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PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACTS OF INDIGENOUS MEDICINAL PLANT *MALLOTUS PHILIPPENSIS*

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ABSTRACT

Mallotus philippensis is an indigenous medicinal plant widely used in traditional medicine because of its diverse therapeutic properties. The present study aimed to perform a qualitative phytochemical screening of the methanolic extract of *Mallotus philippensis* to identify the major bioactive constituents responsible for its medicinal potential. Fresh plant material was collected, shade-dried, powdered, and extracted using 100% methanol. Standard qualitative phytochemical tests were conducted to detect the presence of alkaloids, flavonoids, tannins, saponins, phenolic compounds, glycosides, terpenoids, steroids, carbohydrates, and proteins. The results revealed that the methanolic extract contained significant amounts of flavonoids, tannins, phenolic compounds, saponins, glycosides, and terpenoids, while alkaloids and steroids were detected in lower concentrations. These phytochemicals are associated with antioxidant, antimicrobial, anti-inflammatory, and other pharmacological activities, supporting the traditional medicinal use of the plant. The findings indicate that the methanolic extract of *Mallotus philippensis* is a valuable source of bioactive compounds and may serve

as a promising candidate for further pharmacological investigations and the development of plant-based therapeutic agents.

KEYWORDS: Phytochemical screening, *Mallotus philippensis*, Methanolic extract.

1. INTRODUCTION

Medicinal plants have been used as therapeutic agents since the beginning of human civilization and continue to play a fundamental role in healthcare worldwide. Long before the development of modern synthetic drugs, plants served as the primary source of medicine for treating infectious diseases, inflammatory disorders, gastrointestinal ailments, and various chronic conditions. Despite remarkable advances in pharmaceutical science, medicinal plants remain an essential component of primary healthcare, particularly in developing countries where traditional medicine is often more accessible and affordable. The World Health Organization (WHO) estimates that a large proportion of the global population relies on traditional herbal medicine for primary healthcare, highlighting the continuing importance of medicinal plants in improving human health (World Health Organization, 2013). Furthermore, the increasing incidence of antimicrobial resistance, chronic diseases, and adverse effects associated with synthetic drugs has renewed scientific interest in medicinal plants as valuable sources of novel therapeutic agents.

Plants produce a wide variety of naturally occurring chemical substances known as phytochemicals. These compounds are broadly classified into primary and secondary metabolites. Primary metabolites, including carbohydrates, proteins, lipids, and nucleic acids, are directly involved in plant growth and development. In contrast, secondary metabolites are synthesized primarily as defense mechanisms against herbivores, insects, pathogens, and environmental stresses. Although secondary metabolites are not directly involved in normal plant growth, they possess remarkable biological activities that make them important in medicine and pharmacology. Major groups of phytochemicals include alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, steroids, coumarins, and anthraquinones, many of which exhibit significant pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, antidiabetic, anticancer, antiviral, hepatoprotective, and immunomodulatory activities (Kumar et al., 2021).

Phytochemical investigation has become one of the most important approaches in medicinal plant research because it provides scientific evidence regarding the therapeutic potential of

herbal medicines. Preliminary phytochemical screening is a simple, rapid, and cost-effective analytical technique used to identify the major classes of secondary metabolites present in plant extracts. The presence or absence of these compounds provides valuable information regarding the medicinal value of a plant and serves as the basis for subsequent quantitative analysis, compound isolation, and pharmacological evaluation. Standard qualitative tests such as Mayer's, Wagner's, Dragendorff's, Ferric chloride, Shinoda, Foam, Salkowski, Liebermann–Burchard, and Keller–Killiani tests are commonly employed to detect different groups of phytochemicals in medicinal plants. These preliminary investigations facilitate the identification of promising medicinal species for further phytochemical and pharmaceutical research.

