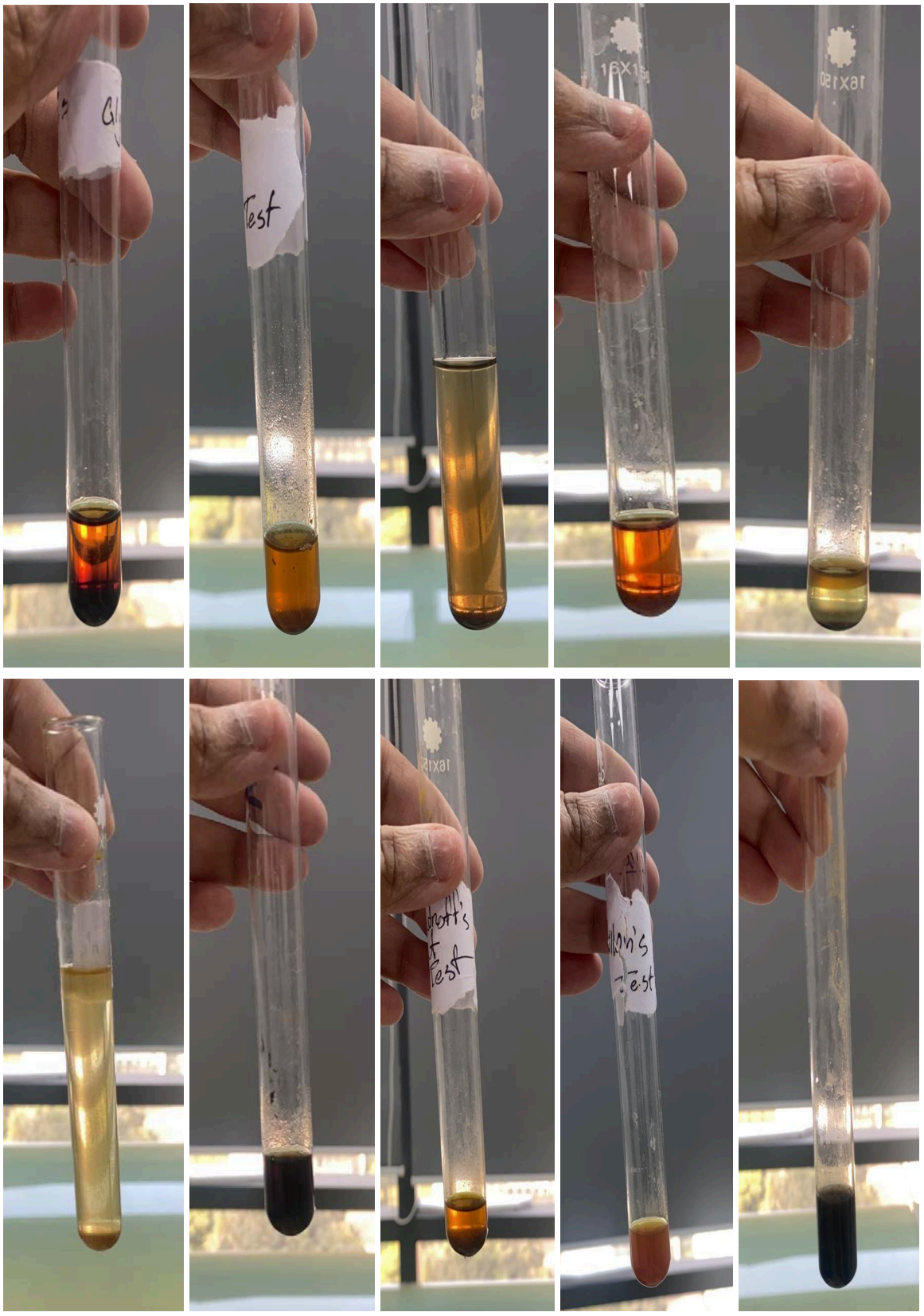

Figure 5 Phytochemical screening test results of P.suberosa leaf

3.2 Antimicrobial Activity Test by Disk Diffusion Method

The antibacterial effectiveness of *P.suberosa* methanolic leaf extract was assessed against selected Gram-positive and Gram-negative bacterial strains utilizing the disk diffusion method (Table 3), with kanamycin (30 µg/disc) as the standard control. Kanamycin had significant antibacterial efficacy, generating inhibition zones between 23 ± 0.49 mm and 27 ± 0.86 mm for all examined microorganisms. Among Gram-positive bacteria, *B.subtilis* exhibited the greatest sensitivity to kanamycin, with a zone of 25 ± 0.60 mm, closely followed by B. cereus at 23 ± 0.57 mm. *K.pneumoniae* CRL demonstrated the most extensive zone of inhibition among Gram-negative bacteria, measuring 27 ± 0.47 mm, whereas *E.coli* and *P.aeruginosa* presented zones of 23 ± 0.49 mm and 25 ± 0.86 mm, respectively. *P. suberosa* extract exhibited modest antibacterial activity, characterized by narrower inhibition zones. The extract inhibited *B.subtilis* and *E.coli*, producing zones of inhibition measuring 10 ± 1.53 mm and 10 ± 2.56 mm, respectively. The extract exhibited minimal efficacy against *P.aeruginos*a, *K.pneumoniae*, and *B.cereus*, with inhibition zones measuring 8 ± 1.57 mm, 9 ± 1.10 mm, and 9 ± 0.58 mm, respectively. The standard deviations for the extract's activity were predominantly elevated, signifying heterogeneity in its antibacterial efficacy. *P.suberosa* demonstrates notable antibacterial capabilities; nonetheless, its effectiveness is inferior to that of kanamycin, indicating opportunities for further enhancement.

