

# ***In vitro* antimicrobial activity and phytochemical analysis of different solvent extracted samples from medicinally important *Litsea glutinosa***

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**Abstract:** The present research work investigate the antimicrobial activities of crude methanolic extracted samples from the leaves of *Litsea glutinosa* against different microorganisms through disc diffusion assay applied in two different concentrations of 1 and 2mg disc<sup>1</sup>. The tested microbial species included *B. subtilis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *C. albicans*. The crude methanolic extract was applied in two different concentrations of 1 and 2mg disc<sup>1</sup>. Analysis of the data revealed that crude methanolic extracted samples showed different ranges of antimicrobial activities against all the tested microbes at both concentrations. Maximum growth inhibition was measured against gram negative *Pseudomonas aeruginosa* followed by the fungal specie *Candida albicans*. In case of Petroleum extracted fractions maximum growth reduction was measured in *Candida albicans* at higher concentration. Similarly, growth inhibition was more in *Pseudomonas aeruginosa* at higher concentration of aqueous extracted samples. Different solvent extracted samples showed the presence of alkaloids, flavonoids, proteins, fats, oils, tannins, carbohydrates, sterols and saponins.

**Keywords:** Antimicrobial, solvent, phytochemical analysis, *Litsea glutinosa*.

## **INTRODUCTION**

Herbal medicines are in use by human beings since ancient times. These herbal medicines as therapeutic agents possessed many advantages i.e. easy availability, local historical and cultural aspects, personal preferences, local demand for natural and organic products, being economical and with out side effects. However, there is need to test these herbal medicines not only by ethno-pharmacological evidences but also by scientific research to confirm their therapeutic use (Carmona and Soares, 2013). It is reported that about 70-95% of the world's population uses herbal medicines mostly plant extracts or their active components for their primary health care (Sardesai, 2002; Robinson and Zhang, 2011). World Health Organization concluded that nearly 28% of medicines currently available in the market are plant based products or its derivatives (Newman *et al.*, 2003). Historically herbal products as such or their derivatives have remained as a valuable source of novel therapeutic agents. Ethno botanical and ubiquitous plants will serve as a rich repository of natural drugs for research and development (Kong *et al.*, 2003). The increasing resistance of various microbes to available antibiotics resulted in the search for newer, more effective, affordable and easily available drugs (Adekunle and adekunle, 2009). Therefore, medicinal plants are one of the richest sources of therapeutic agents, nutra-ceuticles, food supplements, folk medicines, pharmaceutical intermediates and bio-molecules for synthetic drugs (Hammer *et al.*, 1999). The beneficial effects of herbal medicines are due to the secondary bio-active compounds

present in the plant (Briskin, 2000). Presently many pharmaceutically important bio-active agents are isolated from wild or cultivated plants (Caldentey and Inze, 2004). There are 2600 plant species of which more than 700 are noted for their uses as medicinal herbs including antimicrobial potential (Ali- Shtayeh and Abu, 1999; Bakht *et al.*, 2017; Mediha *et al.*, 2018; Bilal *et al.*, 2018).

*Litsea glutinosa* belongs to the family Lauraceae, is an aromatic evergreen medium-sized tree found in the Western Ghats of India. Extracts from different parts of the plant are used for the treatment of diarrhea, dysentery, wound healing, respiratory disorders, sexual disorder and rheumatism (Ghani, 2003; Rout and Thatoi, 2009). Essential oils are known to act as antimicrobial, antispasmodic, carminative and antiviral agents and have analgesic, antipyretic, and anti-inflammatory potential. Phytochemical analysis of the stem bark showed the presence of tannin,  $\beta$ -sitosterol, and actinodaphnine, boldine, norboldine, laurotetanine, n-methylaurotetanine, n-methylactinodaphnine, quercetin, sebiferine, litseferine etc (Chatterjee and Pakrashi, 1994). The use of *Litsea* species in traditional herbal medicines is due to the presence of monoterpenoids and different structural types of phenolic compounds in their essential oils. The present study investigates the antimicrobial activity of crude methanolic extracted samples from the leaves of *L. glutinosa*.