The extraction process represents one of the most critical steps in phytochemical analysis because the nature and concentration of extracted compounds depend largely on the solvent employed. Solvents differ considerably in polarity, thereby influencing their extraction efficiency for various classes of phytochemicals. Water is traditionally used for herbal preparations such as decoctions and infusions, whereas organic solvents including methanol, ethanol, acetone, chloroform, and ethyl acetate are widely employed in laboratory investigations (Azwanida, 2015). Among these solvents, methanol has gained considerable attention due to its ability to dissolve a broad spectrum of polar and moderately non-polar phytochemicals. Methanolic extraction is particularly effective in isolating flavonoids, tannins, alkaloids, phenolic compounds, glycosides, and terpenoids, often resulting in higher extraction yields compared to many other solvents (Dai & Mumper, 2010). Consequently, methanol is regarded as one of the preferred solvents for qualitative and quantitative phytochemical investigations.

Among the numerous medicinal plants investigated worldwide, *Mallotus philippensis* (Lam.) Müll. Arg. has attracted considerable scientific attention because of its extensive traditional uses and diverse pharmacological activities. This species belongs to the family Euphorbiaceae and is commonly known as Kamala, Kampillaka, Kamela, or Red Kamala. It is widely distributed throughout the tropical and subtropical regions of South and Southeast Asia, including Bangladesh, India, Nepal, Pakistan, Sri Lanka, Myanmar, Thailand, Malaysia, and southern China. The plant is commonly found in deciduous forests, hilly regions, and open woodlands where climatic conditions are favorable for its growth (Kumar et al., 2021).

Mallotus philippensis is a medium-sized evergreen tree that usually grows between 5 and 15 meters in height. The plant is characterized by broad ovate leaves, smooth greyish bark, and distinctive fruits covered with reddish-orange glandular hairs. These glandular hairs, commonly known as Kamala powder, represent one of the most valuable medicinal parts of the plant and have been extensively utilized in traditional systems of medicine. Different plant parts including the leaves, bark, fruits, seeds, roots, and glandular hairs have been employed in indigenous healthcare systems for centuries. Traditional practitioners prescribe these plant materials for the treatment of intestinal helminth infections, diarrhea, dysentery, skin diseases, wounds, ulcers, inflammation, bronchitis, fever, parasitic infections, and liver disorders. Such widespread ethnomedicinal applications indicate the presence of multiple bioactive compounds responsible for the plant's therapeutic properties.

Rana, Prakash, and Sagar (2016) included their study that medicinal plants have long been used for treatment of many ailments and such traditional knowledge is the foundation of discovery of many drugs and novel molecules. Nature has been a source of medicinal agents for thousands of years and numerous drugs have been isolated from plants, many based on their use in traditional healing properties. Medicinal plants have always had an important place in the therapeutic world of human beings. According to WHO approximately 80% of world population depend on medicinal plants for their primary health care needs. Out of the 350,000 plant species known so far about 35,000 (some estimate up to 70,000) are used worldwide for their medicinal properties and about less than about 0.5% of these have been investigated for their phytochemical and pharmacological potential worldwide. Nowadays in India interest of people has been diverted to traditional medicines (Ayurveda) from allopathy. At least 25% of the medicines prescribed issued in the USA and Canada contain bioactive constituents that are derived from plant natural products. Many pharmaceutical industries are showing interest in chemicals isolated from plants for therapy of many pathological diseases. Number of plants has been screened for their medicinal properties and their products are playing major role in healing various diseases. Kumar et al., (2021) told their study that Kamala tree (*Mallotus philippensis*) is traditionally used by different ethnic groups to treat a variety of diseases and health ailments. However, these traditional uses need to be scientifically investigated and validated in order to develop drugs from this tree. The tree possesses more than 50 different types of important phytochemicals of natural origin. The wide array of phytochemicals possesses fascinating biological activities like anthelmintic, antibacterial, anti-inflammatory, antioxidant, anti-cancerous, anti-tuberculosis, anti-parasitic,

analgesic, anti-urolithiasis and anti-viral activities (Kumar et al., (2021).

