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

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

School of Pharmacy
BRAC University
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing 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 that 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.

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Approval

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Ethics Statement

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

Abstract

Polyalthia suberosa is a medicinal plant widely used in traditional Asian medicine for its anti-inflammatory, analgesic, and antimicrobial properties. Its bioactive compounds have attracted interest for potential pharmaceutical applications. The present study was conducted to find out the phytochemical constituents and antimicrobial effect of the methanolic extract of the stem part (MPSS). The phytochemical screening was performed following the standard methods. Antimicrobial activity was tested against 5 pathogenic bacteria *Bacillus cereus, Bacillus subtilis, Escherichia 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 in tested microorganisms. Although the experimented extract showed an antimicrobial activity against the tested pathogenic microbes. but we still don't know which components is responsible for this effect; thus, further work regarding this can be done.

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

Dedication

I would like to dedicate this project paper to my parents, my teachers and my friends.

Acknowledgement

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

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List of Acronyms

WHO World Health Organization

BNH Bangladesh National Herbarium

THP Traditional Health Practitioners

SE Stem Extract

M.H Mueller-Hinton

ICDDRB International Centre for Diarrhoeal Disease Research, Bangladesh

Chapter 1: Introduction

Since the inception of human civilization, individuals have harnessed diverse environmental resources, including sustenance and medicinal substances. Plants significantly contribute to these resources by providing critical services and sustaining environmental equilibrium. Knowledge of medicinal plants has been transmitted through centuries, driven by the growing need for these plants. This information got increasingly comprehensive and exact with the advancement of civilization. Medicinal plants are of significant value in diverse cultures today (Jamshidi-Kia et al., 2017). The phrase "medicinal plant" refers to many plants possessing notable therapeutic characteristics. Prior to recorded history, early humans utilized plants throughout various aspects of daily life, including clothing, housing, food, and fuel. Medicinal plants constitute one of the oldest scientific subjects in nations such as China, Greece, Egypt, and India. In ancient Persia, medicinal plants were frequently utilized as pharmaceuticals, disinfectants, and aromatic substances, signifying their application in disease treatment since the early phases of human existence (Hamilton AC., 2004). Plants have historically been the primary therapeutic option for mankind from the dawn of civilization. Recently, more than 50,000 plant species, nearly one-tenth of all plant species, are extensively utilized in the pharmaceutical and cosmetic industries (Halberstein RA., 2005). Medicinal plants, due to their vital therapeutic properties, can facilitate the development of innovative pharmaceuticals. Plant components such as seeds, roots, leaves, bark, flowers, fruits, or the complete organism may exert physiological impacts on living entities. The interaction of these chemicals may provide both advantageous and detrimental outcomes. The growing awareness of the toxicity and harmful effects associated with conventional and allopathic medicines has heightened the desire for the safe utilization of herbal remedies, thereby diminishing the reliance on chemical pharmaceuticals (Jack DB., 1997). Notwithstanding the importance of medicinal plants, their distribution and utilization

are uneven globally. These are predominantly sourced from wild origins. The demand for these natural sources has increased by 8%-15% annually in Europe, North America, and Asia in recent years (Verma et al., 2008). Conversely, nations such as Malaysia, Korea, France, Chile, and Nepal exhibit deficiencies in both the quantity and proportion of medicinal plant species. Moreover, the populations of undeveloped continents such as Asia, Africa, and Latin America are perceived as lacking progress and, consequently, exhibit a deficiency in innovation to make or utilize current pharmaceuticals through the application of new technologies. In these developing countries, herbal medicine serves as the sole remedy for illnesses and a means of generating income through imports (Adodo, 2013). Consequently, this medication serves as a rallying cry to combat any impediments. Bangladesh is located on the fertile Bengal delta in South Asia. Bangladesh boasts not only rich, fertile soil but also a conducive climate that fosters the growth of several medicinal plants, which hold substantial importance in the country's traditional healthcare practices. These plants have been utilized in several ancient medicinal practices, including Ayurveda, Unani, and herbal medicine, as remedies for diverse diseases and disorders. Among several therapeutic plants, notable examples include Neem, Turmeric, Aloe Vera, and Tulsi. These medicinal herbs are essential for addressing minor health concerns as well as serious conditions such as diabetes, hypertension, and cancer, underscoring their significant role in healthcare (Rahman et al., 2022). The future of therapeutic plants is significant, as over five hundred thousand plant species worldwide remain undiscovered. These unexamined species may have valuable elements that are crucial for innovative therapies in the future. As we continue to explore this specific area, we may uncover that these plants has therapeutic potential for other diseases, thereby altering public perceptions of healthcare and medicine.

