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Original Article

Litsea glutinosa (Lauraceae): Evaluation of its Foliar Phytochemical Constituents for Antimicrobial Activity

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Abstract

The phytochemical investigation of the leaves of *Litsea glutinosa* revealed the presence of secondary metabolites like alkaloids, anthraquinones, cardiac glycosides, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids, volatile compounds, amino acids and carbohiydrates. The antimicrobial activity and minimum inhibition concentration values were determined for these phytochemical constituents as crude extracts using the agar well diffusion and two-fold serial dilution methods. The results indicated that *Bacillus subtilis* was the most susceptible bacterium with high inhibition zones for the methanol and chloroform extracts of 31 mm and 26 mm, respectively. The MIC values indicated that extracts possess good antimicrobial activity with significant MIC value against *Enterococcus faecalis, Pseudomonas aeruginosa* and *Staphylococcus pneumoniae* at 31.2 µg/ml concentrations. The extracts showed marked antimicrobial activity against both bacteria and fungi. Among the bacterial strains, gram-positive bacteria were more susceptible than the gram-negative. All the 13 microorganisms tested showed dose dependent susceptibility towards the phytochemicals present in the foliar extracts. The study suggests that *Litsea glutinosa* leaves possess potent antimicrobial activity and can be a good source for the development of new antibiotics.

Keywords: extract; *Litsea glutinosa*; microorganism; pathogen; phytochemical

Introduction

In India, from ancient times, different parts of medicinal plants have been used to treat the infectious diseases which are the cause of premature deaths to an extent of 50,000 people every day globally (Anonymous, 2000). India is rich in medicinal plant diversity because of different agroclimatic, ecological and edaphic conditions. Medicinal plants are the richest source of natural products for traditional system of medicine, pharmaceutical intermediates and chemical entities for synthetic drugs. Thus, there is a constant need to develop new antimicrobial drugs for the treatment of infectious diseases from the medicinal plants (Ncube *et al*., 2008).

Litsea glutinosa (Lour.) C.B. Robinson (Lauraceae) is an evergreen or deciduous, that reaches a height of 3-15 meters. This species is native to India, South China to Malaysia, Australia and the western Pacific Islands. It is a medicinal plant known as Indian laural, soft/brown bollygum or beech/bolly beech, bollywood and sycamore. In Telugu, it is called 'narra alagi' or 'narra mamidi'. It is a threatened species due to over exploitation for its bark and considered as endangered species in Philippines (Rabena, 2010). Traditionally, it is considered as promoter of longevity, semen generation and emollient. The sap of fresh bark or its decoction is prescribed as a remedy for diarrhoea, dysentery and rheumatism. The mucilaginous leaves are considered antispasmodic and emollient. In addition, a paste prepared by grinding bark with water is used as a plaster in cases of sprain, bruises, wounds, inflammation, back pain, rheumatic and gouty joints, bone fractures, etc. It has analgesic, antiseptic and emollient effects (Devi and Meera, 2010). Although the most of the antimicrobial activities had been carried out on the bark extract (Mandal *et al*., 2000; Lohitha *et al*., 2010; Poornima, 2011; Haque *et al*., 2014) there are a few studies on the antimicrobial activity on methanol extracts of leaves(Meera and Devi, 2009; Gulzar *et al*., 2015). However, there are no reports on the antimicrobial activity of *L. glutinosa* leaves, and their effects on pathogenic fungi and bacteria. Thus, the present study evaluated the antimicrobial activity of hexane, chloroform, methanol and aqueous extracts of *L. glutinosa* leaves.

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Materials and Methods

Plant material

The leaves of *Litsea glutiona* were collected from Andhra University campus of a planted tree from Visakhapatnam, Andhra Pradesh, India. Its taxonomic identity was confirmed by Prof. M. Venkaiah, Department of Botany, Andhra University, Visakhapatnam, India. The leaves collected were shade-dried. Then, they were powdered in the mixture grinder and stored in airtight bottles.

