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Journal of Advancement in Pharmacognosy
Volume 5 Issue 1, 2025
ISSN: 2582-8401

Phytochemical, Biochemical and Antibacterial Efficacy of Tulsi leaves

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ABSTRACT

Tulsi (Ocimum sanctum L.) is an aromatic and a native plant of India. Ocimum sanctum also known as tulsi or holy basil is an aromatic plant and belongs to the Lamiaceae family. It is also known for its medicinal properties in Ayurveda. The present study was aimed to analyze the various phytochemical and biochemical constitutents of the plant as well as to study the antimicrobial properties of tulsi leaves. Phytochemical screening of the plant leaf extracts revealed the presence of saponins, alkaloids, flavonoids, steroids, phenols, tannins and glycosides. Biochemical analysis of the leaf extracts of tulsi plant confirmed presence of essential nutrients like carbohydrates and lipids. The plant showed antimicrobial properties against two bacteria. Observations indicate the use pharmacognistic of tulsi leaves in drug formulations as well as in therapeutics.

Keywords: *Anti-inflammatory, Essential oils, phytochemicals, biochemical analysis*

INTRODUCTION

The Tulsi plant, also known as Holy Basil, is an indigenous and revered herb in Hindu culture and is often grown in Indian households and is believed to bring blessings and protection. The use of Tulsi as an ayurvedic medicine has been a traditional culture among the Indian tradition. Tulsi plant is well known for its spiritual significance and medicinal properties. (Kakurde, 2024). The leaves of tulsi plant are used in conventional medicine for its powerful health benefits such as anti-inflammatory, antioxidant, and antimicrobial properties. It's often used to support the immune system, relieve stress, and in the recovery of respiratory health. Tulsi leaves are used in teas, soups, and even as a garnish in some dishes. Essential oils and extracts from the plant are also used in various health and beauty products. Tulsi plant is scientifically known as *Ocimum sanctum or Ocimum tenuiflorum* and classified under the mint family Lamiaceae (Nahak etal., 2011). It is an erect bushy plant and has several varieties and the main ones include Rama Tulsi with green leaves and a mild flavor, Krishna Tulsi having purple leaves with a stronger taste and aroma and Vana Tulsi which is a wild variety with a slightly different flavor profile. (Kumar, etal., 2018). The growth conditions for this plant is a moderately warm climate with plenty of sunlight. (George etal., 2021).

Table 1: Varieties of tulsi

Plant Variety	Features
Rama Tulsi or normal green variety	*Ocimum Americanum* Linn. - whitish green leaves
Krishna Tulsi or Dark green or Black Variety	(*Ocimum tenuiflorum*) - black variety-purple leaves
Vana Tulsi Wild Green leaf tulsi	(*Ocimum basilicum*) - lush-green leaves with fresh look

Journal of Advancement in Pharmacognosy
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Fig. 1. Rama, Krishna, and Vana Tulsi plants

Tulsi plant is used in diverse types like dried or fresh leaves can be brewed into a healthy and flavorful tea, as supplements that are available in capsule, tablet, and liquid forms, essential oil used in aromatherapy and topical applications, culinary herbs adding a unique flavor to cuisines and as an ayurvedic medicine in various formulations.

Table 2: Morphology of tulsi plant

Characters	**Observations**
Colour	Externally purplish brown to black Internally cream coloured
Odour	Faintly aromatic
Taste	Characteristic or sour taste
Height:	A subshrub that is 30–60 cm (12–24 in) tall
Leaves	Simple, petioled, ovate leaves with a slightly toothed margin that are green or purple
Flowers	Purplish flowers in close whorls on elongated racemes
Stems	Hairy stems that are thick and woody
Fruits	Nutlets that produce numerous seeds with small ovules

Tulsi, or Holy Basil, is renowned for its broad range of medicinal properties. Here are some of its key medicinal benefits such as

1) **Anti-Inflammatory** property: Tulsi contains compounds like eugenol and rosmarinic acid, which have anti-inflammatory properties. This can help reduce inflammation in the body, which is beneficial for conditions like arthritis, mild edema etc. Crushed tulsi leaves along with other ingredients like ginger reduce the pain and inflammation. (Bhadra, & Sethi 2020).
2) **Antioxidant**: The plant is rich in antioxidants, such as flavonoids and phenolic compounds. Polyphenolic compounds present in tulsi are known to show powerful antioxidant properties because of their ability to produce free radicals. Thus excessive oxidative damage of the body can be prevented. These antioxidants also help combat oxidative stress and protect cells from damage caused by free radicals (Kumar etal., 2010).
3) **Adaptogen**: Tulsi is known as an adaptogen, meaning it helps the body adapt to stress and maintain balance. It can support mental health by reducing anxiety and stress levels.Eugenol and caryophyllene preset in tulsi control frequent mood swings and aid mental peace. It is also found to minimize the risk of mental problems due to ageing. They also help in reduction of stress causing factors like corticosteroids. (Gavin 2001).

