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Phytochemical Analysis and Antimicrobial Evaluation of *Litsea salicifolia* (Roxb. ex Nees) Hook. f. of Lauraceae from Assam, India

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Abstract: ***The present work deals with the study of phytochemical and antimicrobial evaluation of one of the important phytopesticidal and ethnomedicinal plant, Litsea salicifolia (Roxb. ex Nees) Hook. f. of Lauraceae from Assam, India. The plant has wide traditional and cultural uses in Assam as medicine. Qualitative phytochemical screening of the leaves was found to exhibit positive tests for different phytoconstituents. The total phenol and flavonoid contents in the leaf are 47.06 mg/g, gallic acid equivalent, and 21.45 mg/g, quercetin equivalent, respectively. Crude Leaf extract of the plant exhibits antimicrobial activity against Bacillus subtilis, Staphylococcus aureus, S. epidermidis, and Enterococcus faecalis with zones of inhibition ranging from 8.3±0.47 to 12±1.41 mm. The result indicates the plants' medicinal value and validates its traditional claim.***

Keywords: *Litsea*, Phytochemical, Antimicrobial, Assam

1. Introduction

Application of plants as Phyto-remedies in and around the habitat place is the oldest practice of human society. Phyto-remedies or plant-based remedies are considered the most effective drugs without any side effects. Historically, all medicinal preparation was derived from plants, whether in the simple form of raw plant materials or the refined form of crude extracts, mixtures, etc. (Krishnaraju et. al., 2005). Plants largely used to cure diseases are recognized as drug-yielding or medicinally important. Various recent studies reinforce the enduring role of phyto-remedies, particularly in India (Vendrapati et al., 2024); Pandey et al. 2025)

The medicinal value of drug plants is mainly due to the presence of some chemical substance or substances in the tissues. These chemical substances, if administered into the human body, produce a definite physiological action, i.e., either beneficial effects in the treatment of diseases or harmful effects. Phytochemical investigations are the most important chemical as well as biological investigations in modern-day plant research. It helps us to know about the presence of various chemical constituents in a specific plant or plant parts. Knowledge of the chemical constituents is desirable for the discovery of therapeutic agents as well as new sources of economic materials.

The Assam plain has a tremendous wealth of medicinal plants scattered all over a vast area. Assam is rich in flora; there are an estimated 3895 species of flowering plants found in Assam, (Barthakur, 1996). In the rural areas of Assam, there has been the practice of using some plant formulations though in crude forms for different diseases. *L. salicifolia* is a widely used but less studied ethnomedicinal plant of Assam. It is an evergreen small tree, about 8-9 m tall. Leaves alternate, elliptic-oblong, size varies from 10-30 x 3-7.5 cm. Flowers 5-8 mmx4-8 mm, pedicel 1 -2 mm long. It is traditionally used by various peoples in the North Eastern regions. This plant is an integral part of the Rongali bihu festival of Assam. During the Rongali Bihu season, the plant is used as a mosquito & fly repellent herb. Twigs of the plant, along with the flowering twigs of *Flemingia strobilifera* are used especially in cow worship or Cow's Bihu. During this festival, cows in the village units are taken to a nearby river, pond, or similar water bodies for a thorough bath and gently beaten with these plants, and wishing for their healthy growth.

2. Literature Review

The plant is used by different ethnic communities of the study site as folkloric medicine for its different pharmacological activities. The bark of the plant is used in asthma, and leaf decoction is given in Dysentery (Buragohain, 2011). The Atapani tribes of Arunachal Pradesh use the fruit of the plant in the treatment of bone fractures and stomach disorders (Kala, 2005). The plant is locally known as 'Dighloti' in Assam and is one of the many plants used as phytopesticide traditionally by various tribes of Assam (Phukan and Kalita, 2005, Barukial and Sarmah, 2011). Traditionally, extracts of this plant's bark, fruits, and leaves have been used to treat various diseases including chronic inflammation (Puppala et al., 2023).

Recent studies also confirm its mosquito-repellent and larvicidal properties against *Aedes aegypti* further validate its folkloric use during the Rongali Bihu festival (Konwar et al., 2025). *L. salicifolia* are cultivated to rear silkworms since the leaves are a good feed for the latter (Anonymous, 1956). As the plant has wide traditional and cultural use in the study site, an attempt was made to qualitative and quantitative phytochemical analysis along with an antimicrobial study of the leaves of *L. salicifolia* in order to validate the traditional knowledge of the ethnic tribes of Assam regarding the application of this plant.

