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SCREENING OF PHYTOCHEMICALS AND ANTIBACTERIAL POTENINAL OF *LAURUS NOBILIS*

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ABSTRACT

The plants provide food, the whole part of the plant is claimed to possess the medicinal properties in the traditional medicinal system. In the present investigation different solvent extracts of *Laurus nobilis* were used to screen the phytochemicals following bioactive potential. Butanol extracts containing remarkable positive results of phytochemical compared to other solvent extracts. The butanol extracts showed maximum of alkaloids flavonoids and tannins. The flavonoids and alkaloids have important antibacterial potential efficiency. Butanol solvent shows remarkable antibacterial activity when compared to other solvent extracts and standard antibiotics. The higher concentration (100 µg /ml) of butanol extract shows 12, 12, 8, 8 and 11 cm of inhibition against *S. aureus*, *K. Pneumonae*, *V. Cholreae*., *S. typhi* and *E. coli* respectively. The active fraction obtained from this plant is an attractive material for further studies leading to possible drug development.

Keywords: *L. nobilis, antibacterial activity and Phytochemical screening.*

INTRODUCTION

Medicinal plants have an important role in our normal life. Curing disease through medicinal plants is an age old practice. The drugs from plants have cured, healed, the ailments of thousands of cowherds, hermits, hunters and those who live in forest just by eating roots and tubers. Some of the plants have the medicinal value in all over the body. But some have only in root stem, leaves, flowers and plants. Nowadays medicinal plants constitute a group of industrially important crops which bring appreciable income to the country by way of valuable foreign exchange. Medicinal plants have influenced the culture and civilization of man in many countries (Diwan and Tilloo, 1982). There is no report on the antibacterial activities of this plant. The extract from this whole plant body is given to cure alimentary tract infections and diabetics in human system (Clark and Plenum, 1996). Phytochemical or biological works were scanty for *Laurus nobilis*. In order to know the antibacterial activity, tests were conducted against human pathogenic bacteria.

MATERIALS AND METHODS

Laurus nobilis was chosen as a plant sample for screening antibacterial activities. The leaves were collected from the local market Manavalanagar, Tiruvallur District, Tamil Nadu, India. Fresh samples of small healthy leaves were collected from plant in early morning for investigation. The collected leave samples immediately transferred to the laboratory for shadow dry.

Preparation of plant sample: The plant material was dried in shadow place, after complete dry, the dried sample, powdered with the help of mixer grinder. The powdered sample used for preparation of extract. In the present study the plant extract prepared with different organic solvents (low polar to high polar). Hexane, butanol, ethanol, chloroform and aqueous solvents used for extract preparation by Pestle and Soxhlet method.

Preliminary phytochemical screening: The different solvent extracts of *L nobilis* were used to screen the following phytochemicals like sterol, sugar, alkalods, phenolic compounds, Flavanoids, tannins, saponins, aminoacids and ascorbic acids by standard methods.

Screening of antibacterial activity: Antibacterial efficiency of *Laurus nobilis* was screened by disc diffusion method.

Disc diffusion method: The sterile nutrient agar plate was prepared and dried. The pathogenic cultures of gram positive bacteria (*S. aureus*) and gram negative bacteria (*E. coli*, *V. Cholreae, K. Pneumonae and S. typhi*) were selected for analysis. The bacterial strains subcultured in sterile nutrient broth periodically (maintain purity) and incubate at 37°C for 18-24 hours. Each young cultures lawn into dried sterile nutrient agar plates separately. Whatman No-1 filter paper was used to prepare disc with the help of punching machine. The disc papers sterilized in autoclave and loaded with suitable concentration of test extracts. Test extracts loaded disc placed in bacterial culture lawn nutrient agar plates. Then the plates were incubated at 37°C for 2-3 days. After incubation period the zone of inhibition was measured and recorded. Similarly instead of plant extracts a standard antibiotic, tetracycline used to detect antibacterial activity and compared to plant extracts.

Thin layer chromatography studies: TLC plate washed in distilled water and dried. Then the TLC

plate was coated with silicate. After coating with silica gel, the plate was incubated at 100°C in hot air oven for activation. The activated TLC plate marked in bottom and upper region. The bottom region makes a mark offer sample load. TLC studies in successive extraction of hexane, butanol and aqueous prepared by both mortar and pestle and Soxhlet method. Each test extracts spotted in TLC plates. The sample spotted TLC plate placed in solvent for sample movement. The sample reach upto top marking region then sprayed ninhydrin solution for determination of spots. The Rf value was measured and recorded Rf value was calculated by the following formula

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

RESULTS AND DISCUSSIONS

In vitro antibacterial assay to asses the efficacy of *L. nobilis* to inhibit the growth of pathogenic microbes showed that butanol extract of the plant had broad spectrum antibacterial potential. The antibacterial activity and phytochemical screening of *L. nobilis* was analyzed and recorded.