Figure 1 P.suberosa (Asian plant,2018)

1.1 Overview of *P.suberosa*

P.suberosa is a medicinal plant from the Annonaceae family, extensively found in tropical areas of South and Southeast Asia, including India, Bangladesh, and Sri Lanka. This plant has historically been utilized in folk medicine to address symptoms including fever, discomfort, ulcers, wounds, and inflammation. Indigenous communities frequently utilize leaf and bark extracts topically or ingest decoctions for internal health concerns. Phytochemical investigations of *P.suberosa* have identified a diverse array of secondary metabolites, including as alkaloids, flavonoids, tannins, saponins, and glycosides. These chemicals are linked to many biological functions, including antibacterial, antioxidant, anti-inflammatory, and cytotoxic properties. Scientific research has validated certain traditional applications, indicating that extracts from *P.suberosa* exhibit considerable efficacy in suppressing microbial proliferation and neutralizing free radicals (Rahman et al., 2020). Due to its varied pharmacological qualities and the persistent global interest in natural product-derived treatments, *P.suberosa* has garnered attention as a potential source of innovative bioactive

chemicals. Further research is necessary to identify specific components, clarify methods of action, and determine its safety and efficacy through clinical assessments.

1.2 Classification of the Medicinal Plant

Medicinal plants are broadly classified based on their therapeutic properties, morphological characteristics, and chemical constituents. One widely used classification is based on their active phytochemical components, such as alkaloids, flavonoids, tannins, glycosides, terpenoids, and saponins. These compounds are responsible for the plants' medicinal activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects. Another approach categorizes plants by the part used, such as leaves, roots, bark, seeds, or flowers, which often determines their specific applications in traditional and modern medicine. Additionally, medicinal plants can be grouped according to their ethnobotanical uses—such as in Ayurveda, Traditional Chinese Medicine, or African traditional systems—which highlights their cultural significance and long-standing usage in treating various ailments. Taxonomically, plants are classified into families, genera, and species to aid in scientific identification and standardization. The pharmacological classification further divides them by the systems they affect, such as cardiovascular, gastrointestinal, or nervous systems. This multifaceted classification system facilitates research, conservation, and pharmaceutical development, ensuring a systematic understanding of plant-based medicine (Kumar et al., 2013).

1.3 Global medicinal use of *P. suberosa* stem

P.suberosa is utilized in traditional medicine for different portions of the plant, particularly its stem. The therapeutic attributes of *P.suberosa* stems have been employed in numerous nations throughout Asia, especially in India, Bangladesh, Sri Lanka, and Southeast Asia. The stem

bark and wood are frequently utilized for their analgesic, anti-inflammatory, and antibacterial qualities. In conventional Ayurvedic medicine, the stem is utilized to address ailments such as fever, cephalalgia, and arthralgia. The bark and stems are frequently cooked or processed into a decoction that is ingested to relieve pain and diminish inflammation, rendering it effective in the treatment of arthritis and muscle discomfort (Choudhury et al., 2009). In Southeast Asia, the stem of *P. suberosa* has been conventionally employed in the treatment of gastrointestinal ailments, including diarrhea and dysentery. The stem possesses antibacterial and antiseptic characteristics, rendering it effective in treating illnesses, particularly those associated with the digestive tract (Xue et al., 2012). The stem is occasionally utilized in poultices for topical treatment to address wounds, cuts, and bruises, since it is thought to possess therapeutic characteristics that facilitate expedited recovery. The medicinal potential of P. suberosa stem has garnered interest due to its bioactive components, including alkaloids and flavonoids, recognized for their efficacy in combating inflammation and microbial infections. Although widely utilized, the scientific validation of these traditional assertions is ongoing, necessitating additional research to comprehensively elucidate its medicinal effects.