The therapeutic importance of *Mallotus philippensis* has been scientifically supported through numerous phytochemical and pharmacological investigations. Studies have revealed that the plant contains a diverse range of secondary metabolites that contribute to its medicinal properties. Different parts of the plant, including the fruits, leaves, bark, seeds, roots, and glandular hairs, contain varying concentrations of flavonoids, phenolic compounds, tannins, alkaloids, terpenoids, steroids, glycosides, phloroglucinol derivatives, chalcones, and resinous compounds (Gangwar, Goel & Nath, 2014). Among these constituents, rottlerin (mallotoxin) is considered the principal bioactive compound isolated from the glandular hairs of the fruit. Rottlerin has attracted considerable attention because of its antioxidant, anti-inflammatory, antimicrobial, antiparasitic, anticancer, and enzyme-modulating properties. In addition to rottlerin, compounds such as isorottlerin, kamalins, cinnamic acid derivatives, bergenin, and various flavonoids have also been reported, indicating the remarkable chemical diversity of the species (Kumar et al., 2021).

Phenolic compounds and flavonoids represent two of the most abundant classes of phytochemicals identified in *Mallotus philippensis*. These compounds are recognized as powerful natural antioxidants capable of scavenging free radicals, chelating transition metals, and preventing oxidative damage to biological macromolecules. Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms of the body. Excessive ROS production contributes to lipid peroxidation, DNA damage, protein oxidation, and cellular dysfunction, thereby increasing the risk of chronic diseases such as diabetes mellitus, cardiovascular disorders, neurodegenerative diseases, inflammatory conditions, and cancer. The presence of abundant phenolic compounds in *Mallotus philippensis* therefore supports its traditional use in preventing and managing oxidative stress-related disorders (Gangwar, Goel & Nath, 2014).

Flavonoids exhibit a broad spectrum of biological activities beyond their antioxidant effects. Numerous experimental studies have demonstrated that flavonoids possess anti-inflammatory, antimicrobial, antiviral, hepatoprotective, cardioprotective, antidiabetic, and anticancer properties. These compounds inhibit inflammatory mediators, modulate cellular signaling pathways, and protect tissues from oxidative injury. Likewise, tannins possess strong antimicrobial and astringent activities, making them useful in the treatment of diarrhea, wounds, skin infections, and gastrointestinal disorders. Saponins contribute to

immunomodulatory, cholesterol-lowering, and antimicrobial effects, whereas alkaloids are well known for their analgesic, antimicrobial, and neurological activities. Terpenoids and steroids have also been associated with anti-inflammatory, antimicrobial, anticancer, and hepatoprotective properties. The coexistence of these diverse phytochemical groups suggests that the pharmacological activities of *Mallotus philippensis* result from the synergistic interaction of multiple bioactive constituents rather than a single compound (Kumar et al., 2021).

Modern pharmacological investigations have validated many of the traditional medicinal claims associated with *Mallotus philippensis*. Extracts prepared using methanol, ethanol, aqueous solvents, and other organic solvents have demonstrated significant antioxidant activity in several in vitro assays, including DPPH radical scavenging, ABTS radical cation decolorization, ferric reducing antioxidant power (FRAP), and reducing power assays. These antioxidant effects are generally attributed to the high concentration of phenolic compounds and flavonoids present in the extracts. Since oxidative stress is implicated in the pathogenesis of numerous chronic diseases, antioxidants derived from medicinal plants are increasingly being investigated as safer alternatives to synthetic antioxidants.

The antimicrobial activity of *Mallotus philippensis* has also been extensively investigated. Various studies have reported inhibitory effects of plant extracts against Gram-positive bacteria, Gram-negative bacteria, and several pathogenic fungal species. The antimicrobial mechanisms of these phytochemicals include disruption of microbial cell membranes, inhibition of essential enzymes, interference with nucleic acid synthesis, and suppression of microbial biofilm formation. Such findings are particularly important in view of the global increase in antimicrobial resistance, which has reduced the effectiveness of many conventional antibiotics. Plant-derived antimicrobial compounds may therefore serve as valuable candidates for the development of new therapeutic agents against resistant microorganisms (World Health Organization, 2023).

In addition to antioxidant and antimicrobial activities, *Mallotus philippensis* has demonstrated promising anti-inflammatory properties. Inflammation is a protective physiological response to tissue injury or infection; however, prolonged or excessive inflammation contributes to the development of numerous chronic diseases, including arthritis, cardiovascular diseases, diabetes, inflammatory bowel disease, and cancer. Experimental studies suggest that extracts of *Mallotus philippensis* suppress inflammatory

mediators by modulating cytokine production and reducing oxidative stress. These findings provide scientific support for the traditional use of the plant in treating inflammatory disorders and painful conditions.

