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Research Article

ANTIBACTERIAL ACTIVITY DIFFERENCE IN SINGLE AND GRADIENT EXTRACTIONS OF *LITSEA LIGUSTRINA* (NEES) HOOK. F.

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ABSTRACT

Leaves of *Litsea ligustrina* (Nees) Hook.f. (Lauraceae) evaluated for its antibacterial activity in 80% ethanolic extract and compared with its activity in gradient extraction using various solvents of increasing polarity. *Litsea ligustrina* showed a prominent antibacterial activity towards all the nine strains of bacteria in ethanolic extract. Antibacterial principle isolation is usually done in a process starting with gradient extraction. Antibacterial study of *L. ligustrina* was done using petroleum ether, acetone and ethanol as extracting solvents in the gradation of increasing polarity. However, these solvent extracts didn't show any antibacterial activity. From this result it was inferred that, antibacterial activity of *L. ligustrina* exhibited in ethanol extract might be due to some heat liable compounds which may be lost during drying process and powdering. Preliminary phytochemical evaluation revealed the presence of alkaloids, phenolics and flavonoids in *L. ligustrina* which may be the reason for its antibacterial activity. But there may be some heat liable compounds which may be lost during processing which may cause some reduction in antibacterial activity.

Keywords: *Litsea ligustrina* , antibacterial, disc diffusion, phytochemical, gradient extraction

INTRODUCTION

Litsea ligustrina (Nees) Hook.f. belongs to the family Lauraceae, its synonyms are *Darwinia quinqueflora* Dennst., *Litsea quinqueflora* (Dennst.) Suresh in Nicolson et al., *Tetranthera ligustrina* Nees in Wall., and *Actinodaphne quinqueflora* (Dennst.) M.R. Almeida & S.M. Almeida.[1] Medicinally relevant part is leaf of the plant. The plant is used for Anti-inflammatory and wound healing activity. *L. ligustrina* is an important medicinal plant endemic to Western Ghats. Kani tribals of Thirunelveli Hills Tamil Nadu use powder of leaf, stem, bark and flower of *L. ligustrina* along with leaves of *Vitex altissima, Hygrophila auriculata* and *Pavetta indica* are mixed and heated with water and taken internally to treat snake and scorpion bites[2]. Ethanolic and aqueous extractions are more common and less toxic in practical extraction technique[3]. Most extracts of plant species from Lauraceae genus showed antioxidant activity and revealed great free radicals of DPPH scavenger properties[4]. Methanolic extract of *Litsea acubeba* showed remarkable antioxidant activity and contained powerful natural antioxidant compounds[5]. The methanolic extract of the powdered leaves showed significant anti-inflammaroty activity in a dose dependent manner[6].

The genus *Litsea* has been used in traditional and indigenous chinese medicines for the treatment of diarrhea, stomachache, dyspepsia, gastroenteritis, diabetes, edema, cold, arthritis, asthma, pain, traumatic injury, etc. for a long history. The extensive literature survey reveals that *various Litsea* species form a group of important medicinal plants used for the ethnomedical treatment of gastrointestinal diseases, diabetes and microbial infections. Pharmacological investigations have supported the use of some *Litsea* species in the traditional medicines[7]. The present study tells that contradictory results can be obtained in antibacterial activity in single and gradient extraction methods.

MATERIALS AND METHODS

Preparation of Plant Extract

Fresh specimens (Leaves of *Litsea ligustrina*) were collected in the month of December from Kottayam District of Kerala State, India. A voucher specimen (AC 1220) was deposited at the herbarium of St. Thomas College Palai. 20 gm of fresh leaves of the plant were washed properly, cut into small pieces 50 ml of 80% ethanol was added to each sample and kept for 48 hours for preparing extracts. After 2 days the liquid part was filtered out, was used as extracts. Antibacterial activity of *L. ligustrina* was further evaluated by gradient extractions in petroleum ether, acetone and ethanol. For this, the leaves were washed properly, air dried and powdered well. About 50 gm of powdered material was successively extracted in petroleum ether, acetone, and ethanol by keeping the powder in each solvent for 24 hours[8].

Bacterial Strains Used

Test organisms were collected from the culture collection of the Institute of Microbial Technology (IMTECH) Chandigarh. These include *Vibrio parahaemolyticus, Salmonella typhi, Bacillus cereus, Enterobacter sps. Salmonella paratyphi, Vibrio cholerae, Staphylococcus aureus, Escherichia coli and Streptococcus haemolyticus.* These bacteria were sub-cultured on nutrient agar slants, incubated at 37^{o}C for 4 hours and stored at 4^{o}C in the refrigerator to maintain the stock culture.

In vitro Antibacterial Assay

The disc diffusion method was used to determine the growth inhibition of bacteria by plant extracts[9]. Sterile liquid Mueller Hinton Agar media (pH 7.4 ± 2) was poured into sterile petridish and after solidification; the bacteria (1 ml broth of approximately 10^5 CFU) were swabbed with a sterile swab

under aseptic conditions. Sterile discs prepared using Whatman No. 4 Filter Paper, of 5-mm diameter were used in the study. The original solvent in which the extract prepared was used as a control. Test materials were dissolved in the respective solvent to obtain a stock solution of concentration of 100 mg/ml. 10 µL of the solution was loaded per disc to attain a concentration of 1 mg/disc. The discs (including control) were used after drying them in an incubator at 50°C to remove any trace of solvent. Discs including controls were also prepared in the same way as those with extracts. Discs were introduced onto the surface of the medium. The plates were incubated overnight at appropriate incubation temperatures. Microbial growth was determined by measuring the diameter of zone of inhibition. Experiments were conducted in more than three replicates and average inhibitory zone diameter was determined along with standard deviation.