1.4 Traditional uses of *P.suberosa* stem in Bangladesh

P.suberosa, recognized for its therapeutic properties in Bangladesh, is significant in traditional healing methods. The stem and bark of this tree are specifically utilized in traditional medicine for their medicinal benefits. P. suberosa is located in many areas of Bangladesh, particularly in the hill tracts and mixed forests of Chattogram and Sylhet, where indigenous tribes have historically depended on its therapeutic properties. The stem is acknowledged for its anti-inflammatory, analgesic, and antibacterial properties, rendering it a significant asset for addressing many health issues. Traditionally, the stem of *P.suberosa* is utilized to alleviate pain and inflammation, especially in ailments such as arthritis and

myalgia. The stem is frequently boiled to produce a decoction, which is used as a treatment to alleviate pain and inflammation (Choudhury et al., 2009). This decoction is utilized to alleviate headaches and general bodily discomfort, with the stem's anti-inflammatory characteristics being pivotal to its application. Furthermore, the stem and bark are utilized in the treatment of wounds and dermatological illnesses. A paste derived from the stem's bark is topically applied to cuts, wounds, and abrasions to expedite healing and avert infection, due to its antibacterial characteristics (Xue et al., 2012).

1.5 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

1.6 Aim of the study

The aim of the study is to screen out the phytochemical constituents and antimicrobial activity of *P.suberosa*.

1.7 Objectives of the study

The objectives of this study are:

To conduct a phytochemical screening and evaluate the antimicrobial activity of the methanolic extract of *P.suberosa* stem, with the aim of identifying bioactive constituents to treat different diseases or find a novel compound to make cure for chronic disease and assessing its potential effectiveness against selected pathogenic microorganisms.

Chapter 2: Methodology

2.1 Collection of the plant part and Identification

We painstakingly gathered fresh *P.suberosa* leaves from the Savar Botanical Garden in Dhaka, Bangladesh. This garden is famous for its extensive plant collection, which includes rare and therapeutic species. Samples were transferred to Mirpur, the home of Bangladesh's National Herbarium, to ensure the accuracy of the plant identification. After confirmation, the leaves were carefully rinsed under running water to eliminate any debris that could have contaminated them, ensuring they were prepared for the subsequent laboratory procedures.

2.2 Extraction Process

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

2.2.1 Processing of the *P.suberosa* stem part

Stem 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.

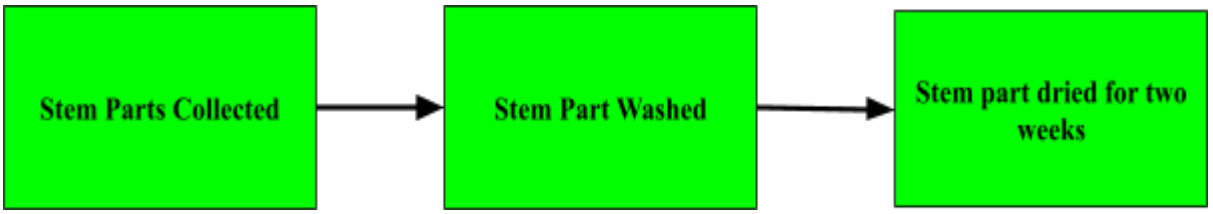

Figure 2: Steps of preparation and drying

2.2.2 Stem Size Reduction

The dried out Stems are now cut down into smaller pieces to reduce their sizes. The Stem are broken down into small pieces of two or three inches length. Next, these smaller pieces are granulated into fine powders individually and weighed. one clean air tight glass container were labeled with necessary information and the crude powder were stored in them.

2.2.3 Maceration of the stem in solvent

Afterwards, maceration of extraction process was performed where methanol was used as the organic solvent. 1000 gm of stem powder was weighed and completely soaked into 1.5L of methanol in glass container for 14 days at normal room temperature (22-25 °C). Regular stirring was done to soak the plant part properly in the solvent.