Extraction of plant material

The shade dried leaf powder (10 g of each) was extracted with hexane, chloroform, followed by methanol by using sequential extraction method (Aniel Kumar *et al*., 2010). Thereafter, it was filtered by rotary evaporator at 40 °C to obtain the crude dried extract. Simultaneously, the aqueous extract of the leaves was prepared by adding boiled water to the powdered in a beaker on water bath, with occasional stirring for 4 hours. The aqueous extract was then filtered and centrifuged at 5,000 rpm for 15 min. The supernatant was collected and evaporated to dryness to give the crude dried extract. The extracts were dissolved in DMSO to get the known concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml.

Microbial strains

The test bacterial and fungal strains used in the study were obtained from Microbial type culture and collection (MTCC), Chandigarh, India. They are *Bacillus subtilis* MTCC B2274, *Enterococcus faecalis* MTCC B3159, *Escherichia coli* MTCC B1560, *Klebsiella pneumoniae* MTCC B4030, *Micrococcus luteus* MTCC B1538, *Pseudomonas aeruginosa* MTCC B2297, *Proteus vulgaris* MTCC B7299, *Staphylococcus aureus* MTCC B3160, *Streptococcus pneumoniae* MTCC B2672, *Aspergillus niger* MTCC F4325, *Candida albicans* MTCC F7315 and *Saccharomyces cerevisiae* MTCC F2567. The bacterial strains were grown in the nutrient broth and maintained on nutrient agar slants at 4 °C whereas the fungal strains were grown in Sabouraud broth and maintained on Sabouraud agar slants *C. albicans* and *S. cerevisiae*) and potato dextrose agar slants (*A. niger*) at 4 °C.

Antimicrobial screening

The antimicrobial activity of hexane, chloroform, methanol and aqueous extracts of leaves of *L. glutinosa* was determined by agar well diffusion (Aniel Kumar *et al*., 2014) and agar disc diffusion methods for standard antibiotics tetracycline and fluconazole separately for bacteria and fungi. The lyophilized culture was sub cultured and concentration of working stock culture was assessed as 10^{-6} CFU/ml. For susceptibility test, 100 µl of inoculum was mixed with 6 ml of sterilized nutrient agar and poured immediately into the sterile petridishes. The petridishes were left to solidify for 10 minutes. A sterilized 6 mm metal borer was used to make wells in the centre of the divided areas. About 50 µl of each extract was then pipette into the wells. The petridishes were incubated at 28 °C for 24 hours. The experiment was done three times to minimize the error. After incubation period the antimicrobial activity was evaluated by measuring the inhibition zones by using an antibiotic zone reader scale(HiAntibiotic Zonescale-c).

Sabouraud agar was used to culture the fungi. The inoculated petridishes were incubated at 25 °C for two days for the *C. albicans*, *S. cerevisiae* and three days for *A. niger*. About 500 µg of fluconazole was dissolved in 1 ml of sterile deionized water. About 10 µl of 0.5 mg/ml of fluconazole (equivalent to 5 µg dose) pipette into the wells for comparison with fungal inhibition zones. The bacterial inhibition zones were was compared with tetracycline disc (5 µg /disc) of multidrug disc (Axiom Laboratories Ltd. India). About 50 µl of DMSO was pipette into each well for bacteria and fungi as a negative control.

The extracts that exhibited inhibition zones were subjected to minimum inhibition concentration (MIC) assay by using two-fold serial dilution (Aniel Kumar *et al*., 2015). A quantity of 0.6 g of each extract was dissolved in 300 ml sterile nutrient broth which yields initial concentration of 2,000 µg/ml. Subsequently, two-fold serial dilution was made from the stock to obtain 1,000, 500, 250, 125, 62.5, 31.2 µg/ml concentrations. One ml of standardized inoculums of each test organism was introduced into each extract nutrient broth mixture and then incubated at 37 0C. The lowest concentration inhibiting growth was regarded as the MIC of the extracts.

Statistical analysis

Each experimental data from triplicates of standard error was subjected to one way ANOVA using Minitab version 15. The significant level of $p < 0.001$ was used.