The plant has also attracted considerable attention for its antidiabetic potential. Diabetes mellitus is a major global public health problem characterized by chronic hyperglycemia resulting from impaired insulin secretion, insulin action, or both. Experimental studies have indicated that extracts of *Mallotus philippensis* may lower blood glucose levels through antioxidant mechanisms, enhancement of insulin activity, inhibition of carbohydrate-digesting enzymes, and protection of pancreatic β-cells from oxidative damage. Although further clinical investigations are required, these preliminary findings highlight the therapeutic promise of the plant in managing metabolic disorders (Gangwar et al., 2014; Kumar et al., 2021).

Another important pharmacological property of *Mallotus philippensis* is its antiparasitic activity. Traditionally, the reddish glandular powder obtained from the fruits has been widely used as an anthelmintic agent for the treatment of intestinal worm infestations in humans and livestock. Scientific investigations have confirmed that extracts and isolated compounds from the fruits exhibit significant activity against several parasitic worms (Singh et al., 2015). This traditional application has contributed substantially to the medicinal reputation of the plant in Ayurvedic and other indigenous healthcare systems. Furthermore, hepatoprotective, analgesic, wound-healing, immunomodulatory, and anticancer activities have also been reported in experimental studies, indicating the plant's broad pharmacological potential (Kumar et al., 2021; Sharma & Verma, 2018).

The phytochemical composition of medicinal plants is influenced by numerous environmental and biological factors. Geographic location, climatic conditions, soil composition, altitude, rainfall, harvesting season, plant age, and post-harvest processing can significantly alter the concentration and distribution of secondary metabolites. Similarly, extraction techniques and solvent polarity greatly influence the recovery of phytochemicals. Consequently, phytochemical screening of locally available *Mallotus philippensis* populations is essential to establish baseline information regarding their chemical composition and medicinal value. Such investigations contribute to quality control, authentication, standardization, and the rational utilization of medicinal plant resources.

Among the available extraction solvents, methanol remains one of the most widely employed in phytochemical research because of its excellent ability to extract a broad spectrum of biologically active compounds. Methanolic extracts generally contain higher concentrations of phenolic compounds, flavonoids, tannins, alkaloids, and glycosides than many aqueous extracts, making them particularly suitable for preliminary phytochemical investigations. Consequently, qualitative phytochemical screening of the methanolic extract of *Mallotus philippensis* provides valuable baseline information regarding the presence of important secondary metabolites and establishes the foundation for future quantitative analyses, bioactivity-guided fractionation, compound isolation, and pharmaceutical development.

Although considerable progress has been made in understanding the pharmacological properties of *Mallotus philippensis*, several aspects of its phytochemical composition remain insufficiently explored. Many published studies have focused primarily on the fruit glandular hairs because they contain rottlerin, the principal bioactive constituent. Comparatively fewer investigations have comprehensively evaluated the phytochemical profile of methanolic extracts prepared from locally collected plant materials. Moreover, variations in geographical origin, climatic conditions, soil characteristics, harvesting season, plant maturity, and extraction methods can significantly influence the qualitative and quantitative composition of phytochemicals. Consequently, findings reported from one geographical region cannot always be generalized to plants growing in another region. These variations highlight the need for continued phytochemical investigations using standardized extraction procedures and analytical methods to establish reliable phytochemical profiles for medicinal plants.

Qualitative phytochemical screening serves as an essential preliminary step in medicinal plant research. The identification of major classes of secondary metabolites provides baseline information that supports subsequent quantitative estimation, chromatographic separation, structural characterization, and pharmacological evaluation of individual compounds. Furthermore, preliminary phytochemical investigations contribute to the quality control and standardization of herbal medicines by ensuring consistency in the chemical composition of plant materials. Such information is particularly valuable for pharmaceutical industries involved in the development of herbal formulations, nutraceuticals, and plant-derived therapeutic products.