3. Materials and methods

3.1. About the study site

The Upper Brahmaputra Valley (South) zone of Assam comprises the districts of Tinsukia, Dibrugarh, Sibsagar, Jorhat, Golaghat, Charaideo, and Majuli covering an area of 16,013 sq.km, accounting for 20.40 percent of total area of Assam. It is extended between 26.45° and 27.15° N latitudes and 94.25° and 95.25° E longitudes. It has an elevation of 86.6 m. The average annual rainfall is 2183 mm, and temperatures vary between 15°-35°C. Soil is alluvial and suitable for cultivation. Semi-evergreen deciduous forest and grassland are the dominating vegetation types of the study site. The Climate is characterised by very wet summer months followed by sunny winters (Das and Saikia, 2018)

3.2. Collection of plant materials:

The leaves of *L. salicifolia* were collected locally from different localities of two districts of the Upper Brahmaputra Valley of Assam, India viz. Sivasagar and Jorhat districts. The plant material was identified and authenticated in the department of Botany, Jagannath Barooah University Jorhat, Assam, India. The plant samples were washed and unwanted materials were discarded, and the collected plant materials were shade dried for 30 days, well dried small pieces of leaves were grinded into powder by using a mortar & pestle. The powder was stored in an airtight container and kept in a cool dark place for analysis.

3.3. Preparation of plant extract:

20 gm of leaf powder was macerated overnight with 150 ml of three different solvents based on their polarity as polar (methanol), dipolar (acetone), and non-polar (ethyl acetate), separately. Then, the macerated material was extracted in the Soxhlet apparatus at 50º C for 5 hours. Then, the extract was collected and concentrated by evaporating, and the extracts were kept in the refrigerator at 4ºC until use.

3.4. Preliminary phytochemical screening:

Preliminary phytochemical screening was done by using standard procedures (Harborne, 1998; Edoga et al., 2005; Kokate, 2005; Jaradat et al., 2015) for the detection of the presence of alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, carbohydrates, steroids, etc.

Carbohydrates:

Fehling's Test: A mixture having equal volumes of Fehling's solution A and B was added to the crude extract and then heated. The appearance of a brick-red precipitate indicated the presence of reducing sugars.

Benedict's Test: To 2 ml of Benedict's reagent, the plant extract was added, and the mixture was boiled. A reddish-brown coloration was observed, confirming carbohydrates.

Molisch's Test: The extract was mixed with 2 ml of Molisch's reagent, after which concentrated sulfuric acid was gently poured along the test tube wall. The appearance of a violet ring at the interface confirmed carbohydrates.

Alkaloids: The extracts were dissolved in dilute hydrochloric acid, filtered, and tested as follows:

- **Mayer's Test:** Addition of Mayer's reagent to the filtrate resulted in a yellow precipitate, suggestive of alkaloids.
- **Wagner's Test:** Treatment with Wagner's reagent is added to the crude extract; if it produces a brownish-red precipitate, it confirms as alkaloids.

Flavonoids: Approximately 0.2 g of the extract was dissolved in a 10% sodium hydroxide solution. The development of a yellow colour indicated the presence of flavonoids.

Phenols: Equal volumes (2 ml each) of the extract solution and alcohol were mixed, followed by a few drops of ferric chloride. The development of a green or bluish colour indicated phenolic compounds.

Tannins: 0.5 ml of extract was taken, and subsequently added 1 ml of distilled water and then a few drops of ferric chloride. Appearance of green or bluish colour signified the presence of phenolics.

In the second test, 2 ml of each, i.e., extract and diluted distilled water, are taken, and then lead acetate. The appearance of white turbidity or a precipitate was taken as evidence for tannins.

Terpenoids and Steroids (Salkowski Test): 2 ml chloroform was added to 4-5 ml of extract, and then 3 ml concentrated H_2SO_4 was added. A reddish-brown colour at the interface indicated terpenoids, while a red coloration in the lower layer suggested steroids.

Steroids (Liebermann–Burchard Test): The crude extract was mixed with chloroform and concentrated sulfuric acid. Red colouration in the chloroform layer validate steroids existance.

Saponins: 2 gm. of plant extract mixed with 10 ml of distilled water and agitated constantly. Persistent frothing was considered a positive result for saponins.

Cardiac Glycosides (Keller–Killiani Test): Glacial acetic acid containing ferric chloride was added to the extract, and then added concentrated H_2SO_4. The appearance of a green colour confirmed cardiac glycosides.