## **MATERIALS AND METHODS**

### ***Plant materials***

Aerial parts of *Litsea glutinosa* were collected from the Department of Botany, University of Dhaka, Bangladesh.

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The collected plants were thoroughly washed with tap water to remove the dirt and soil particles.

#### Crude extract preparation

Shade dried aerial parts of *Litsea glutinosa* were chopped and grinded to obtain dried fine powder. About 450 grams of dried powder were soaked in methanol and the solution was then kept at room temperature for about 6 days. The solution was filtered using Whatman filter paper. Fresh methanol was mixed with the residues and the process was repeated three times. The filtered methanolic solution was subjected to the rotary evaporator for drying below 45°C. About 65 grams of dried crude (methanol) extract was prepared in this manner.

#### Fractionation of extract

The dried crude extract was dissolved in 300 ml distilled water and mixed with equal volumes of petroleum ether in separatory funnel, gradually shaken and allowed to stand for 10 minutes until two discrete layers were formed. The upper layer petroleum ether was collected and the lower aqueous phase was again partitioned with fresh petroleum ether. The whole process was repeated three times. All the petroleum ether fractions were pooled, filtered through Whatman No. 1 filter paper and dried to a semi- solid material under vacuum pressure by rotary evaporator. After the completion of the whole process, the lower aqueous phase was collected and dried as described earlier.

#### Disc diffusion susceptibility assay

The antibacterial activity of different solvent extracted samples from the leaves of *Litsea glutinosa* was carried by disc diffusion assay as described in Bauer *et al.* (1966) and antifungal activity by Ramdas *et al.* (1998) against different bacterial and fungal strains (tables 1).

#### Positive controls

For Gram-positive bacteria; Ciprofloxacin 50µg per 12µl  
For Gram negative-bacteria; Ciprofloxacin 50µg per 12µl  
For Fungal strain; Fluconazole 50µg per 12µl.

#### Phytochemical analysis

Phytochemical analysis was carried out for the existence of proteins, alkaloids, carbohydrates, flavanoids, terpenoid (Siddiqui and Ali, 1997), phytosterols, tannins (Iyengar, 1995), saponins and glycoside (Harborne, 1984).

### STATISTICAL ANALYSIS

For statistical analysis, MSTATC computer software was used (Russel and Eisensmith, 1983). Least Significant Difference (LSD) test was employed upon obtaining significant difference among means (Steel *et al.*, 1997).

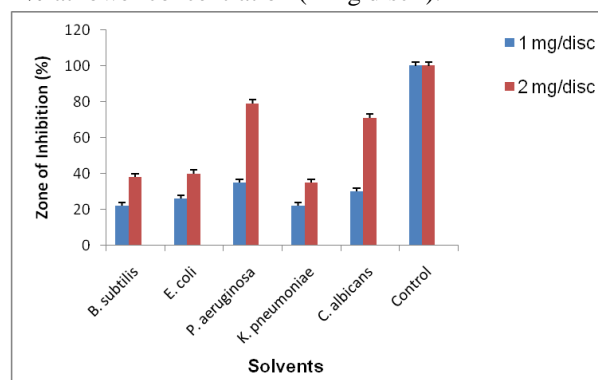
### RESULTS

The antibacterial activity of crude methanolic extract from the leaves of *Litsea glutinosa* against different

bacterial and fungal species by disc diffusion susceptibility method is shown in fig. 1. Analysis of the data revealed that all the tested bacterial species (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*) and a fungal specie (*Candida albicans*) were susceptible to crude methanolic extract obtained from the leaves of *Litsea glutinosa* and showed different ranges of antimicrobial activities at 1 and 2 mg disc<sup>-1</sup> concentrations. Our results revealed that increasing concentration of the extract also increased growth reduction. The data showed that crude methanolic extract was most effective to reduce the growth of *Pseudomonas aeruginosa* and measured 35 and 79% inhibitory effect at 1 and 2mg disc<sup>-1</sup> concentrations respectively when compared with other microbes and controls. Similarly, *Candida albicans* also showed susceptibility to the same extract measuring 30 and 71% ZI at 1 and 2mg disc<sup>-1</sup> concentrations respectively. The data further suggested that crude extract methanolic extract was least effective against *Klebsiella pneumoniae* at both concentrations compared with other microorganisms and control. The growth of the same bacterium was reduced by 21% at minimum concentration (1mg disc<sup>-1</sup>) and 30% at higher concentration (2 mg disc<sup>-1</sup>).