The increasing global demand for plant-based medicines has intensified research aimed at discovering safe, effective, and affordable natural products. Medicinal plants are increasingly

recognized as valuable reservoirs of bioactive compounds capable of addressing major public health challenges, including antimicrobial resistance, chronic inflammatory disorders, metabolic diseases, oxidative stress-related conditions, and cancer. As synthetic drugs may produce undesirable adverse effects or lose efficacy because of microbial resistance, natural products continue to provide promising alternatives for the discovery of novel therapeutic agents. Consequently, systematic phytochemical evaluation of medicinal plants such as *Mallotus philippensis* remains an important area of pharmaceutical and biomedical research.

The present study was designed to investigate the phytochemical constituents present in the methanolic extract of the indigenous medicinal plant *Mallotus philippensis*. Standard qualitative phytochemical tests were employed to detect the presence or absence of major classes of secondary metabolites, including alkaloids, flavonoids, tannins, phenolic compounds, saponins, glycosides, terpenoids, steroids, carbohydrates, proteins, and other phytochemical groups. Identification of these constituents is expected to provide scientific evidence supporting the traditional medicinal uses of the plant while establishing a foundation for future pharmacological and phytochemical investigations.

The findings of this study are expected to contribute to the growing body of knowledge regarding the medicinal value of *Mallotus philippensis*. In addition to supporting its traditional applications, the results may facilitate the development of standardized herbal formulations, promote quality assurance of medicinal plant materials, and encourage further investigations into the isolation and characterization of bioactive compounds. Ultimately, comprehensive phytochemical evaluation of *Mallotus philippensis* may contribute to the discovery of novel therapeutic agents for the management of infectious diseases, inflammatory disorders, metabolic diseases, and other health conditions.

2. AIM OF THE STUDY

The primary aim of this study is to qualitatively evaluate the phytochemical constituents present in the methanolic extract of the indigenous medicinal plant *Mallotus philippensis*.

2.2. Specific Objectives

1. To prepare a methanolic extract of *Mallotus philippensis* using standard extraction procedures.
2. To perform qualitative phytochemical screening of the methanolic extract.

3. To determine the presence or absence of major phytochemical groups, including alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, terpenoids, steroids, carbohydrates, and proteins.
4. To provide scientific evidence supporting the traditional medicinal use of *Mallotus philippensis*.

3. Plant Profile of *Mallotus Philippensis*

This genus *Mallotus,* includes approximately 150 species distributed in tropical and sub-tropical regions in Asia (Cambodia, China, India, Laos, Malaysia, Sri Lanka, Thailand, Vietnam). A few species are found in the North and East of Australia and the Pacific-Ocean Archipelago (the East of Fiji). Only two species are found in Africa and Madagascar (Schatz, 2001).

Scientific classification of *Mallotus Philippensis* (CABI, 2021)

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Euphorbiales

Family: Euphorbiaceae

Genus: Mallotus

Species: Mallotus Philippensis

Figure 3.1: *Mallotus Philippensis.*

4.0. MATERIALS AND METHODS

4.1. Extraction of *Mallotus Philippensis*

The extraction was carried out as per procedure described by Marjhan and Mannan, 2024. The fruit glands and hairs of *Mallotus Philippensis* were taken from the medicinal plant garden of Hamdard University Bangladesh. After thoroughly cleaning the plant parts allowed to dry in the shade. Then the plants parts were grinded in to powder by mechanical grinder and pass through a 100 mm sieve for fine powder. To get a clear filtration, 100 g of the fine powder was soaked in 400 ml of methanol for 72 hours while being stirred. Then the mixture was filtered through two layers of muslin, centrifuged for 10 minutes at 9000 rpm, and then filtered once more through Whatman filter paper No. (1). Using water bath, the filtrate was evaporated and dried at the temperature of 45^{o}C. The yield of the extract was measured and subsequently stored in a small bottle in the refrigerator at 5°C. The calculation of the percentage yield was performed using the formula: Extract yield % = R/S × 100, Where, R signifies the weight of the extracted plant residues and S indicates the weight of the raw plant sample (Marjhan and Mannan, 2024).