2.2.4 Filtration of the crude extract

After that, the methanol soaked solutions were first filtered using a clean cotton cloth and then filtered using the Filter papers to obtain a clear solution with the crude extract.

2.2.5 Evaporation of the Methanol

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

2.2.6 Extraction of the plant extract

The extraction procedure of the plant *P.suberosa* stem is described in the (Figure. 4)

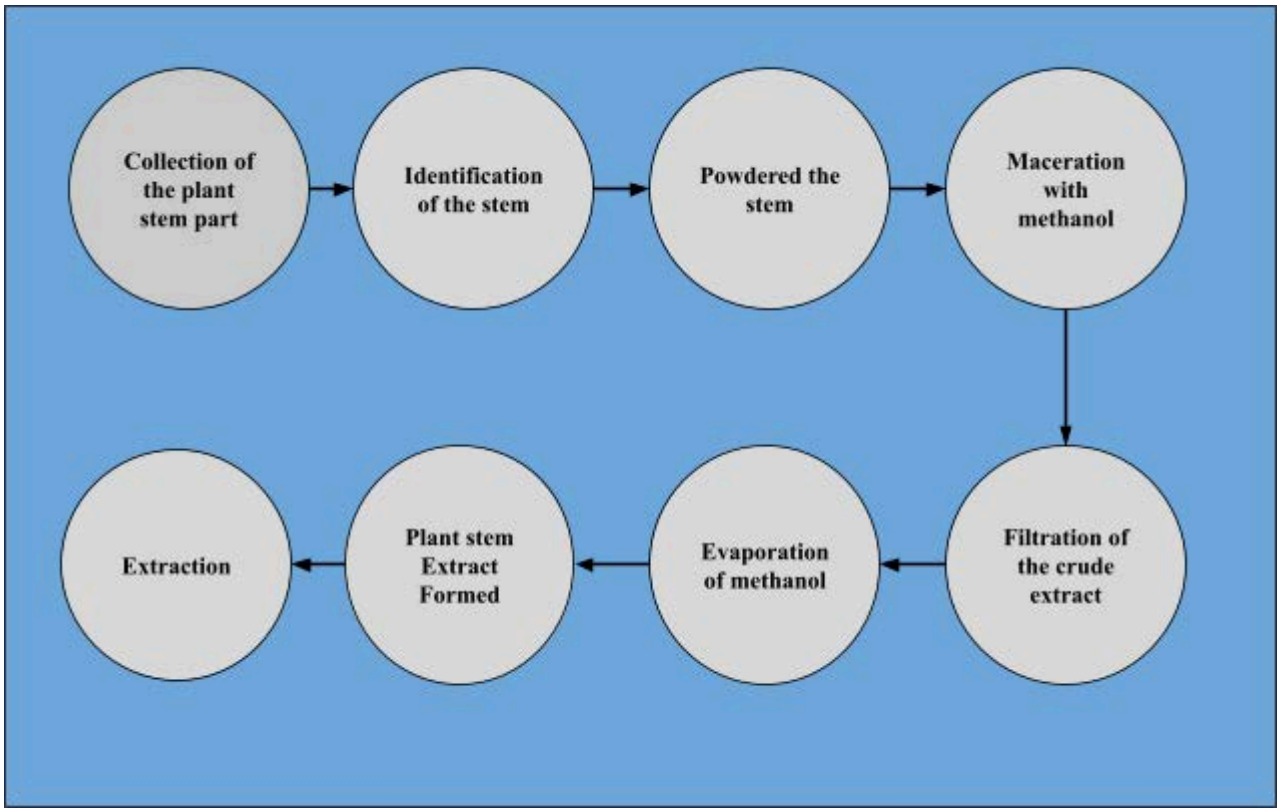

Figure 3: Extraction procedure

2.3 Preliminary screening of *P.suberosa* stem

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

2.3.1 Detection of Alkaloids

Alkaloids form precipitates when reacted with reagents containing heavy metal salts. A portion of the methanolic extract was treated separately with Mayer's reagent (potassium mercuric iodide) and Wagner's reagent (iodine in potassium iodide). The formation of a

cream-colored (Mayer's) and reddish-brown (Wagner's) precipitate confirmed the presence of alkaloids in strong intensity (+++) (Harborne, 1998; Sofowora, 1993).