Similar growth reduction was also noted for *E. coli* at both concentrations (i.e. 26% and 40% ZI at 1 and 2 mg disc<sup>-1</sup> concentrations respectively). From these results it can be concluded that crude methanolic extracts from the leaves of *Litsea glutinosa* effectively reduced the growth of *Klebsiella pneumoniae* and *Candida albicans* at both concentrations when compared with other microbes under study and controls.

It is also clear from the data that crude methanolic extract was almost equally effective against *Bacillus subtilis* and *E. coli* at both concentrations. The data revealed that crude methanolic extract inhibited the growth of *Bacillus subtilis* by 38 at higher concentration (2 mg disc<sup>-1</sup>) and 22% at lower concentration (1 mg disc<sup>-1</sup>).



**Fig. 1:** Antimicrobial activity of crude methanolic extracts from the leaves of *Litsea glutinosa* against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans* by disc diffusion assay (Bar represent LSD value at p<0.5).

**Table 1:** Microbial strains tested during the present experiment

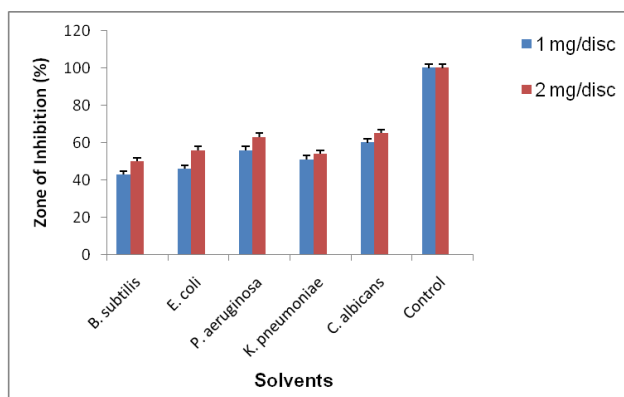
| Microbial Species             | Gram strain type | Details of the Microbial strains used   |
|-------------------------------|------------------|---|
| <i>Klebsiella pneumoniae</i>  | Negative         | Clinical isolate obtained from Microbiology Laboratory, Quaid-I-Azam University Islamabad, Pakistan |
| <i>Pseudomonas aeruginosa</i> | Negative         | ATCC # 9721   |
| <i>Bacillus subtilis</i>      | Positive         | Clinical isolate obtained from Microbiology Laboratory, Quaid-I-Azam University Islamabad, Pakistan |
| <i>Escherichia coli</i>       | Negative         | ATCC # 25922  |
| <i>Candida albicans</i>       |                  | ATCC # 10231. Plant Pathology Department KPK The University of Agriculture Peshawar, Pakistan       |

**Table 2:** Phytochemical profile of solvent extracted samples from shoots of *Litsea glutinosa*.

| Extract          | Alkaloids | Proteins | Tannins | Carbohydrates | Sterols | Flavonoids | Saponins | Glucosides |
|------------------|-----------|----------|---------|---------------|---------|------------|----------|------------|
| Crude Methanolic | +         | +        | +       | +             | —       | +          | +        | —          |
| Petroleum Ether  | +         | +        | +       | +             | —       | +          | +        | —          |
| Aqueous          | +         | +        | +       | +             | —       | +          | +        | —          |