Bacteria Strain	**Kanamycin 30µg/Disk zone of inhibition (mm)**	***P. suberosa (1000µg/Disk)* zone of inhibition (mm)**
Gram- Positive Bacteria		
B.cereus	23± 0.57	9± 0.5773
B.subtilis	25± 0.60	10± 1.527
Gram- Negative Bacteria		
K.peunomonia CRL	27± 0.47	9± 1.10
P.aeruginosa	25± 0.86	8± 1.57
E.coli	23± 0.49	10± 2.56

Table 3 Antimicrobial test results of P.suberosa leaf

Chapter 4: Discussion

The phytochemical analysis of the methanolic leaf extract of *P.suberosa* (PSML) identified the presence of various significant secondary metabolites, such as alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, glycosides, phenols, resins, and carbohydrates, with proteins being missing. These results correspond with earlier research demonstrating that species in the Annonaceae family are abundant in bioactive chemicals possessing medicinal potential (Chakraborty et al., 2020). Alkaloids, validated by Mayer's and Wagner's assays, exhibit diverse pharmacological actions, encompassing antibacterial, analgesic, and anticancer properties (Smith et al., 2020). Flavonoids and phenolic substances, identified using alkaline reagent and ferric chloride assays respectively, are linked to antioxidant, anti-inflammatory, and cardioprotective effects (Jones & Patel, 2019; Ravi et al., 2019). The detection of tannins, confirmed by the ferric chloride test, indicates astringent and antibacterial properties, corroborating the plant's traditional applications in infection treatment (Miller et al., 2018). Saponins, identified using the froth test, are extensively recognized for their antibacterial and anticancer properties (Kumar & Singh, 2021). The identification of terpenoids and steroids by the Salkowski reaction reinforces the extract's pharmacological potential, especially in anti-inflammatory and antioxidant mechanisms (Williams & Brown, 2017). Glycosides, validated using the Keller-Killiani test, are particularly pertinent for cardiovascular applications (Chakraborty et al., 2020). The lack of proteins indicates that the noted biological activity are primarily due to non-protein phytochemicals. This experiment also revealed its antibacterial properties. The analysis and comparison of the zone of inhibition test findings indicate moderate potency against all five tested pathogenic microorganisms. The five selected microorganisms predominantly exhibit antibiotic resistance; yet, they demonstrate moderate antibacterial effectiveness against those microbes. These findings offer scientific validation for the conventional medical application

of *P.suberosa*, emphasizing its extensive phytochemical composition. The existence of several bioactive chemicals indicates possible therapeutic uses, especially in antibacterial and antioxidant treatments. Nevertheless, more research involving compound isolation, structural elucidation, and in vivo pharmacological assessment is necessary. Subsequent investigations will ascertain the plant's clinical significance and safety profile. *P.suberosa* constitutes a promising resource for natural drug discovery.

Chapter 5: Conclusion and Future work

This investigation revealed that the methanolic extract of *P.suberosa* leaves included various pharmacologically significant phytochemicals, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds. The extract demonstrated modest antibacterial efficacy against both Gram-positive and Gram-negative bacteria, corroborating its traditional application in the treatment of infections and inflammation. Despite its inferior efficacy compared to the conventional antibiotic, the findings indicate that *P.suberosa* possesses therapeutic potential. Additional study is required to identify specific active chemicals, elucidate their modes of action, and evaluate their efficacy via in vivo investigations, which may facilitate the advancement of innovative, plant-derived pharmaceuticals.

Future research on the leaf's of *P.suberosa* holds significant potential for uncovering new pharmacological and phytochemical properties. Initial investigations have identified many bioactive ingredients, such as alkaloids, flavonoids, tannins, and saponins; nevertheless, extensive research is necessary to isolate and identify the specific chemicals responsible for the reported antibacterial action. Both in vivo and in vitro investigations are crucial for evaluating not only antibacterial efficiency but also potential anti-inflammatory, antioxidant, anticancer, and antidiabetic effects. Toxicological assessments are essential to determine safe dose levels and to identify any possible harmful effects related to its use.

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