2.3.2 Detection of Flavonoids

Flavonoids react with alkaline reagents to produce a transient yellow coloration, which disappears upon acidification. The extract was treated with a few drops of sodium hydroxide solution followed by dilute hydrochloric acid. A yellow coloration that turned colorless upon acidification indicated the moderate presence (++) of flavonoids (Harborne, 1998).

2.3.3 Detection of Tannins

Ferric chloride reacts with tannins to form colored complexes due to the presence of phenolic groups. A few drops of 5% ferric chloride solution were added to the extract. A dark green coloration confirmed the strong presence (+++) of tannins (Harborne, 1998).

2.3.4 Detection of Saponins

Saponins exhibit surfactant properties and generate stable froth when shaken with water. The extract was vigorously shaken with distilled water and left to stand. The formation of persistent froth indicated a moderate presence (++) of saponins (Sofowora, 1993).

2.3.5 Detection of Steroids

Steroidal compounds react with concentrated sulfuric acid in chloroform to produce characteristic color changes. The extract was mixed with chloroform, followed by the careful addition of concentrated sulfuric acid. A faint reddish-brown coloration at the interface indicated a mild presence (+) of steroids (Harborne, 1998).

2.3.6 Detection of Terpenoids

Terpenoids react similarly to steroids under Salkowski's conditions, yielding characteristic coloration. The same procedure as for steroids was followed. A slight brownish-red coloration at the interface indicated a mild presence (+) of terpenoids (Sofowora, 1993).

2.3.7 Detection of Glycosides

Cardiac glycosides containing deoxy sugars react with glacial acetic acid and ferric chloride, followed by concentrated sulfuric acid, to produce a reddish-brown ring at the interface. The extract was mixed with glacial acetic acid and ferric chloride, followed by slow addition of concentrated sulfuric acid. The formation of a reddish-brown ring confirmed the moderate presence (++) of glycosides (Harborne, 1998).

2.3.8 Detection of Phenolic Compounds

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 (Harborne, 1998).

2.3.9 Detection of Carbohydrates

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 (Harborne, 1998).

2.3.10 Detection of Proteins

Millon's test was performed to detect proteins in the extract of *P. suberosa*. Upon addition of Millon's reagent followed by gentle heating, a red coloration was observed, indicating the presence of tyrosine-containing proteins in the plant. However, no color change was observed, indicating the absence (–) of proteins in the extract (Sofowora, 1993).

2.3.11 Detection of Resin

The extract was dissolved in alcohol and then treated with water. The appearance of turbidity or precipitate confirmed the presence of resinous compounds in the sample (Sofowora, 1993).

2.6 Antimicrobial Activity test by Disk diffusion method

The Disk diffusion assay is a widely recognized and standardized procedure for assessing the antimicrobial activity of various compounds. In this method, discs impregnated with the test extract are positioned on agar plates previously inoculated with specific bacterial strains. The presence and diameter of inhibition zones surrounding the discs are used as indicators of the extract's antibacterial efficacy.

2.6.1 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.

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

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

2.6.3 Sterilization procedure

All the needed devices for the test including the beakers, cottons, conical flasks and forceps were sterilized by the autoclave and stored in an aseptic place before use. All the works were performed under the laminar air flow hood and the UV light was lit before starting of the experiment for 1 hour in order to maintain the control environment. One time useable sterilized petri-dishes were used to perform the experiment.

2.6.4 Sample Preparation

In total 1 stock solution were prepared for the test from which sample solution from each having the concentrations of 1000µg/ml were prepared.