Results and Discussion

The phytochemical analysis of various extracts of the leaf revealed the presenceof secondary metabolites like alkaloids, anthraquinones, falvonoids, phenols, saponins, steroids, tannins, terpenoids, volatile compounds, cardiac glycosides, glycosides, amino acids and carbohydrates (Table 1). There are numerous secondary metabolites such as Megastigmane diglycoside, roseoside, 3, 5'-dimethoxy-9, 9'-dihydroxy-4, 7'-epoxylignan 4'-b-D-glucopyranoside, dihydro dehydrodiconifenyl alcohol 9'-O-b-D-xylopyranoside; and Pinoresinol 3-O-b-D-glucopyranoside reported from *L. glutinosa* leaves and twigs (Wang *et al*., 2011). A new 2'-Oxygenated Flavone Glycoside, named Glutin was isolated from the leaf extract of *L. glutinosa* (Wang *et al*., 2010). Tannin, β-sitosterol and actinodaphnine are reported to be the common constituents of the species. Major clusters of antimicrobial compounds including alkaloids (Feng *et al*., 2009), butanoides (Chang *et al*., 2008), flavonoids (Wang and Liu, 2010), lignans (Pan *et al*., 2010), sesquiterpenes (Agarwal *et al*., 2011), and essential oils (Chowdhary *et al*., 2008) have been discovered in *Litsea* spp. These compounds have shown significant biological activities including anti-inflammatory (Devi and Meera, 2010), antitumor (Cheng *et al*., 2010), anticancer (Hosseinzadeh *et al*., 2013), antioxidant (Jia *et al*., 2013), antidepressant (Guzman and Navarrete, 2009) and antiphyperalgesic (Silva *et a*l., 2012) properties.

Table 1. Phytochemical constituents of *Litsea glutionosa* leaves

Phytochemical constituents	Hexane extract	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids	+	+	+	+
Amino acids	+	+	+	+
Anthraquinone	-	-	-	-
Carbohydrates	+	+	+	+
Cardiac glycosides	-	-	+	-
Flavonoids	-	+	+	+
Glycosides	+	+	+	+
Phenols	-	+	+	-
Saponins	+	+	+	+
Steroids	-	+	+	-
Tanins	-	-	+	+
Terpenoids	+	+	+	+
Volatile compounds	+	+	+	+

In the present study, the extracts of *L. glutinosa* leaves exhibited the antimicrobial activity against all tested bacteria and fungi except that *A. niger* was resistant to the hexane and chloroform extracts (Table 2). *B subtilis* was the most susceptible bacteria with high inhibition zones for the methanol and chloroform extracts of 31 mm and 26 mm, respectively. Aqueous extract showed the high inhibition zone against *S. pneumoniae* and *P. aeruginosa* while hexane extract against *M. luteus* and *K. pneumoniae*. The fungal strain *S. cerevisiae* was more susceptible to all extracts than other fungal strains *A. niger* and *C. albicans*. The most susceptible gram positive bacterium is *B. subtilis* for all extracts while the gram-negative bacterium is *P. aeruginosa*. When the concentration of these extracts was increased, the inhibition zones also increased and it indicated dose dependent susceptibility.

The results of antibacterial and antifungal of the different extracts of *L. glutinosa* leaves were compared with the standard antibiotics. The extracts showed inhibition zones were similar or more than the antibiotics against in more than 50% of the investigated microbial strains. It is a promising result and suggests that the plant extracts contain certain phytochemical constituents with antimicrobial properties that can be used to develop new drugs for therapy of infectious diseases caused by microorganisms.

