

Phytochemical Screening and Evaluation of In vitro Anti bacterial Activity of *Litsea Glutinosa* (L) bark Ethanolic Extract.

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Summary

In vitro antibacterial activity of ethanolic extract of *Litsea glutinosa* (L) medicinal plant were screened against multidrug resistant bacteria including *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli*, isolated from clinical specimen. Ethanolic extract of *Litsea glutinosa* (L) showed antibacterial activity when compared with Gentamicin. Phytochemical studies on bark extract of *Litsea glutinosa* (L) revealed the presence of alkaloids, saponins, Cardiac glycosides, and tannins.

Keywords: Antimicrobial activity, *Litsea glutinosa* (L), multidrug resistant pathogens, phytochemical studies

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Introduction

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in AIDS and cancer patients^{1, 2}. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities³. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms⁴.

The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. However, very little information is available on such activity of medicinal plants^{5,6}.

Materials and Methods

Morphology of plant

An evergreen shrub or tree, upto 25 m in height and 1.5m in girth, branches and peduncles softly pubescent. Bark somewhat corky, viscid inside, brownish grey, and wood yellowish grey to grayish brown. Leaves aromatic, elliptic ovate or oblong-lanceolate, pubescent, variable in size veins 10-12 pairs. Flowers white or yellowish, fruit globose, black or purple, 0.64cm diameter⁷.

Phytochemical Constituents

Tannins, β - sitosterols and actinodaphnine, boldine, nor-boldine, laurotetanine, N-methyl laurotetanine, N-methylactinodaphnine, quercetine, sebiferine, litsiferine⁹. Kaempferol-3-glucoside, amino acids, quercetin-3-rhamnoside, Kaempferol-7-aminoglucoside, Pelargonidine-5-glucoside, naringenin-7-monorhamnoside, mono and sesquiterpenes, β -amirine acetate⁷.

Traditional medicinal uses

The bark of *Litsea glutinosa*, which according to Kirtikar and Basu, “is one of the most popular of native drugs”, is considered to be capable of relieving pain, arousing sexual power and also producing a soothing effect on the body, good for the stomach and are considered to be mildly astringent, other uses of the bark include treatment of diarrhoea and dysentery. The methanolic extract of the bark of *Litsea glutinosa* (L) showed antibacterial activity comparable to chloramphenicol, against 16 tested microorganisms⁸.

Litsea glutinosa is widely available throught India. The fresh dried stem barks were collected from the forests of Ananthanahalli near Harapanahalli, Karnataka in the month of June 2008. Herbarium was prepared by processing the plant for 10 days. The specimen was further identified and authenticated in Department of Botany, by botanist Prof. S.A. Kappali, Basaveshwar Science College, Bagalkot, Karnataka. Voucher specimen (B.sc./Bot/07/08-09) was deposited in the herbarium of the same college. The bark was subjected to coarse powdered (#: 44) to obtain uniform texture. Gentamicin was purchased from local market (Batch No: 91215, Concord Drugs Limited), all chemicals and reagents purchased were analytical grade.

The sieved powder was stored in airtight and high density polyethylene containers before extraction. The sieved powder was subjected to hot continuous soxhlet extraction with petroleum ether and ethanol for 24 hours cycle at 70⁰C¹⁰. Excessive solvent was removed by solvent distillation apparatus and residue was concentrated by using Lyotrap dryer. The brownish solid masses of extract were preserved in aseptic condition before performing the experiment.

Preliminary Phytochemical Analysis

The leaf extract was screened for the phytochemical bases using the standard method^{10,11}. The phytochemical components analyzed were alkaloids, steroids, starch, proteins, anthraquinone glycosides, saponins, flavonoids, tannins, and cardiac glycosides.

Preparation of bacteria

The bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia Coli* were purchased from M.T.C.C Institute of Microbial Technology, Chandigarh, India (Invoice No. 9/7/5790). The ability of the various extracts to inhibit growth of clinical bacteria and fungi isolates was determined using the agar disc diffusion method. Sterile filter paper discs, 11 mm in diameter were impregnated with each extract concentration and dried at 30° C in the static incubator. They were then carefully placed aseptically with a forceps on the surface of the Nutrient Agar (NA) plates that were preinoculated with the 24 h culture of bacteria and 0.1 ml spore suspension (1×10^5 spores/ml). The control antibiotics disc containing gentamicin (40 µg/ml) was placed on each of the inoculated plates of nutrient agar. The plates were left on the bench undisturbed for few minutes, after which the bacterial culture plates were incubated at 37° C for 24 h. The external diameters of visible zones of growth inhibition were measured after incubation¹².

Results and Discussion

The results of phytochemical screening of the bark of *Litsea glutinosa* for the bioactive components are presented in Table 1. Bark contains glycosides, alkaloids and tannins with variable amounts of saponins. The result for antibacterial screening to determine diameters of zone inhibition is given in Table 2.

The bark of *Litsea glutinosa* is found to contain alkaloids, tannins and phenolics, carbohydrates, proteins, cardiac glycosides and saponins. The presence of some of the phytochemical components like saponins, tannins and phenolic compounds have been attributed to the antibacterial activity of the ethanolic extract of the bark of *Litsea glutinosa* and the different concentrations of extract were found to be effective against some strains of *E.Coli*, *P.aeruginosa*, *B.cereus* and *S.aureus* in dose dependent manner when compared with Gentamicin which was measured in terms of Zone Of Inhibition (ZOI).