3.2. Reagents and chemicals

Reagents and chemicals used in this study included Wagner's reagent (iodine in potassium iodide), Hager's reagent (saturated picric acid solution), Mayer's reagent (potassium mercuric iodide solution), Dragendorff's reagent (potassium bismuth iodide solution), Molisch's reagent (alcoholic α-naphthol solution), Benedict's reagent, Fehling's solution, Salkowski reagent, concentrated sulfuric acid, 10% lead acetate solution, 0.1% ferric chloride solution, dilute sodium hydroxide (NaOH), ammonia solution, dilute hydrochloric acid (HCl), distilled water, and methanol, which were used for qualitative phytochemical screening following standard procedures (Harborne, 1998; Trease & Evans, 2009).

3.3. Phytochemical Analysis

The crude extract was subjected to different qualitative chemical tests to determine the presence of phytochemicals, which were identified based on characteristic color changes following standard procedures. Tests for alkaloids were carried out according to Harborne (1973), steroids following Trease and Evans (1989), phenolics and flavonoids as described by Awe and Sodipo (2001), saponins and cardiac glycosides according to Sofowora (1993), tannins following Odebiyi and Sofowora (1978), and terpenoids as well as saponins according to Obadoni and Ochuko (2001).

3.3.1. Detection of alkaloids: Extract was dissolved in dilute Hydrochloric acid and filtered.

3.3.1.1. Mayer's Test: Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow-colored precipitate indicates the presence of alkaloids.

3.3.1.2. Wagner's Test: Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

3.3.1.3. Dragendroff's Test: Filtrate was treated with Dragendroff's reagent (Solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

3.3.1.4. Hager's Test: Filtrate was treated with Hager's reagent (Saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

3.3.2. Detection of carbohydrates: Extract was dissolved in 5 ml distilled water and filtered. The filtrate was used to test for the presence of carbohydrates.

3.3.2.1. Molisch's Test: Filtrate was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

3.3.2.2. Benedict's Test: Filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

3.3.2.3. Fehling's Test: Filtrate was hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3.3.3. Detection of glycosides: Extract was hydrolyzed with dil. HCl, and then subjected to test for glycosides.

3.3.3.1. Modified Borntrager's Test: Extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treat with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

3.3.4. Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

3.3.5. Detection of saponins

3.3.5.1. Froth Test: Extract was diluted with distilled water to 20 ml, and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

3.3.5.2. Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced and persists for ten minutes, it indicates the presence of saponins.

3.3.6. Detection of phytosterols

3.3.6.1. Salkowski's Test: Extract was treated with chloroform and filtered. The filtrate was treated with few drops of Concentrated Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

3.3.6.2. Libermann Burchard's test: Extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled, and cooled. concentrated Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

3.3.7. Detection of phenols

3.3.7.1. Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

3.3.8. Detection of tannins

3.3.8.1. Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

3.7.9. Detection of flavonoids

3.3.9.1. Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which became colorless on addition of dilute acid, indicates the presence of flavonoids.

3.3.9.2. Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

3.3.10. Detection of proteins and amino acids

3.3.10.1. Xanthoproteic Test: The extract was treated with few drops of concentrated Nitric acid. Formation of yellow color indicates the presence of proteins.

3.3.10.2. Ninhydrin Test: To the extract, 0.25% ninhydrin reagent ($C_9H_6O_4$) was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

4. Results: Phytochemical Screening of *Mallotus philippensis*

Table 4.1: Results of alkaloids in methanolic extract of *Mallotus philippensis.*

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Alkaloids	Mayer's Test	Present
2		Wagner's Test	Present
3		Dragendroff's Test	Present
4		Hager's Test	Present

Table 4.2: Results of carbohydrates in methanolic extract of *Mallotus philippensis.*

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Carbohydrates	Molisch Test	Present
2		Benedict's Test	Present
3		Fehling Test	Present

Table 4.3: Results of glycoside in methanolic extract of *Mallotus philippensis.*

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Glycoside	Modified Borntrager's Test	Present
2		Legal's Test	Present

Table 4.4: Results of saponins in methanolic extract of *Mallotus philippensis.*

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Saponins	Forth Test	Absent
2		Foam Test	Absent

Table 4.5: Results of phytosterol in methanolic extract of *Mallotus philippensis.*

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Phytosterol	Salkowski's Test	Present
2		Libermann Burchard's Test	Absent