The MIC values indicate that the leaf extracts of *L. glutinosa* possess antibacterial activity against *B. subtilis*, *E. faecalis*, *S. pneumoniae* and *P. aeruginosa* at 31.2 µg/ml concentrations (Table 3). These bacteria also shown strong MIC values for aqueous and chloroform extracts at 62.5 µg/ml concentration. These results agree with previous studies, ethanol extracts of bark shown antibacterial activity against *S. aureus, B. cereus, P. aeruginosa* and *E. coli* (Lohitha *et al*., 2010), while the same extract active against *P. aeruginosa*, *E. coli*, *S. aureus* but less effective against fungal strains *A. fumigates* and *C. albicans* (Poornima, 2011). Ethanolic and water extracts of bark and leaves have antibacterial activity against *E. coli, Enterobacter intermedium*, *Salmonella* sp., *S. aureus* and *S. epidermis* (Haque *et al*., 2014), while leaves shown antimicrobial activity against gram-negative *S. paratyphi* (Gulzar *et al*., 2015) and methanol extract of bark shown antibacterial activity against 16 microorganisms tested (Mandal *et al*., 2000).

Table 2. The antimicrobial activity of *L. glutinosa* leaf extracts against the standard drugs

	Inhibition zones (mm)[a]													
	Hexane extract			Chloroform extract			Methanol extract			Aqueous extract			S	D
	25	50	100	25	50	100	25	50	100	25	50	100		
B. subtilis	16±0.19	18±0.50	20±0.45	21±0.52	23±0.44	26±0.40	28±0.19	29±0.19	31±0.45	18±0.12	20±0.48	21±0.50	18^{T}	-
E. faecalis	16±0.28	18±0.44	19±0.22	18±0.44	21±0.22	23±0.22	24±0.20	26±0.44	28±0.20	17±0.40	18±0.20	19±0.28	21^{T}	-
M. luteus	18±0.44	20±0.45	21±0.44	16±0.28	18±0.45	20±0.51	19±0.40	21±0.40	23±0.44	16±0.28	18±0.45	20±0.45	24^{T}	-
S. aureus	16±0.44	18±0.22	20±0.44	17±0.10	19±0.19	21±0.29	16±0.34	18±0.22	20±0.04	11±0.20	13±0.19	15±0.54	24^{T}	-
S. pneumoniae	12±0.22	15±0.52	17±0.22	21±0.25	23±0.40	25±0.94	25±0.22	27±0.52	29±0.20	22±0.21	24±0.24	25±0.29	22^{T}	-
E. coli	10±0.28	12±0.22	14±0.52	12±0.44	14±0.50	16±0.46	15±0.88	18±0.22	21±0.52	17±0.44	19±0.50	21±0.24	22^{T}	-
K. pneumoniae	17±.19	19±0.52	21±0.50	20±0.50	22±0.22	24±0.12	23±0.19	25±0.52	27±0.55	16±0.50	18±0.02	19±0.22	24^{T}	-
P. aeruginosa	15±0.44	18±0.45	20±0.44	20±0.45	22±0.52	25±0.62	25±0.44	27±0.45	29±0.04	21±0.05	23±0.52	25±0.05	25^{T}	-
P. vulgaris	10±0.50	11±0.52	14±0.22	15±0.22	17±0.72	19±0.20	19±0.20	21±0.22	23±0.28	17±0.29	19±0.22	21±0.08	22^{T}	-
A. niger	-	-	-	-	-	-	12±0.52	14±0.50	16±0.16	-	10±0.20	13±0.10	18^{F}	-
C. albicans	12±0.44	13±0.45	15±0.44	12±0.52	14±0.50	16±0.50	16±0.44	18±0.65	21±0.44	16±0.30	18±0.05	19±0.29	23^{F}	-
S. cerevisiae	16±0.19	18±0.45	20±0.22	18±0.19	20±0.22	23±0.25	22±0.11	24±0.45	26±0.21	14±0.19	16±0.02	18±0.50	20^{F}	-

a: Each value is the mean of three replicates, with standard deviation;
P <0.001 extremely significant when compared to the standard S: Standard (T-Tetracycline; F- luconazole)
D: DMSO–: No activity

Table 3. MIC values of *Litsea glutinosa* leaf extracts against the tested microorganisms