Journal of Advancement in Pharmacognosy
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4) **Immune Support**: The herb is believed to boost the immune system. It has antimicrobial and antiviral properties that can help the body fight off infections and illnesses. The vital tulsi components serve as immunomodulators and helps in the speedy recovery thereby balancing body's immune system (Chatterjee (2001).
5) **Digestive Health**: Tulsi supports digestion by stimulating appetite, improving digestion, and reducing bloating and gas. It can also help with stomach ulcers and other gastrointestinal issues. (Sarin etal., 2013).
6) **Blood Sugar Regulation**: Some studies suggest that Tulsi may help regulate blood sugar levels, which can be beneficial for people with diabetes or those at risk of developing diabetes. (Pandey & Madhuri 2010). 'Eugenol'. Present as the main ingredient responsible for controlling the blood sugar levels in the body. It prepares the beta cells of the pancreas and as a result enhances the secretion of insulin thus lowering the blood sugar.
7) Respiratory Health: Tulsi leaves aid respiratory health by improving symptoms of coughs, colds, and asthma. It helps in driving out of mucus and may reduce the severity of respiratory infections. Decocton of tulsi leaves with honey and licorice are known to cure cough and sore throat. It also helps to get relief from fever and mild body ache (Shuman, Raju, & Jogdeo, 2018).

Chemical Composition of Tulsi

The sacred basil contains many nutrients and other bioactive components. These components may be affected and vary from species to species and also their constitution may be affected by factors promoting growth conditions. Tulsi is known to contain a variety of phytochemicals that are not yet well understood.

Some of the essential compounds present in tulsi are Essential oils like eugenol and cineole (Eucalyptol) present in the leaf, linalool that offers calming effects and has antimicrobial properties, carvacrol showing antibacterial, antifungal, and anti-inflammatory properties, flavonoids like apigenin and luteolin which are antioxidants with anti-inflammatory and neuroprotective effects and quercetin that provides antihistamine effects.

Tusli leaves also contain phenolic compounds like rosmarinic acid and caffeic acid, triterpenoids in the form of oleanolic acid with hepatoprotective properties and ursolic acid. The constitution of tulsi leaves also includes s**aponins** showing immune-modulating effects, tannins with astringent properties, and helping in wound healing and reducing inflammation, terpenoids like beta-caryophyllene with analgesic effects that support cardiovascular health, vitamins (A and C) and minerals like calcium, iron and magnesium all contributing to overall health and wellness.

The above phytochemicals all together add on to Tulsi's medicinal potency, making it an important herb in traditional and modern herbal practices.

The current work was aimed to comprehensively evaluate the phytochemical properties including but not limited to alkaloids, flavonoids, terpenoids, saponins, and phenolic compounds, biochemical analysis such as carbohydrates, proteins, lipids, and minerals and to assess the antioxidant capacity of the plant material, and antimicrobial properties of Tulsi leaves extract against a panel of pathogenic microorganisms with the goal of elucidating their potential therapeutic applications. The study also contributes to the scientific understanding of Tulsi and provides a foundation for further research into their therapeutic applications.

Journal of Advancement in Pharmacognosy
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METHODOLOGY

Extraction and Recovery of Phytochemicals

Fresh leaves of tulsi plant were collected and thoroughly washed with distilled water to remove impurities like dust and other materials. After the water was completely drained off, these leaves were weighed 5 g using a digital balance. The leaves were then transferred to a suitable container and then ground into a fine paste using a mixer, by add 100 ml of phosphate buffer (50mM, pH 7). The initial extract was then filtered using cheese cloth and incubated at 37°C for 30 min by shaking on a rotary shaker. This ensured recovery of the ingredients in the extract from plant cells. The homogenate was then further filtered through whatmann paper and the clear extract was stored in a suitable airtight container at cold conditions and used for further studies.

The homogenate was further mixed thoroughly by incubating the contents at room temperature in a rotary shaker for 30 min at 150 rpm. The homogenate was filtered through cheese cloth and centrifuged at 10,000 rpm for 15 min at 4 °C and the clear supernatant obtained and was assayed for protease inhibitor activity and protein content. The homogenate was further mixed thoroughly by incubating the contents at room temperature in a rotary shaker for 30 min at 150 rpm. The homogenate was filtered through cheese cloth and centrifuged at 10,000 rpm for 15 min at 4 °C and the clear supernatant obtained and was assayed for protease inhibitor activity and protein content.