2.6.5 Experimental Procedure

7g of nutrient broth was weighed and dissolved in 250 ml of distilled water to prepare the broth for culturing the bacteria. Next 1 conical flasks were taken with 50 ml of the prepared broth and 5 mentioned bacterial strains were added. These conical flasks were stored in the incubator in shaking position for the next 24 hours at 37 ° C temperature. After 24 hours, the conical flasks were taken out from the incubator and were kept in a controlled environment for later use. The agar medium was prepared next by using 3.3 g of M.H. agar and it was dissolved in 100 ml of distilled water. The prepared agar was then placed on patriotic plates and kept for cooling in room temperature. After the agar medium was solidified in the petri-dishes, the prepared bacterial strains of the conical flasks were spread out carefully on them by the cotton bars. Kanamycin discs were used as the standard in all the petri-dishes and placed carefully. Likewise the samples' discs prepared and blanks were introduced in the petri-dishes and kept in the incubator at a temperature of 37 ° C for next 24 hours to provide the optimal environment for the bacterial growth. After 24 hours, the petri-dishes were collected from the incubator and the inhibition zone of the standard and the sample discs were measured.

Chapter 3: Result

3.1 Preliminary screening tests result of *P.suberosa* stem

A series of standard qualitative assays was employed to conduct phytochemical analysis of the methanolic extract of *P.suberosa* stem in order to detect the presence of a variety of bioactive compounds. The affirmative result was indicated by the detection of alkaloids using both Mayer's and Wagner's tests. The Alkaline Reagent Test was employed to verify the presence of flavonoids, while the Ferric Chloride Test was employed to identify tannins. The Froth Test indicated the presence of saponins, and Salkowski's Test confirmed the presence of steroids and terpenoids. Glycosides were detected by the Keller-Killani Test, and the Ferric Chloride Test further confirmed the presence of phenolic compounds, resulting in a positive result. An abundance of carbohydrates was indicated in the extract by the detection of carbohydrates using Molisch's Test. Nevertheless, the findings of Millon's Test were negative, indicating that proteins were not present. Ultimately, the resin content was verified through the Acetone Water Test, which yielded a positive result. In general, the extract demonstrated a diverse array of secondary metabolites, many of which are recognized for their pharmacological importance, which lends credence to its potential therapeutic applications.

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	+
Terpinoids	Salkowski's Test	+
Glycosides	Keller-Killani Test	+
Phenolic Compounds	Ferric Chloride Test	+
Carbohydrates	Molisch's Test	+
Proteins	Millons Test	-
Resin	Aceton water Test	+

Note: In this Table, Positive or **(+)** indicates the presence of the compound and Negative or **(-)** indicates the absence of the compound.