Screening of antibacterial activity:

Antimicrobial screening of the leaf extract were analyzed in disc diffusion and well plate methods.

Phytochemical screening: Among the solvent extracts of *L. nobilis* studied for the antibacterial activity responds against pathogens. The study encourages screening the preliminary phytochemical analysis of leaf extract. The results were analyzed and recorded in Table 1.

Butanol extracts containing remarkable positive results of phytochemical compared to other solvent extracts. The butanol extract shows alkaloids flavonoids and tannins. The flavonoids and alkaloids have important antibacterial potential efficiency. It contains in butanol extract. So, the butanol extract can effective antibacterial agent against test pathogens. In this study clear from the results that leaf butanol extracts of *Laurus nobilis* adversely affect the growth of microorganisms. However the extent of concentration was more incase of test pathogens. Where as incase of low concentration this indicates the presence of some toxic compounds in leaf extracts. The alkaloids and flavonoids were bioactive compounds, butanol soluble growth inhibitors that also play significant role in plant microbe's interactions (Gabel *et al*., 1997). The presence of phytochemical determined in leaf extract ascorbic acid increase in other solvent extracts. This accounts for the increase inhibitory effect on the growth of the test organisms in response to the other solvents of leaf extracts.

Disc diffusion method: Antibacterial activity of *L. nobilis* plant leaf was screened by disc diffusion method. The extract sample loaded to disc with different concentration of 25, 50 and 100 μg/ml and results were noted in Table 2. In butanol extract of *L.nobilis* shows maximum zone of inhibition against pathogenic test organisms.

The other extracts of hexane and aqueous not much effect on bacterial growth butanol extract of plant shows predominant inhibition against grain positive bacteria (*S. aureus*) compared to gram negative bacteria (*S. typhi. K. Pneumonae V. Cholreae* and *E. coil*). Increment of extract concentration leads to maximum inhibition compared to lower concentration of extracts.

From the result inhibition of bacterial growth by extract is a dose dependent manner. Butanol solvent shows remarkable antibacterial activity when compared to other solvent extracts and standard antibiotics (Table 2). The higher concentration (100 μg /ml) of butanol extract shows 12, 12, 8, 8 and 11 cm of inhibition against *S. aureus*, *K. Pneumonae*, *V. Cholreae*., *S. typhi* and *E. coli* respectively.

In developing countries the peoples very often suffer from diabetes and the major cause of this disease is uncontrolled diet with the aim to wiping out the problem of diabetes, which leads an increasing stage in developing countries, the World Health Organization (WHO, 1964) has constituted a diabetic disease control program which includes studies on traditional medicinal practices, together with evaluation of health education and prevention approaches (Anonymous, 1979). In traditional system of medicine, plant materials are used as stomachic, demulcent, emetic and also used in diabetes, diarrhoea, gonorrhoea, eye troubles, liver and kidney complaints (Kiritikar and Basu, 1935 and Nadkarni and Nadkarni, 1976). On the basis of traditional use of the plants as a potent antibacterial and immunomodulate agent, the present study was carried out with butanol extract of *Laurus nobilis* plant materials to substantiate the folklore claim using different experimental models.

Among the different antibacterial screening butanol extract shows maximum antibacterial activity against *S. aureus* followed by *K. Pneumonae, E. coli*, *S. typhi* and *Vibrio sp*. *Laurus nobilis* have been used traditionally by tribal people for the treatment of gastro intestinal system and respiratory system in animals. But hitherto, no work has been carried out scientifically to prove the efficacy of this plant to inhibit the growth of microbes. Hence in the present study five pathogenic bacterial strains were chosen to find out the antibacterial activity of *L.nobilis*. The study clearly indicates that *L. nobilis* had a board spectrum of antibacterial substances in their body. Antibacterial activity of different solvent extracts of *L. nobilis* indicated that butanol extract had greater inhibition against tested microbes than the other extracts.

This result is in agreement with the earlier reports of Handa *et al.,* (1989) stated that the dicot plants are producing certain alkaloids which faithfully control the growth of microbial pathogens, further the dicot leaves were found to have wider antibacterial activity when compared to other plant parts. Hence, the leaf extract was used as a broad spectrum antimicrobial agent (Easmon and Glynn, 1975). From the results, it can be concluded that the antibacterial activity of medicinal plants is varied from species to species. It clearly emphasizes that the efficiency of antimicrobial activity of the plant should be determined by the physiological biochemical synthesis of antimicrobial principles.