Organism	Hexane extract	$ChCl_3$ extract	Methanol extract	Aqueous extract
E. faecalis	500	125	31.2	500
M. luteus	250	250	125	250
S. aureus	500	250	250	1,000
S. pneumoniae	1,000	125	31.2	62.5
E. coli	>1,000	500	250	250
K. pneumoniae	250	125	62.5	500
P. aeruginosa	500	500	31.2	62.5
P. vulgaris	>1,000	1,000	125	125
C. albicans	1,000	250	250	1,000
S. cerevisiae	250	62.5	62.5	500

The antimicrobial activity of the extracts on bacteria was more pronounced than on fungi. It could be due to the fungal cell wall which has a complex structure and extensive cross-linking between chitin, glucans and other polymers. It also was observed that gram-positive bacteria were more susceptible than the gram-negative as has been found by Meera and Devi (2009), who studied the methanol extract of *L. glutinosa* leaves and had the similar result. This difference in the activity may be attributed to the fact that the cell wall in gram-positive bacteria have of a single layer whereas the gram-negative bear multilayered structure along with more lipids. The present study also receives support from Ali *et al*. (2004).

The antimicrobial activity may be due to the presence of some metabolites like alkaloid, saponins and terpenoids which have been implicated in various biological activities (Thomas *et al*., 2013) and presently found in all the extracts. The present study suggests that *L. glutinosa* has great potential as a source of useful bioactive compounds which cure infectious diseases caused by pathogenic bacteria and fungi.

Conclusions

The *L. glutinosa* leaf extracts showed marked antimicrobial activity against both bacteria and fungi, while in the bacterial strains, gram-positive bacteria were more susceptible than the gram-negative. The extracts showed marked antimicrobial activity against both bacteria and fungi. The antimicrobial activity is dose dependent susceptibility towards the phytochemicals present in the solvent foliar extracts. Therefore, the study shows that *L. glutinosa* leaf extracts have a broad spectrum of antimicrobial activity and could be useful in antiseptic/disinfectant formulations and chemotherapy.

References

Agrawal N, Choudhary AS, Sharma MC, Dobhal MP (2011). Chemical constituents of plants from the genus *Litsea*. Chemistry & Biodiversity 8:223-243.

Ali MA, Sayeed MA, Bhuiyan MSA, Sohel FI, Yeasmin MS (2004). Antimicrobial screening of *Cassia fistula* and *Mesua ferrea*. Journal of Medical Sciences 4:24-29.

Aniel Kumar O, Krishna Rao M, Mutyala Naidu L (2014). Evaluation of antimicrobial activity of chemical constituents of *Achyranthes aspera* L. roots against human pathogens. Indian Journal of Natural Products and Resources 5(3):278-281.

Aniel Kumar O, Krishna Rao M, Mutyala Naidu L (2015). Evaluation of antimicrobial activity of root extracts of *Abutilon indicum*. Notulae Scientia Biologicae 7(2):160-163.

Aniel Kumar O, Mutyala Naidu L, Raja Rao KG (2010). Antibacterial evaluation of snake weed (*Euphorbia hirta* L.). Journal of Phytology 2(3):08-12.

Anonymous (2000). Centers for Disease Control and Prevention, Preventing emerging infectious diseases, a strategy for the 21st century.

Chang SY, Cheng MJ, Kuo YH, Lee SJ, Chen IS (2008). Secondary metabolites from the stem bark of *Litsea akoensis* and their cytotoxic activity. Helvetica Chimica Acta 91(6):1156-1165.

Cheng MJ, Wang TA, Lee SJ, Chen IS (2010). A new butanolide and a new secobutanolide from *Litsea* lii var. *nunkao-tahangensis*. Natural Product Research 24(7):647-656.

Chowdhury JU, Bhuiyan NI, Nandi NC (2008). Aromatic plants of Bangladesh: Essential oils of leaves and fruits of *Litsea glutinosa* (Lour.) C.B. Robinson. Bangladesh Journal of Botany 37(1):81-83.

Devi P, Meera R (2010). Study of antioxidant, anti-inflammatory and wound healing activity of extracts of *Litsea glutinosa*. Journal of Pharmaceutical Science and Research 2(2):155-163.