3.5. Quantitative phytochemical analysis:

Determination of Total Phenolic Content: Estimation of total phenol content in the plant extract was measured spectrophotometrically by Folin–Ciocalteu colorimetric method (Nabavi, 2003; Kamtekar, 2014; Sahu and Saxena, 2013) using Gallic acid as the standard and Total phenol value is expressed in terms of gallic acid equivalent (GAE) as mg/g of of sample. A calibration curve of gallic acid was prepared (Figure - 1). 1ml of standard solution of 0.01, 0.02, 0.04, 0.06, .08, and 0.1 mg/ml concentration of gallic acid were prepared in methanol. Concentration of 0.1 and 1mg/ml of plant extract

was also prepared in methanol and 0.5ml of each sample was introduced into test tubes, Folin-Ciocalteu reagent 5ml (1:1 diluted with distilled water) and mixed thoroughly. After five minutes 5ml of 10% Na2CO3 solution was added. This solution was heated up to a minute and allowed to cool. The absorbance of the reaction mixtures was recorded at 760 nm with UV Vis. spectrophotometer.

Determination of the Total Flavonoid: Aluminium chloride method was used for flavonoid determination (Das and Saikia, 2018; Harborne, 1998). Quercetin was used as standard. The quantitative value of flavonoids was measured in the form of quercetin equivalent. A calibration curve of quercetin was also prepared (Figure - 2). 1ml of standard solution of concentration 0.01, 0.02, 0.04, 0.06, .08 and 0.1 mg/ml of quercetin were prepared in methanol. Concentration of 0.1 and 1mg/ml of plant extract was also prepared in methanol. 1ml of standard solution of 0.01, 0.02, 0.04, 0.06, .08, and 0.1 mg/ml concentration was taken into 10ml volumetric flask, containing 4ml of distilled water. 0.3ml of 5%NaNO2 was added to the flask. 0.3ml 10%$AlCl_3$ was added to the mixture after some time. And 2ml of 1M NaOH was added, and its volume was made up to 10ml with distilled water. The absorbance was noted at 510nm using a UV-Vis spectrophotometer.

3.6. Determination of antibacterial activity:

Bacterial strains, namely *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis* were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. The strains were maintained on nutrient agar slant and kept in the refrigerator. Antibacterial activities were determined by the Agar well diffusion method. Phytochemical analysis of the species indicates the presence of the maximum number of phytochemicals in the methanolic extract in comparison to other extracts, these results clearly indicate that organic solvents were more suitable for the extraction of the active principles responsible for antimicrobial efficacy. Therefore, the methanolic extract was considered for the antimicrobial assay. After that, 15 mg/ml concentration of each plant extract was prepared in DMSO. 20 ml of pre-sterilized nutrient agar media was poured on sterile glass petri plates having size of 90 mm. The plates were allowed to solidify. Inoculating a loopful of bacteria, bacterial suspension was made. The pure form of bacterial suspension was spread on the media using a sterile glass spreader and allowed to dry.

A sterile cork borer (9 mm) was used to make cups in an agar plate, and it was labelled properly. With the help of a micropipette, 0.1 ml of the prepared extract was placed in each cup. DMSO was used as a negative control and standard streptomycin was used as a positive control. All the plates were incubated at 37ºC for 24 hours. The zone of inhibition was measured and compared with the standard. All the observations were repeated twice and the average of two independent observations was recorded.

4. Result and Discussion

The qualitative phytochemical screening of the leaf of *L. salicifolia* was found to exhibit the positive tests for Carbohydrate Alkaloid, Flavonoids, Phenol, Tannin, Saponin, and negative test for Terpenoid, Steroid, and Glycosides in methanolic extract. Acetone extract exhibited positive test for Carbohydrate Alkaloid, Flavonoids, Phenol and negative test for Tannin, Terpenoid, Steroid, Saponin and Glycosides. Ethyl acetate extract exhibited positive test for Carbohydrate and Tannin only. These results clearly indicate that organic solvents were more suitable for the extraction of the active principles responsible for antimicrobial efficacy. The results indicate that methanol was the most efficient solvent for extracting phytoconstituents from *L. salicifolia* leaves. This may be attributed to the high polarity of methanol, which facilitates the dissolution of both polar and moderately nonpolar compounds (Do et al., 2014). Several previous studies have also shown that methanol extracts generally contain higher amounts of biologically active phytochemicals compared to other organic solvents (Parekh & Chanda, 2007; Cowan, 1999).

Table 1: Qualitative Phytochemical screening of leaf and root of *Litsea salicifolia* (Roxb. ex Nees) Hook.