Phytochemical Screening

The aim of the study of phytochemical screening is to identify and compare the potential therapeutic compounds in Tulsi, laying the groundwork for drug discovery and development. The prepared extract of Tulsi plant was used to test various phytoconstituents present in it. Phytochemical screening was the term given to these various qualitative tests. (Alamzed et al. 2013, Thusa & Mulmi, 2017, Talukdar & Chaudhary, 2010).

1) Test for tannin / polyphenol or Ferric Chloride Test: Ferric chloride or (FeCl3) is a specific test for tannins. 3-4 drops of 10% $FeCl_3$ was added to a few drops of the crude extract. The presence of tannin turned the solution green or bluish green.
2) Test for reducing sugar: To 0.5 ml of plant extract 1ml of water and 5-8 drops of Fehling's solution was added and heated at 100°C for 5 min. The appearance of a brick-red precipitate signaled the presence of reducing sugar.
3) Test for quinine: 1 ml of freshly prepared $FeSO_4$ was added to the extract along with 1 ml of ammonium thiocyanate then conc. H_2SO_4 was added dropwise. The presence of quinine was indicated by deep red coloration.
4) Test for glycosides: Molisch's Test: 5 ml of Molisch's reagent was added to the tulsi extract followed by the addition of dropwise concentrated H_2SO_4. The presence of glycosides was indicated by violet colored ring at the junction of the two liquids.
5) Test for flavonoids (Shinoda test): To 4 ml of the extract solution, 1.5 ml of 50% methanol solution a small zinc chunk was added and warmed. 5-6 drops of concentrated HCl was also added and the presence of flavonoids was observed by the formation of red color.
6) Dil. NH_3 test: To 5 ml of the extract dilute NH_3 solution was added along with the addition of conc. H_2SO_4. The presence of yellow-colored precipitation indicated presence of flavonoids.
7) Test for terpenoids: 0.2 ml of the sample was mixed with 2 ml chloroform and 3 ml conc. H_2SO_4. The presence of terpenoids was indicated by reddish-brown coloration.

Journal of Advancement in Pharmacognosy
Volume 5 Issue 1, 2025
ISSN: 2582-8401

8) Test for alkaloids/ Meyer's test: To 2 ml of tulsi extract, 1 ml of Meyer's reagent was added. The presence of alkaloids was indicated by pale-yellow precipitate.
9) Dragendroff's reagent test: 2 ml of the tulsi extract was warmed with 2 ml of 2% H_2SO_4. Few drops of Dragendroff's reagent were added. Orange-red precipitate indicated the presence of alkaloids.
10) Test for saponins: The tulsi filtrate was shaken vigorously with 5 ml of distilled water for about five minutes. Presence of saponins was indicated by frothing over the solution.
11) Test for volatile oils: Test was carried out by taking 2 ml extract and treating it with 1ml of 10% NaOH followed a small amount of diluted HCl. The formation of white precipitate indicated the presence volatile oils.
12) Test for cardiac glycosides: 5ml extract was treated with 2 ml of glacial acetic acid and 2 ml of 1% $FeCl_3$ solution for the detection of cardiac glycosides and observed for the formation of a violet ring or a pale greenish colouration.
13) Test for steroids: To 1ml of the plant extract a few drops of acetic acid and a drop of conc. H_2SO_4 was dissolved. The formation of green color indicated the presence of steroids.

Biochemical Analysis

1. **Test for Carbohydrates:** Sugars are biomolecules that occur naturally in plants. These carbohydrates can be as simple sugars (monosaccharides) and also from abundant polysaccharides. In order to test the presence of these compounds in a plant extract, several qualitative tests can be carried out. Molisch test and Anthrone tests stand out to be the most common and authentic.
    a. **Molisch's Test:** In a test tube, 2 ml of the plant extract was taken and two drops α-naphthol in ethanol (Molisch reagent) was added to it. Few drops of concentrated sulfuric acid were then added dropwise carefully (down the side of the test tube).
    b. **Anthrone Test:** To 1 ml of the plant extract 4 ml of anthrone reagent was added in excess and the colouration obtained was noted. Presence of blue green color (purple to orange) meant the presence of carbohydrates and in particular polyscaccharides.

2. **Test for Protiens or Biuret Test:** 2 ml of the plant extract was treated with 1 ml of Biuret reagent. The contents were mixed well and let stand for a few minutes. Presence of violet color indicated the presence of proteins.

3. **Test For Lipids:**
    a. **Test for unsaturation:** To 1ml of the sample, a few drops of 1% $K_2Cr_2O_7$ solution was added in a test tube. The presence of lipids was indicated by decolorization or fading of the original colour.
    b. **Test for saponification:** To a few drops of the sample 2 ml of soap solution was added. The appearance of thick emulsion indicated the presence of lipids.