The presence of some of the phytochemical components like saponins, tannins and phenolic compounds have been attributed to the antibacterial activity of the crude drugs observed. Tannins, glycosides and alkaloids were effective against some strains of *E.coli*, *P.aeruginosa* whereas tannins, alkaloids, steroids and cardiac glycosides were demonstrated to inhibit the growth of *B.cereus* and *S.aureus*. The presence of these bioactive components in the crude drugs have been linked to their activities against disease causing microorganisms and also offering the plants themselves protection against infection by pathogenic micro-organisms¹³.

Conclusion

In conclusion ethanolic extract of *Litsea glutinosa* (*L*) bark was assessed in this study. The results seem to justify their continued use in the treatment of microbial infections. The inhibition of growth of the test organisms that are known to cause nosocomial infections and displaying multidrug resistance to most antibiotics and non-antibiotic antimicrobial agents justify the continued use of these plants in folk and traditional medical practice. Studies should therefore be done in order to identify the active phytochemical constituents and evaluate their effectiveness in vitro so that they can be synthesized and commercial production begins in earnest.

Table1: Phytochemical screenings of *Litsea glutinosa* bark ethanolic extract.

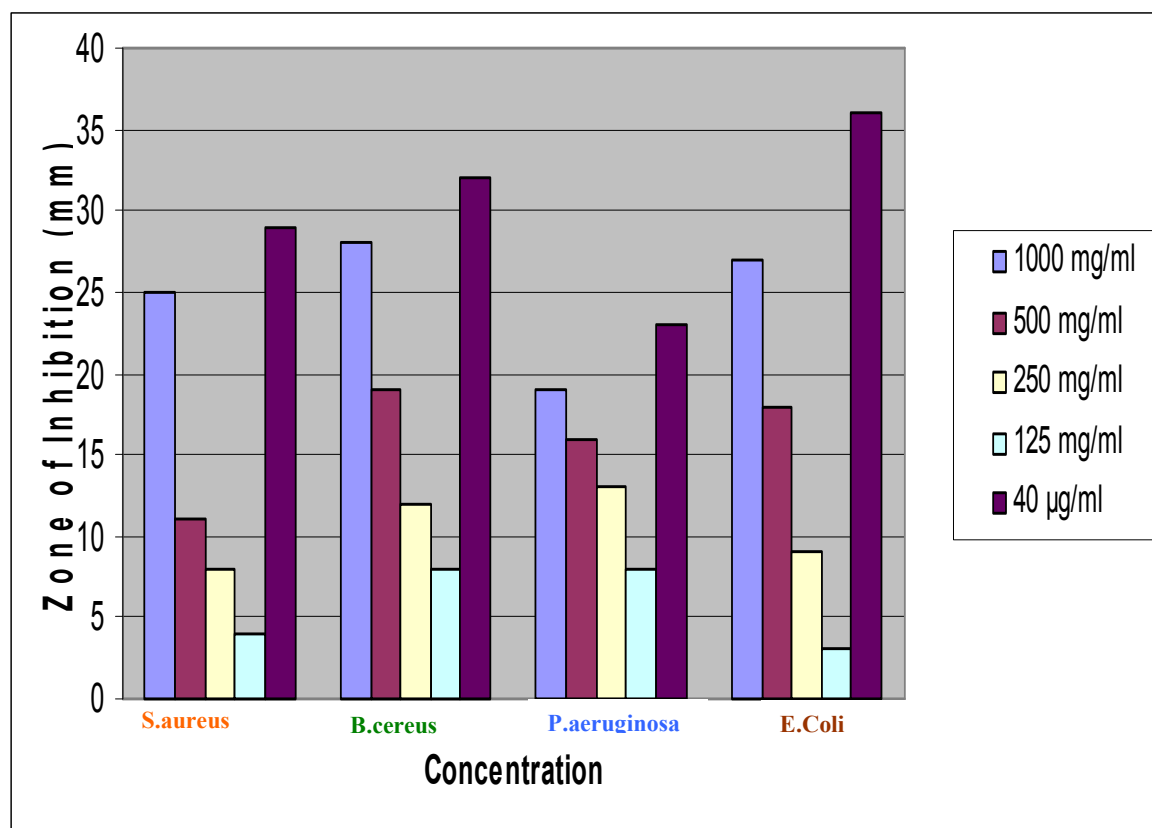
Constituent	Ethanolic extract
Alkaloids	+
Sterols	-
Glycosides	+
Carbohydrates	+
Mucilage	-
Starch	
(a) Iodine test	-
(b) Tannic acid test	-
Proteins	+
Cardiac glycosides	+
Saponins	+
Tannins and Phenolics	+
Flavonoids	-

“+” indicates presence of active constituents

“-” indicates absence of active constituents

Table2. Diameters (mm) of Zones of inhibitions produced By *Litsea glutinosa* (L) bark ethanolic extract.

Microorganism Species	Standard 40µg/ml Gentamicin ZOI in mm	1000mg/ml ethanolic extract ZOI in mm	500mg/ml ethanolic extract ZOI in mm	250mg/ml ethanolic extract ZOI in mm	125mg/ml ethanolic extract ZOI in mm
<i>Staphylococcus aureus</i>	29	25	11	8	4
<i>Bacillus cereus</i>	32	28	19	12	8
<i>Pseudomonas aeruginosa</i>	23	19	16	13	8
<i>Escherichia Coli</i>	36	27	18	9	3



- Indicates 1000mg/ml of Ethanolic extract
- Indicates 500mg/ml of Ethanolic extract
- Indicates 250mg/ml of Ethanolic extract
- Indicates 125mg/ml of Ethanolic extract
- Indicates 40µg/ml of Gentamicin

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