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Research Article

Bactericidal activity of *Lagenandra ovata* (Linn.) Thw. rhizome oil

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Abstract

Lagenandra ovata* (Linn.) Thw.** syn. *L. toxicaria* Dalz. of Araceae family was evaluated for antibacterial activity against the Gram positive bacterium, *Staphylococcus aureus* and the Gram negative bacteria, *Escherichia coli, Klebsiella pneumoniae*, *Proteus mirabilis*, *P. vulgaris* and *Pseudomonas aeruginosa*. Methanol extract of the rhizome and the oil fractions obtained from the methanol extract through column chromatography were screened. The rhizome oil was found to be active against the pathogens, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* and this activity was compared to the standard antibiotic, Chloramphenicol. The rhizome oil of ***L. ovata possesses potential antibacterial activity and can be exploited as an antiseptic agent against the susceptible organisms.

Keywords: *Lagenandra ovata*, Antibacterial activity, Gram positive bacteria, Gram negative bacteria, Methanol extract, Rhizome oil.

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Introduction

Medicinal plants have wide spectrum of biological activity. The therapeutic effect of the drugs are due to the presence of low volume high value secondary metabolites such as alkaloids, cyanogenic glycosides, terpenoids, saponins, steroids, coumarins, tannins, flavonoids and other phenolic compounds. Recently, more than a hundred species of therapeutically important plants and many aromatic oils have been listed for their antimicrobial activity. The antimicrobial activity against a variety of human and plant pathogenic bacteria and fungi have well been investigated.

Chopra *et al*[1] studied some essential and fixed oils from Indian medicinal plants for antimicrobial activity, using filter paper disc method for measuring the zone of inhibition. Geda and Bokadia[2] screened antimicrobial activity of essential oils at different dilutions. Geda[3] reported the bactericidal activity of essential oils from ***Psoralea corylifolia* Linn.**, ***Blumea laciniata* DC.**, ***Oenanthe javanica* (Blume) DC.** syn. *O. stolonifera* Wall. ex DC. and ***Albizia lebbeck* Benth.** The efficacy of the oils was compared with standard antibiotic drugs.

***Lagenandra ovata* (Linn.) Thw.** syn. *L. toxicaria* Dalz. (Family: Araceae) is commonly found in South West India[4]. It is a semi-aquatic herb found in marshes and along water courses, often growing gregariously in semi-evergreen forests. Traditionally, the rhizome is used in renal and cardiac ailments. Rhizome is considered carminative, tonic, and diuretic and used in bilious complaints[5]. Plant is used in the preparations of ointments for skin itch. Juice of the fresh plant is applied to wounds for quick healing[6]. Paste of the plant is applied on swelling to reduce pain and inflammations[7]. However, the plant has not been subjected to any scientific evaluation so far. Effective utilization of any information requires its systematic evaluation. This paper focuses on the systematic study of the antibacterial activity of the rhizome of this *hitherto* unexplored plant.

Materials and Methods

The rhizomes of ***L. ovata*** were collected from the Papanasam hills of Tirunelveli district, Tamil Nadu, in the month of March. Authentic specimen is deposited in the Department of Botany, St. John's College, Palayamkottai, Tamil Nadu. The rhizomes were cleaned with water, chopped, dried and ground into powder; 300g of the powder was extracted thrice with two litres of methanol for 6 hours at 65°C using a three litre round bottom flask fitted with a water condenser. After distilling the solvent the extract was concentrated under reduced pressure.

The brown viscous methanol extract (20g) was loaded onto a silica gel

Rhizome of *Lagenandra ovata*

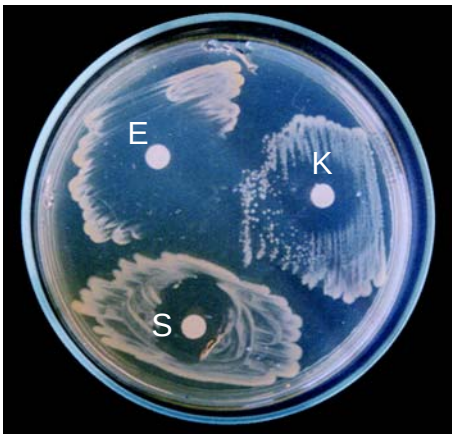

Fig. 1 : Fraction 7 of rhizome oil

Fig. 2 : Fraction 8 of rhizome oil

E= *Escherichia coli*, K= *Klebsiella pneumoniae*, S= *Staphylococcus aureus*

(60-120 mesh) column (4 × 25 cm) and eluted with nine different eluents of increasing polarity. The polarity of the eluents was increased, starting from n-hexane, n-hexane : ethyl acetate (4:1), n-hexane : ethyl acetate (3:2), n-hexane : ethyl acetate (2:3), n-hexane : ethyl acetate (1:4), ethyl acetate : methanol (3:1), ethyl acetate : methanol (1:1) to absolute methanol (each 500 ml). The fractions were collected separately in 100 ml portions. A total of 45 fractions were obtained. The fractions from n-hexane : ethyl acetate (4:1) eluent, numbered 7 and 8 yielded greenish yellow oil (LT-oil), which was monitored for antibacterial activity. Methanol extract (1%) of the rhizome was also screened for antibacterial activity.