Thin layer chromatography analysis: From the phytochemical screening and antibacterial analysis shows number of phytochemical presence in the test plant sample. The phytochemical confirmed with help of TLC analysis. The results of TLC also show potential antibacterial compounds, it was recorded in Table 3. In TLC analysis colour development occurs in plate sprayed with ninhydrin, it confirmed the presence of phytochemical and Rf value of sample indicate the presence of different phytochemical in sample.

CONCLUSION

The phytochemical screening of leaf extracts found flavonoids alkaloids sugars sterol and tannins. Amino acids and saponins were absent in all extracts. The butanol extracts of *Laurus nobilis* leaves showed moderately antibacterial activity against *S. aureus*, *K. Pneumonae*. *V. Cholreae*. *S. typhi* and *E. coli*. The higher dose of butanol extracts activity almost similar to standard antibiotics. The active fraction obtained from this plant is an attractive material for further studies leading to possible drug development. This fraction can be used as such for phytomedicine development with further studies to establish safety and efficacy. Development of phytomedicines is relatively inexpensive and less time consuming; it is more suitable to our economic conditions compared to allopathic type of drug development.

REFERENCE

Anonymous. 1979. Diarrhoea disease control program. Weekly Epidemic Rec. 16: 121.

Clark, R.A.F and M. Plenum. 1996. The molecular and cellular biology wound repair. *Journal of Wound Care*. 276(4): 3-50.

Diwan, P.V and L.D.Tilloo. 1982. Influence of Tridax procumbens on wound healing. *Indian Journal of Medical Research*. 75: 450-454.

Easmon, C. S and A.A. Glynn. 1975. Cell-mediated immune responses in *Staphylococcus aureus* infections in mice. *Immunology* 29, 75–85.

Gabel, T., Kevekordes, S., Par, K., Edenharder, R and Dunkelberg. 1997. *In vivo* genotoxicity of selected herbicides in mouse bone-marrow micronucleus test. *Arch. Toxicol*, 71: 193-197.

Handa, S.S., Chowla, A. S and S. Maninder. 1989. Hypoglycaemic plants: a review. *Fitoterapia*. 60: 195-202.

Kirtikar, K.R and B.D. Basu. 1935. Indian Medicinal plants. Dehradun: Bishen Singh and Mahendra pal singh. 3: 1647.

Nadkarni, K.M. and A.K. Nadkarni. 1976. Indian Matera Medica. Popular Prakashan, Bombay. 1175.

WHO. 1964. In: WHO Technical report series of enteric infection. Geneva. 288.

Table 1. Preliminary screening of phytochemical obtained in *L.nobilis.*

S.No	Phytochemical	*Laurus nobilis* extracts				
		Hexane	Butanol	Ethanol	Chloroform	Water
1.	Sterol	+	+	-	+	-
2.	Reducing sugar	+	+	+	-	-
3.	Sugar	-	+	+	+	-
4.	Alkaloid	+	+	-	-	+
5.	Phenolic compound	-	+	+	+	+
6.	Flavanoids	+	+	+		+
7.	Tanins	-	+	-		-
8.	Saponin	-	-	-	+	-
9.	Aminoacid	-	+	-	-	-

Table 2. Screening of antibacterial activity of *L. nobilis* against pathogenic bacteria.

S.No	Solvent	Conc. (µg/ml)	Average size of inhibition zone (cm) against pathogenic bacteria				
			E. coli	*K. Pneu monae*	*S. typhi*	*V. chlorae*	*S. aureus*
1	Control	0	0	0	0	0	0
2	Tetracycline	50	10.4	13.5	11.8	10.2	14
3	Hexane	25	2.3	2.6	2.6	3.1	2.3
		50	4.8	3.5	3.6	4.2	3.5
		100	4.9	4.2	4.1	4.6	4.6
4	Butanol	25	9.0	4.6	4.5	4.8	5.2
		50	10.8	8.8	6.2	6.8	9.1
		100	11	12	8.0	8.0	12
5	Ethanol	25	5.0	3.2	4.2	2.1	3.2
		50	5.6	3.8	5.1	3.6	4.5
		100	6.1	4.1	6.2	4.2	5.4
6	Chloroform	25	4.1	3.6	2.1	2.6	2.9
		50	4.6	4.1	3.6	3.8	3.4
		100	5.0	4.9	3.9	4.5	5.1
7	Water	25	6.1	3.2	4.1	3.4	3.8
		50	6.8	4.2	5.1	4.6	4.9
		100	7.1	5.2	5.8	5.2	6.1

Table 3. Screening of TLC analysis.

Observations	Extracts of *Laurus nobilis*				
	Hexane	Butanol	Ethanol	Chloroform	Water
Colour	Light green	Dark brown	brown	green	Dark green
Rf Value	0.86	0.98	0.85	0.78	0.93

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