Solvents / Constituents	Ethyl acetate	Acetone	Methanol
Carbohydrate	+	+	+
Alkaloid	-	+	+
Flavonoids	-	+	+
Phenol	-	+	+
Tannin	+	-	+
Terpenoid	-	-	-
Steroid	-	-	-
Saponin	-	-	+
Glycosides	-	-	_-

'+' Positive; '-' Negative

Total amount of phenol and flavonoid contents: Total amount of phenol and flavonoid contents were calculated from gallic acid ($y = 0.0125x - 0.0877$, $R^2 = 0.9774$) and quercetin ($y = 0.0189x - 0.133$, $R^2 = 0.9943$) standard curves (Figure I & II). The total phenol and flavonoid contents in Leaf are 47.06 mg/g, gallic acid equivalent and 21.45 mg/g, quercetin equivalent respectively.

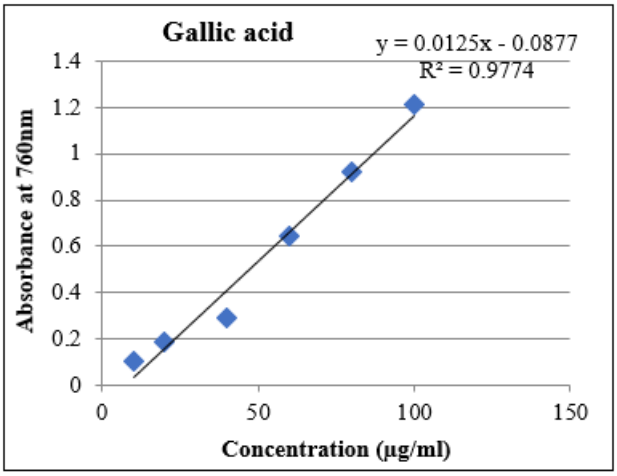

Figure 1: Calibration curve of Gallic acid.

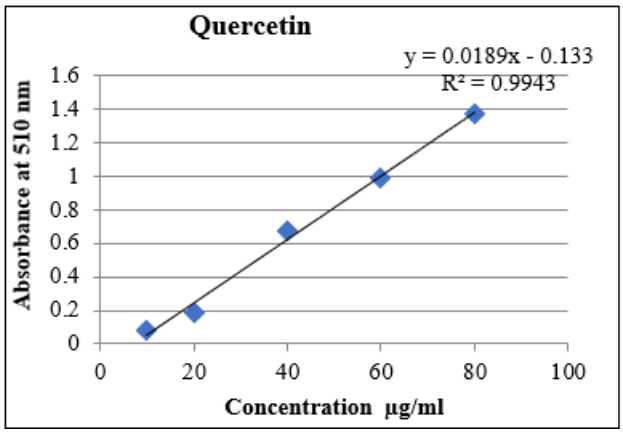

Figure 2: Calibration curve of quercetin.

Table 2: Antibacterial activity of the methanolic and acetone extracts of leaf of *Litsea salicifolia* (Roxb. ex Nees) Hook. f and *C. aromatica* and Streptomycin as positive control.

Sample / Bacteria	Zone of Inhibition (mm)			
	Methanol	Acetone	DMSO (-control)	Streptomycin (+Control)
B. subtilis	12± 1.41	09±0.81	-	21±0.81
S. aureus	11.3±0.47	-	-	23±0.81
S. epidermidis	9.3±1.24	05±0.81	-	20±1.63
E. faecalis	8.3±0.47	-	-	16±.081

Methanolic extracts of both the plant species exhibit antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis* with zones of inhibition ranging from 8.3±0.47 to 12±1.41 mm (Table -5). During MIC test *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis* was observed as 0.9 mg/ml, Enterococcus faecalis has 1mg/ml, and *E. coli* was found as 1.2 mg/ml. Alkaloids and flavonoids have been reported to interfere with bacterial cell wall synthesis and nucleic acid metabolism, leading to growth inhibition (Cushnie & Lamb, 2005). The antimicrobial assay results, therefore, correlate well with the phytochemical profile of the methanolic extract.

5. Conclusion

L. salicifolia of Lauraceae is a widely used cultural, ethnomedicinal, and an important phytopesticidal plant, however comparatively less studied plant of Assam. The plant is employed by different ethnic communities of the study site to cure various ailments as well as infectious diseases. Preliminary phytochemical screening exhibits positive check for various phytoconstituents like tannic acid, phenols, flavonoids, alkaloids, terpanoids, saponin. Presence of important phytocostituents clearly indicates the healthful properties of the plant species, which validates the standard information of various ethnic communities of the study space that use this plant as a phytomedicine against different common and regularly occurring ailments.

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