Table 2 Phytochemical screening of P.suberosa stem

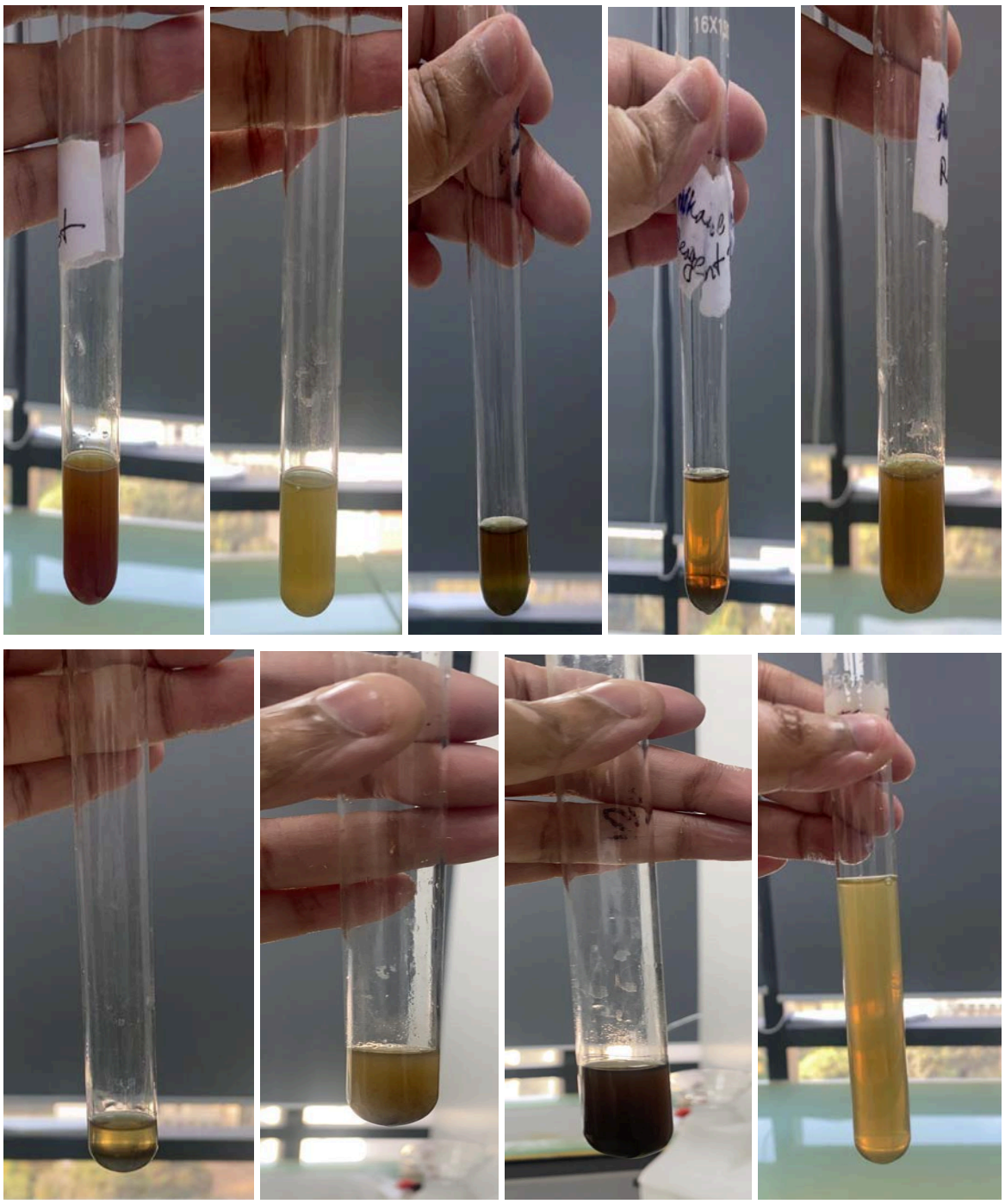

Figure 4 Phytochemical screening tests result of P.subrosa stem

3.2 Antimicrobial Activity Test by Disk Diffusion Method

The antimicrobial efficacy of the methanolic extract of *P.suberosa* stem was evaluated against selected gram-positive and gram-negative bacterial strains using the disc diffusion method. The results were compared with the standard antibiotic kanamycin (30 µg/disc), and the zones of inhibition were measured in millimeters (mm), with corresponding standard deviations provided to ensure statistical reliability. Among the gram-positive strains tested, *B.cereus* exhibited a zone of inhibition of 23 ± 0.47 mm with kanamycin, while the *P. suberosa* extract showed a moderate antibacterial effect, producing a zone of 9 ± 0.83 mm. *B.subtilis* demonstrated a slightly larger inhibition zone with kanamycin (25 ± 0.53 mm), and the plant extract produced a 10 ± 0.57 mm zone, indicating a slightly higher response compared to *B.cereus*. For gram-negative strains, *K.pneumoniae* (CRL strain) showed a 27 ± 0.49 mm inhibition zone with kanamycin, while the extract of *P. suberosa* displayed a 9 ± 1.32 mm zone. *P.aeruginosa* exhibited a 25 ± 0.35 mm inhibition zone with the standard antibiotic, whereas the plant extract yielded a comparatively smaller zone of 8 ± 1.57 mm. *E.coli* showed a 23 ± 0.40 mm zone with kanamycin and a 10 ± 1.97 mm zone with the plant extract, indicating the highest level of susceptibility among the gram-negative strains tested. Overall, the methanolic extract of *P.suberosa* stem demonstrated mild to moderate antibacterial activity against both gram-positive and gram-negative bacteria. Although the zones of inhibition were notably smaller than those produced by kanamycin, the presence of antibacterial activity suggests the presence of bioactive compounds within the extract. These preliminary results support the traditional use of *P. suberosa* in treating infections and justify further investigation into its phytochemical constituents and potential as a source of novel antimicrobial agents.