The microorganisms tested were, the Gram positive bacterium, *Staphylococcus aureus,* the Gram negative bacteria, *Escherichia coli, Klebsiella pneumoniae*, *Proteus mirabilis*, *P. vulgaris* and *Pseudomonas aeruginosa.* Pure cultures of the organisms were obtained from All India Institute of Medical Sciences, New Delhi.

For the evaluation of antibacterial effect, agar disc diffusion technique of Bauer *et al*[8] was adapted. Sterilized Whatmann No.1 filter paper disc (6mm diam.) was dip-drained in the plant samples and simultaneously control (methanol) was maintained. For comparison, standard antibiotic, Chloramphenicol (disc potency 30µg) was used. The pure culture of the organisms tested was grown over Muller Hinton agar medium. The discs with different plant samples tested were placed over the seeded medium along with the control and the standard (3 discs per 9cm plate). After overnight incubation (37°C), the degree of sensitivity was determined by measuring the zones of inhibition and rechecked after further incubation for 18 hours. All the experiments were performed in triplicate and the average zone of inhibition was recorded.

Results and Discussion

Table 1 shows the sensitivity of bacteria to the various plant samples tested. The results were compared with the antibacterial activity of Chloramphenicol antibiotic discs. In the case of crude methanol extract, the zone of inhibition was found to be very little when compared to standard, against *S. aureus*, *E. coli* and *K. pneumoniae.* The crude extract showed no activity towards the other pathogens. So the organisms are considered resistant to the methanol extract of rhizome. No inhibition was found in the control. However, the greenish yellow oils (fractions 7 and 8) of methanol extract of rhizome showed maximum activity, almost equal to the standard antibiotic Chloramphenicol in *E. coli* followed by *S. aureus* and *K. pneumoniae*. The oil fractions 7 and 8 almost showed similar activity in *Proteus mirabilis* and *P. vulgaris*. In the case of *Pseudomonas aeruginosa* the methanol extract and oil fraction 7 did not show any activity, whereas the fraction 8 alone showed less activity. Figures 1 and 2 show the comparison of the sensitivity of three bacterial strains *E. coli, K. pneumoniae* and *S. aureus* to the rhizome oil (fractions 7 and 8). The two fractions showed more or less similar activity. They were pooled and subjected to GC-MS analysis.

Table 1: Antibacterial activity of methanol extract and oil fractions from the rhizomes of *Lagenandra ovata* (Diameter of zone of inhibition in mm)

S. No.	Microorganism	Methanol extract (1%)	Oil fractions		Control	Standard
			No. 7	No. 8	Methanol	Chloramphenicol
1.	*Staphylococcus aureus*	8	20	22	-	23
2.	*Escherichia coli*	9	28	26	-	31
3.	*Klebsiella pneumoniae*	8	15	16	-	24
4.	*Proteus mirabilis*	-	7	8	-	18
5.	*Proteus vulgaris*	-	8	8	-	14
6.	*Pseudomonas aeruginosa*	-	-	7	-	21

The diameter of the zone of inhibition is influenced by a variety of factors such as diffusibility of the drug, disc concentration, the nature and composition of the medium, its thickness, presence of inhibitory or stimulatory substances, *p*H of the medium and the time of incubation[9]. During incubation, the therapeutic agent diffuses out from the disc in all directions. Agents with lower molecular weights diffuse faster than agents with higher molecular weights. Thus, an agent of large molecular weight might be a powerful inhibitor even though it may diffuse only a small zone of inhibition. Moreover, results obtained *in vitro* often differ from those obtained *in vivo*. Metabolic processes in the body of a living organism may inactivate or inhibit an antimicrobial compound[10]. Screening programmes to detect antimicrobial activity in plant extracts have led to the isolation of antimicrobial isoflavonoids from ***Fagonia cretica* Linn.**[11]. The present bioassay guided the identification of chemical constituents of the rhizome oil[12]. The oil fractions of ***L. ovata*** were found to be the same by thin layer chromatographic analysis and hence pooled. The oil was centrifuged at 10,000 rpm for 20 minutes and the clear supernatant oil was subjected to GC-MS analysis using SHIMADZU instrument, GC-MS P5000 (Japan). The chemical constituents present in the oil were identified as methyl ester of 2-hydroxy benzoic acid, diethyl phthalate, oleic acid, palmitic acid ethyl ester and dioctyl ester of 1, 2-benzene dicarboxylic acid. Diethyl phthalate was found to be the major constituent (89.46%). It is evident that any of these compounds or the synergetic effect of these compounds may have the antibacterial activity. The isolation and identification of the active ingredients from the oil will have an impact in medicine in future.

Conclusion

The bactericidal activity of the oil (fractions 7 and 8) of ***L. ovata*** rhizome was found to be more or less equally effective with that of the standard antibiotic Chloramphenicol in the *in vitro* condition. Hence, it can be exploited as an antiseptic agent against the susceptible organisms *S. aureus*, *E. coli* and *K. pneumoniae*.

The plant is poisonous in the crude form hence self-medication with this wild plant is not advisable. The present study may serve as a guide in the selection of the plants with antibiotic activity for further work on the isolation and elucidation of the active compounds. An extensive research and development work should be undertaken for better economic and therapeutic utilization.

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