Table 4.6: Results of phenols in methanolic extract of *Mallotus philippensis.*

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Phenol	Ferric Chloride Test	Present

Table 4.7: Results of tannins in methanolic extract of *Mallotus philippensis.*

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Tannins	Gelatin test	Present

Table 4.8: Results of flavonoids in methanolic extract of *Mallotus philippensis.*

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Flavonoids	Alkaline Reagent Test	Absent
2		Lead acetate Test	Present

Table 4.9: Results of proteins and amino acids in methanolic extract of *Mallotus philippensis.*

Sl.	Detection of Phytochemicals	Name of the test	Result
1	Proteins & Amino acids	Xanthoproteic Test	Present
2		Ninhydrin Test	Present

Figures of Alkaloids Detection

Figure 4.1: Mayer's Test

Figure 4.2: Wagner's Test

Figure 4.3: Dragendroff's Test

Figure 4.4: Hager's Test

Figures of Carbohydrates Detection

Figure 4.5: Molisch Test

Figure 4.6: Benedict's Test

Figure 4.7: Fehling Test

Figures of Glycoside Detection

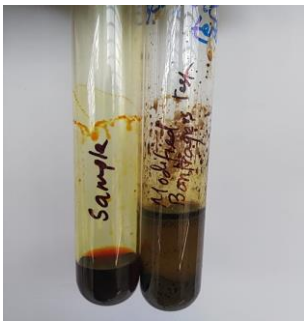

Figure 4.8: Modified Borntrager's Test.

Figure 4.9: Legal's Test.

Figures of Saponins Detection

Figure 4.10: Forth Test

Figure 4.11: Foam Test

Figures of Phytosterol Detection

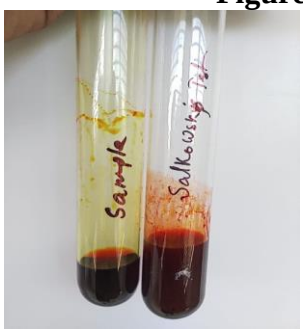

Figure 4.12: Salkowski's Test

Figure 4.13: Libermann Burchard's Test

Figure of Phenol Detection

Figure 4.14: Ferric Chloride Test.

Figure of Tannins Detection

Figure 4.15: Gelatin Test.

Figures of Flavonoids Detection

Figure 4.16: Alkaline Reagent Test.

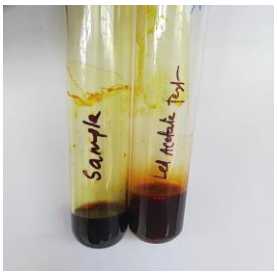

Figure 4.17: Lead acetate Test.

Detection of proteins and amino acids

Figure 4.18: Xanthoproteic Test. **Figure 4.19: Ninhydrin Test.**

4.2. DISCUSSION

The present study was conducted to evaluate the qualitative phytochemical constituents of the methanolic extract of *Mallotus philippensis*. The results demonstrated the presence of several important secondary metabolites, including alkaloids, carbohydrates, glycosides, phenols, tannins, flavonoids, phytosterols, proteins, and amino acids, while saponins were found to be absent. These findings indicate that *Mallotus philippensis* is a rich source of biologically active compounds that may contribute to its traditional medicinal uses.

The detection of alkaloids in all four confirmatory tests (Mayer's, Wagner's, Dragendorff's, and Hager's) strongly suggests a significant presence of alkaloid compounds in the methanolic extract. Alkaloids are well known for their wide range of pharmacological activities, including antimicrobial, analgesic, antimalarial, and anticancer properties. Similar findings were reported by Sharma et al. (2014), who documented the presence of alkaloids in *Mallotus philippensis*, supporting its traditional use in treating infectious and parasitic diseases. The presence of alkaloids may therefore contribute to the medicinal efficacy of the plant.

Carbohydrates were also detected using Molisch, Benedict's, and Fehling's tests. The presence of reducing sugars in the extract is consistent with the findings of Kokate (2005), who reported that plant extracts commonly contain simple sugars and polysaccharides that play roles in energy metabolism and may also exhibit immunomodulatory effects. Although carbohydrates are primarily primary metabolites, they can contribute indirectly to biological activity and formulation stability in herbal preparations.