Bacteria Strain	**Kanamycin 30µg/Disk zone of inhibition (mm)**	***P.suberosa (1000µg/Disk)* zone of inhibition (mm)**
Gram Positive		
B.cereus	23± 0.47	9± 0.83
B.subtilis	25± 0.53	10± 0.57

Table 3 Antimicrobial test results of Gram Positive bacteria

Bacteria Strain	**Kanamycin 30µg/Disk zone of inhibition (mm)**	***P.suberosa (1000µg/Disk)* zone of inhibition (mm)**
Gram Negative		
K.peunomonia CRL	27± 0.49	9± 1.32
P.aeruginosa	25± 0.35	8± 1.57
E.coli	23± 0.40	10± 1.97

Table 4 Antimicrobial test results of Gram negative bacteria

Chapter 4: Discussion

The phytochemical screening of the methanolic extract of *P.suberosa* stem indicates the presence of a diverse range of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, phenolic compounds, terpenoids, and glycosides. These compounds are well known for their pharmacological significance and provide a scientific basis for the plant's traditional medicinal uses (Rahman et al., 2017; Saha et al., 2020). Alkaloids, which were confirmed using Dragendorff's and Wagner's reagents, are recognized for their antimicrobial, analgesic, and central nervous system activities (Kurek, 2019). Their presence supports the stem's application in managing infections and pain-related ailments. Flavonoids and phenolic compounds, identified through standard qualitative tests, are potent antioxidants that help neutralize free radicals and reduce oxidative stress, suggesting potential roles in preventing degenerative diseases such as cancer and cardiovascular disorders (Panche et al., 2016). Tannins, which were detected using ferric chloride, contribute to the plant's astringent and antimicrobial properties, often employed in the treatment of wounds, diarrhea, and oral conditions (Haslam, 1996). Saponins, confirmed through the foam test, have shown immunomodulatory, antifungal, and cholesterol-lowering activities (Sparg et al., 2004). Terpenoids and glycosides, also present in the extract, are associated with anti-inflammatory, anticancer, and cardioprotective properties (Mahato & Sen, 1997). The broad spectrum of these phytochemicals not only supports the ethnobotanical uses of *Polyalthia suberosa* but also indicates its potential as a source of therapeutic agents in modern medicine. The detection of these constituents aligns with previous studies reporting the pharmacological importance of the *Polyalthia* genus and further justifies continued investigation into the bioactive compounds of this species (Shahriar et al., 2018). Future research should focus on the isolation, structural elucidation, and pharmacological testing of these compounds to establish their therapeutic efficacy and safety. Overall, the rich phytochemical profile of

P.suberosa stem underscores its potential as a valuable plant in the development of herbal medicines. From this experiment, we found it has an antimicrobial activity. Analyzing and comparing the results of the zone of inhibition test of antimicrobial activity we can see it is moderate potent against all five pathogenic bacteria. The five microorganisms we have selected are mostly antibiotic resistance but still we can see it show a moderate antimicrobial activity against those microbes.

Chapter 5: Conclusion and Future work

This study demonstrated that the methanolic extract of *P.suberosa* stem contains a variety of pharmacologically important phytochemicals, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds. The extract exhibited moderate antimicrobial activity against both Gram-positive and Gram-negative bacteria, supporting its traditional use in treating infections and inflammation. Although its effectiveness was lower than the standard antibiotic, the results suggest that *P. suberosa* has therapeutic potential. Further research is needed to isolate specific active compounds, determine their mechanisms of action, and assess their efficacy through in vivo studies, which could contribute to the development of novel, plant-based medicines.

Future research on the stem of *P.suberosa* holds significant potential for uncovering novel pharmacological and phytochemical properties. While preliminary studies have indicated the presence of bioactive compounds such as alkaloids, flavonoids, tannins, and saponins, further investigation is needed to isolate and identify which components is responsible for antimicrobial activity. In vivo and in vitro assays should be conducted to evaluate potential therapeutic effects, including antimicrobial, anti-inflammatory, antioxidant, anticancer, and antidiabetic activities. Additionally, toxicological assessments must be carried out to establish safe dosage ranges and identify any adverse effects.

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