Antimicrobial Study of the Crude Tulsi Extract by Agar Well Diffusion Method

Antimicrobial properties of tulsi leaf extract was performed by agar well diffusion method against 2 different bacterial isolates. The crude tulsi extract was filter sterilized through millipore filter (0.22 µm). 20 ml of sterile nutrient agar medium was poured into sterile petri dishes followed by spreading 100 µl of the bacterial culture inoculum. Wells were made on these plates using a sterile well borer and 20 µl of the crude filter sterilized tulsi extract was then loaded into the wells. A positive Filter paper discs loaded with 5 µg of Streptomycin were used as positive control. The plates were kept in the fridge at 4 °C for 2 h to permit plant extracts

Journal of Advancement in Pharmacognosy
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diffusion then incubated at 35 °C for 24 h. The presence of inhibition zones were recorded and considered as indication for antibacterial activity (Mostafa etal., 2018)). The cultures used for this assay were MCC 2052 *Aeromonas hydrophila* and MCC 2076 *Acinetobacter baumannii.*

RESULTS AND DISCUSSION

The nutritional value and pharmacological properties of Tusi plant in its the whole herb in its innate variety poses its valuable outcome and uses in medicine and therapeutics. As this herb is a combination of many phytochemicals which provide synergestic used in medicine, the tulsi extract was subjected to its phytochemical and biochemical analysis. Assessment of various compounds like tannins, flavanoids, terpenoids etc was made using different qualitative tests. Results indicated the presence of tannins, reducing sugars, glycosides, flavanoids, alkaloids, terpenoids, saponins, volatile oils and steroids. The results of Phytochemical Screening of the tulsi extract are indicated in Table 1. Similarly, biochemical analysis of the plant indicated presence of carbohydrates including reducing sugars and lipids like saponins and unsaturated oils as indicated in Table 2. Tulsi plant has essential and volatile oils which are found to show antimicrobial properties by disrupting bacterial cell membranes and inhibiting bacterial growth protein synthesis. As a result, antimicrobial screening of the plant leaf extract was performed using agar well diffusion method against two bacterial isolates namely, MCC 2052 *Aeromonas hydrophila* and MCC 2076 *Acinetobacter baumannii.* A clear zone of inhibition of bacteria around the well indicated antibacterial property of tulsi leaf extract and its use in medicinal formulation. The results are as indicated in Table 3.

Table 3: Phytochemical Analysis of Tulsi leaf Extract

PHYTOCHEMICAL SCREENING													
Plant Extract	Tannins	Reducing sugars	Quinine	Glycosides	Flavanoids	Shinoda Test	Terpenoids	Alkaloids	Dragendroff's Test	Saponin	Volatile oil	Cardiac Glycoside	Steroids
Tulsi	+	++	-	++	++	++	++	++	++	+	++	++	+

Experimental results - indicated absent, + indicated present, ++ indicated highly present

Table 4: Biochemical analysis of Tulsi leaf extract:

BIOCHEMICAL SCREENING							
Plant Extract	Carbohydrates	Reducing sugars	Glycosides	Saponin	Unsaturated oils	Proteins	Amino acids
Tulsi	+	++	++	+	++	-	-

Experimental results - indicated absent, + indicated present, ++ indicated highly present

Journal of Advancement in Pharmacognosy
Volume 5 Issue 1, 2025
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Table 5: Antimicrobial analysis of Tulsi leaf extract:

ANTIMICROBIAL SCREENING		
Plant Extract	MCC 2052 *Aeromonas hydrophila*	MCC 2076 *Acinetobacter baumannii.*
Tulsi	++	++

CONCLUSION

The tulsi plant was assessed for the presence of different phytochemicals including oils and alkaloids. Results indicated presence of almost all the phytochemicals including tannins, reducing sugars, glycosides, flavanoids, alkaloids, terpenoids, saponins, volatile oils and steroids. Biochemical analysis was performed showing the presence of carbohydrates including reducing sugars and lipids like saponins and unsaturated oils. The various oils and bioinhibitory compounds of tulsi contribute to its ability to combat bacterial growth as shown in the results obtained from the study. While Tulsi shows promise as an antimicrobial agent, further investigational studies are needed to be done to fully understand its therapeutic applications.

ACKNOWLEDGEMENT:

The authors are thankful to Dr. D. Y. Patil Biotechnology and Bioinformatics Institute and Dr. D. Y. Patil Vidyapeeth, Pune for the support to carry out this work.

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Journal of Advancement in Pharmacognosy
Volume 5 Issue 1, 2025
ISSN: 2582-8401

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