Glycosides were confirmed using Modified Borntrager's and Legal's tests. Glycosides are important bioactive compounds known for their cardiotonic, laxative, antimicrobial, and anti-inflammatory properties. The presence of glycosides in *Mallotus philippensis* is consistent

with previous phytochemical reports (Evans, 2009), which highlight the occurrence of glycosidic compounds in many Euphorbiaceae family members. These compounds may significantly contribute to the pharmacological potential of the plant.

Saponins were found to be absent in both froth and foam tests. This result differs from some earlier studies where trace amounts of saponins were reported in related extracts. For example, Harborne (1998) noted that saponin content in medicinal plants may vary depending on solvent polarity, plant part used, and geographical conditions. The absence of saponins in this study may be due to extraction with methanol or environmental factors affecting phytochemical composition.

Phytosterols were detected by Salkowski's test but not confirmed by Liebermann–Burchard's test. This partial detection suggests that phytosterols may be present in low concentrations or exist in forms that respond differently to qualitative reagents. Phytosterols are known for their cholesterol-lowering, anti-inflammatory, and anticancer activities. Similar observations were reported in phytochemical studies of medicinal plants where sterol detection varied depending on the sensitivity of the test used (Trease & Evans, 2009).

Phenolic compounds were positively identified using the ferric chloride test. Phenolic compounds are among the most important plant secondary metabolites due to their strong antioxidant properties, and they play a significant role in preventing oxidative stress-related diseases. The presence of phenolic compounds in *Mallotus philippensis* has been widely reported in phytochemical studies, with evidence indicating high phenolic content contributing to its antioxidant activity (Gangwar et al., 2014). This supports the traditional use of the plant in the management of inflammatory and infectious conditions.

Tannins were also detected in the extract. Tannins possess strong astringent, antimicrobial, and wound-healing properties. The presence of tannins in *Mallotus philippensis* is consistent with findings reported by Sofowora (2008), who emphasized that tannin-rich plants are widely used in traditional medicine for treating diarrhea, skin infections, and wounds. Their presence further supports the ethnomedicinal relevance of this plant.

Flavonoids showed mixed results, being positive in the Lead Acetate test but negative in the Alkaline Reagent test. This variation may be due to differences in sensitivity of the tests or low concentration of certain flavonoid subclasses. Flavonoids are widely recognized for their

antioxidant, anti-inflammatory, and antimicrobial properties. Similar variability in flavonoid detection has been reported in other phytochemical studies, where different reagents detect different structural forms of flavonoids (Harborne, 1998).

Proteins and amino acids were confirmed using Xanthoproteic and Ninhydrin tests. While not primary therapeutic agents, amino acids and peptides may contribute to nutritional and metabolic functions. Their presence indicates that the plant contains both primary and secondary metabolites, which may act synergistically in biological systems.

Overall, the phytochemical profile observed in this study aligns well with previous reports on *Mallotus philippensis* and other medicinal plants of the Euphorbiaceae family. The presence of multiple bioactive compounds suggests that the methanolic extract may exhibit a broad spectrum of pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, and therapeutic effects.

5. CONCLUSION

The present study revealed that the methanolic extract of *Mallotus philippensis* contains a wide range of bioactive phytochemicals, including alkaloids, glycosides, carbohydrates, phenols, tannins, flavonoids, phytosterols, proteins, and amino acids, while saponins were absent. These findings scientifically validate the traditional medicinal use of the plant and suggest its potential as a source of natural therapeutic agents. The presence of phenolic compounds, flavonoids, tannins, and alkaloids indicates strong pharmacological potential, particularly in antioxidant, antimicrobial, and anti-inflammatory activities. Variations observed in certain phytochemical tests highlight the influence of extraction methods, solvent polarity, and environmental factors on phytochemical composition. In conclusion, *Mallotus philippensis* can be considered a valuable medicinal plant with significant phytochemical richness. Further studies involving quantitative analysis, compound isolation, and pharmacological evaluation are recommended to fully explore its therapeutic potential and to develop standardized herbal formulations for clinical use.

6. Conflicts of interests

The authors do not disclose any conflicting interests.

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8.0. REFERENCES

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