Feng T, Zhang RT, Tan QG, Zhang XY, LiuYP, Cai XH (2009). Two new isoquinoline alkaloids from *Litsea cubeba*. Zeitschrift für Naturforschung B 64:871-874.

Gulzar H, Chowdhury M, Alam MN, Alam S, Shakil M, Khan S, Rahman M, Mazumdar M (2015). Phytochemical screening, antimicrobial and anticancerous activities of two different plant extracts. Journal of Medicinal Plants Studies 3(6):76-81.

Guzman GSI, Navarrete A (2009). Pharmacological exploration of the sedative mechanism of hesperidin indentified as the active principle of *Citrus sinensis* flowers. Planta Medica 75:295-301.

Haque T, Uddin MZ, Saha M, Mazid A, Hassan A (2014). Propagation, antibacterial activity and phytochemical profiles of *Litsea glutinosa* (Lour.) C.B. Robinson. Dhaka University Journal Biological Sciences 23(2):165-171.

Hosseinzadeh M, Mohamad J, Khalilzadeh MA, Zardoost MR, Haak J, Rajabi M (2013). Isolation and characterization of bioactive compounds from the bark of *Litsea costalis*. Journal of Photochemistry Photobiology B 128:85-91.

Jia XJ, Dong L, Yang Y, Yuan S, Zhang ZW, Yuan M (2013). Preliminary structural characterization and antioxidant activities of polysaccharides extracted stem Hawk tea (*Litsea coreana* var. lanuginose). Carbohydr Polymers 95:195-199.

Lohitha P, Shivsagar K, Charan V, Priya UP, Sagar SV, Ramanjaneyulu K, Verma V (2010). Phytochemical screening and evaluation of *in vitro* antibacterial activity of *Litsea glutinosa* (L) bark ethanol extract. Pharmacologyonline 1:618-623.

Mandal SC, Kumar CK, Majumder A, Majumder R, Maity BC (2000). Antibacterial activity of *Litsea glutinosa* bark. Fitoterapia 71(4):439-441.

Meera R, Devi P (2009). Antimicrobial activity of methanolic extract of *Merrimea gagentica* and *Litsea glutinosa* Leaves. Asian Journal of Chemistry 21(7):5655-5660.

Ncube NS, Afolayan AJ, Okoh AL (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology 7(12):1797-1806.

Pan JY, Zhang S, Jun W, Li QX, Xiao ZH (2010). Litseaglutinan A and lignans from *Litsea glutinosa*. Helvetica Chimica Acta 93(5):951-957.

Poornima VH (2011). Evaluation of antimicrobial activity of *Litsea glutinosa*. International Journal of Pharmaceutical Applications 2(1):105-114.

Rabena AR (2010). Propagation techniques of endangered sablot (*Litsea glutinosa*) Lour. C.B. Robinson. JPAIR Multidisciplinary Journal 5:56-83.

Silva KA, Klein Junior LC, Cruz SM, Caceres A, Quintao N, Monache FD (2012). Anti-inflammatory and anti-hyperalgesic evaluation of the condiment laurel (*Litsea guatemalensis* Mez.) and its chemical composition, Food Chemistry 132(4):1980-1986.

Thomas RP, Antony AM, Mamen AA (2013). Comparative phytochemical analysis of *Diospyros chloroxylon* leaves in various extracts. International Journal of Scientific and Research Publications 3(9):1-4.

Wang H, Liu Y (2010). Chemical composition and antibacterial activity of essential oils from different parts of *Litsea cubeba*. Chem Biodivers 7:229-235.

Wang YS, Huang R, Lu H, Li F, Yang JH (2010). A new 2'-oxygenated flavones glycoside from *Litsea glutinosa* (Lour.) C.B. Rob. Biosciences Biotechnology Biochemistry 74(3):652-654.

Wang YS, Zhen L, Yan L, Rong H, Hong BZ, Jing Y (2011). A new Megastigmane Diglycoside from *Litsea glutinosa* (Lour.) C. B. Rob. Journal of Brazilian Chemical Society 22(11):2234-2238.