Preliminary Detection of Phytochemicals

The crude samples were subjected to phytochemical screening for the detection of alkaloids, phenolics, flavonoids using various spraying reagents, after their separation in silica gel thin layer chromatographic plates as described by Harborne[10].

RESULT

Antibacterial and phytochemical analysis of the leaves of *L. ligustrina* were conducted using fresh leaves in 80% ethanol. The results are reported in Table1. The ethanolic extracts of the plant were tested towards nine bacterial strains. *L. ligustrina* showed prominent antibacterial activity towards all the nine bacterial strains tested. Detailed antibacterial study of *L. ligustrina* was done against all the nine strains of bacteria selected for the study using petroleum ether, acetone and ethanol as extracting solvents in the gradation of increasing polarity (Table 2). These extracts didn't show any antibacterial activity towards all the nine strains of bacteria tested. From this result, it was inferred that, the antibacterial activity of *L. ligustrina* might be due to some heat liable compounds which were lost during heat drying. Preliminary phytochemical analysis of the ethanolic extracts of fresh leaves of the plant was conducted to evaluate the presence of alkaloids, phenolics and flavonoids. Alkaloids, phenolics and flavonoids were detected (Table 3) using various spraying reagents.

Table 1: Antibacterial activity of *L. ligustrina* towards the nine bacterial strains selected for the study

Bacterial strains	Inhibition zone diameter (in millimetres)
Vibrio parahaemolyticus	11 ± 0.85
Salmonella typhi	12 ± 0.96
Bacillus cereus	14 ± 0.84
Enterobacter species	12 ± 0.77
Salmonella paratyphi	13 ± 0.32
Vibrio cholera	12 ± 0.91
Staphylococcus aureus	15 ± 0.67
Escherichia coli	13 ± 0.59
Streptococcus haemolyticus	14 ± 0.48

Values: Mean ± Standard deviation

Table 2: Antibacterial activity of *L. ligustrina* leaf extracts using petroleum ether, acetone and ethanol as extracting solvents towards the nine bacterial strains

Inhibition zone diameter (in millimeters) and extracts used			
Bacterial strains	Petroleum ether	Acetone	Ethanol
Vibrio parahaemolyticus	-	-	-
Salmonella typhi	-	-	-
Bacillus cereus	-	-	-
Enterobacter species	-	-	-
Salmonella paratyphi	-	-	-
Vibrio cholera	-	-	-
Staphylococcus aureus	-	-	-
Escherichia coli	-	-	-
Streptococcus haemolyticus	-	-	-

Values: - no inhibition

Table 3: Phytochemical constituents of *L. ligustrina*

			Phytochemicals		
Name of the plant	Part used	Extract used	Alkaloids	Phenolics	Flavonoids
L. ligustrina	Leaves	Ethanolic	+	+	+
		Petroleum ether	+	-	-
		Actone	-	-	+
		Ethanol	-	+	+

Values: + present, - absent.

DISCUSSION

Single ethanolic extraction method is the common procedure of preparing medicines in Homeopathy[11]. Antibacterial experiments in various medicinal plants show experimental result variation. Certain plants which are said to be medicinally relevant may not give the corresponding results in lab experiments of antibacterial assay. But at the same time some plants which are not at all identified as medicinally relevant may give positive results in antibacterial assay. At the same time, plants which show positive in antibacterial assay of single ethanolic extract may not give the same positive results in gradient extraction. Gradient extraction is essential for separation and isolation of phytochemicals from plants. This is the initial step of isolation of biologically active principles from plants; however certain plants may exhibit contradictory results

in this step. This can be due to the synergistic effects of various compounds found in single ethanolic extraction but which will be separated during gradient extraction. In the present investigation, bioactivity of single extract is better compared to gradient extraction. *Litsea* species are important group of medicinal plants medicinal plants used for the ethnomedical treatment of microbial infections[7]. The present result supported the ethnobotanical uses of the plant in treating microbial infections as reported by Kong et al[7].

CONCLUSION

Antibacterial screening of *L. ligustrina* conducted in 80% ethanolic leaf extracts for preliminary antibacterial activity against nine bacterial strains by disc diffusion method. The leaf extract of showed a prominent antibacterial activity to all the nine strains. Detailed antibacterial activity of *L. ligustrina* was done against all the nine strains of bacteria selected for the study using petroleum ether, acetone and ethanol as extracting solvents in the gradation of increasing polarity. These extracts were taken from the air dried, powdered leaves of *L. ligustrina*. However, these extracts didn't show any antibacterial activity towards any of the nine bacterial strains. From this result it was inferred that, the antibacterial activity of *L. ligustrina* might be due to some heat liable compounds which was lost during drying and powering. Preliminary phytochemical investigation showed Alkaloids, phenols and flavonoids. The present investigation concluded that the *L. ligustrina* contains potential antibacterial components which are heat liable. This kind of contradictory results are common in antibacterial activity in single and gradient extraction.

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