+: Shows presence; - Shows absence. —: Shows complete absence of the compound

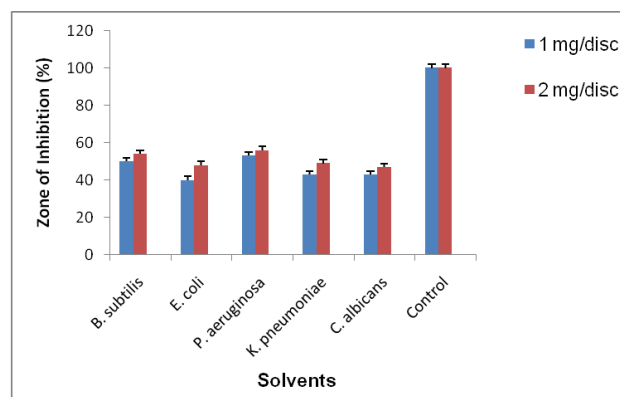
The data also revealed that petroleum extracted fractions also inhibited the growth of different microbes under studies (fig. 2). *Candida albicans* was the most sensitive microbe measuring 63% ZI followed by *Pseudomonas aeruginosa* (63% ZI) at higher concentration of 2 mg disc<sup>-1</sup>. The growth of *Bacillus subtilis* was reduced by 43% at lower concentration when compared with other samples. The results also suggested that the growth of different bacteria under study was variably reduced by aqueous extracted samples. Maximum growth reduction (56%) was observed in *Pseudomonas aeruginosa* at higher concentration while *E. coli* measured minimum growth retardation at lower concentrations (fig. 3).



**Fig. 2:** Antimicrobial activity of Petroleum ether from the leaves of *Litsea glutinosa* against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans* by disc diffusion assay (Bar represent LSD value at p<0.5).

Different solvent extracted samples were also subjected to phytochemical screening for the presence of alkaloids, flavonoids, proteins, fats, oils, tannins, carbohydrates, sterols and saponins according to the standard procedures of analysis (table 2). Crude methanolic extracts were

found rich in tannins, carbohydrates and sterols. Phytochemical analysis of the crude extract also showed moderate quantity of alkaloids, fats, oils, flavonoids and significant traces of proteins, and saponins. The analysis further indicated that n-hexane showed negative results for alkaloids, oils, fats, saponins and tannins. Similarly ethyl acetate was found negative for alkaloids and saponins. The butanol extracted fraction showed tremendous results by exhibiting very good content of all the phytochemicals under study. The results also indicated the good content of alkaloids, tannins, carbohydrates, fats, oils, sterols and moderate content of proteins and flavonoids in the water extracted fractions.



**Fig. 3:** Antimicrobial activity of Petroleum ether from the leaves of *Litsea glutinosa* against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans* by disc diffusion assay (Bar represent LSD value at p<0.5).

## DISCUSSION

The present research investigates the antimicrobial activity of crude methanolic extracts obtained from the leaves of *Litsea glutinosa* through disc diffusion assay

using two different concentrations. Activity of the crude extract against the studied microorganisms increased with increasing concentrations. Our results revealed that crude methanolic extract was effective against all the tested microbes and measured different degree of growth inhibition at both concentrations. Crude methanolic extract significantly reduced the growth of *Pseudomonas aeruginosa* and *C. albicans* at the tested concentrations. The probable reason for good activity of the crude extract against the same microbes may be due to the fact that different bio-active compounds which are effective against these microbes are soluble in methanol.

Moderate activity was recorded against gram negative bacteria *Escherichia coli* at both concentrations when compared with other microbes and controls. However, the tested crude methanolic extract was not very effective against gram positive *Bacillus subtilis* and gram negative bacteria *Klebsiella pneumonia* that measured minimum growth reduction at both concentrations. This may be due to the less solubility of the bio-compounds present in *Litsea glutinosa* in methanol and hence showed less activity against *Bacillus subtilis* and *Klebsiella pneumonia* at both concentrations. Petroleum ether extracted samples reduced the growth of different microbes. *Candida albicans* was the most sensitive microbe at higher concentration. The growth of *Bacillus subtilis* was reduced at lower concentration. Growth inhibition was more in *Pseudomonas aeruginosa* at higher concentration of aqueous extracted samples and *E. coli* measured minimum growth retardation. Phytochemical screening revealed the presence of alkaloids, flavonoids, proteins, fats, oils, tannins, carbohydrates, sterols and saponins in different solvent extracted samples. Li *et al.* (2014) reported that antimicrobial activity of *Litsea cubeba* oil is mainly attributed to the presence of aldehydes, which accounted for approximately 70% in its whole components analyzed by GC/MS. Similar results are also reported by Kumar *et al.* (2011). Our results support the ethno-medicinal uses of *Litsea glutinosa* and require advanced investigation to elucidate responsible compounds as well as their mode of action.

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