ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF SOME COMMON LICHENS FOUND IN KODAYARFOREST (WESTERN GHATS)

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ABSTRACT

The growing population of drug-resistant microorganisms and the problem of treating the infections induced have motivated the search for alternative antimicrobial drugs in lichens. The methanol extracts prepared from some common lichens species were evaluated for antibacterial activity against standard strains (Escherichia coli, Bacillus subtilis, and Staphylococcus aureus). the tested extracts showed more potent inhibitory effects on Gram (+) bacteria than on Gram (-) bacteria.

Keywords: Lichens, Western ghats, E.coli, antibacterial

INTRODUCTION

Lichens have economic benefits to human beings which has antibiotic properties that are valuable commercially for biomedical applications. Lichens and their metabolites yield significant bioactive substances for the treatment of various human diseases caused by different pathogenic microorganisms. There are about 2040 species of lichens present in India [1] in which around 10 lichen species are being used for food, more than 15 lichens used for biomedical applications and few are employed for environmental monitoring and natural dye extraction purposes. Lichens can be efficiently used for monitoring the level of pollution in the atmosphere and analysis of lichen samples can be used to estimate the extent and pollutant emissions around an industry or a particular locality [2] . Lichens provide warning signal before severe damages occur on ecosystem and health. It has been observed that the majority of lichen populations are unexplored for commercial exploitation [3]. Lichens can produce a wide array of both intracellular and extracellular compounds. These lichen compounds are called secondary metabolites which are extracellular in nature often called lichen acids. These are unique to lichens. Since lichens are composed of mycobionts and phycobionts, the former are playing pivotal role for the production of secondary metabolites4 . The quantities of these secondary metabolites may vary up to 30% of the dry weight of the lichen thalli. So far number of lichen compounds have been extracted for various investigations. Lichen secondary metabolites comprise of amino acid derivatives, aromatic compounds, dibenzofurans, depsides and depsidones etc. which exhibited manifold biological activities such as antibiotic, antiinflammatory, analgesic, antipyretic and cytotoxic activities [5,6]. The aim of the present study is to evaluate the antimicrobial activity of methanol extracts of few lichens collected from Kodayar forest, Western Ghats of Tamil Nadu, India.

MATERIALS AND METHODS

Study area: The study area at Kodayar, tropical forests and one of the hot spots of biodiversity centers in India. This forest area has 30 Kani settlements which occupy an area of 6.85 $Km^{2.}$ It is located 400 Km south of Madurai (77°15' E, 8°29' N) and it is at 250-650 m elevation in the Kanyakumari district of Tamil Nadu, South India.

Collection of lichens: Some common Lichens, *U.austriindica G.Aswathi , U. maculata* and *U. cf.nilgirica G. Aswathi* were collected from Kodayar of Western Ghats, Kanyakumari District, during summer season.

Identification of collected lichens: Collected lichens were identified by Dr. Sanjeeva Nayaka, Scientist, Lichenology Laboratory, Plant Biodiversity and Conservation Biology Division, National Botanical Research Institute (NBRI-CSIR), Rana Pratap Marg, Lucknow - 226001, U.P., India

Preparation of lichen extract: Dried lichens were mechanically ground and filtered by the refinery to get a fine powder. Fifty grams of each lichen powder was macerated in 1000 ml of methanol and mixed well. The mixture was poured in a dark well tighten bottle and kept inside the incubator at 40ºC for 3 days, with frequent shaking. Then, the infusion was filtered with Whatman filter paper No.1. The filtrate was left to evaporate inside an incubator for two days to get crude extract.

Bacterial strains:*Escherichia coli* (ATCC – 25922), *Staphylococcus aureus* (ATCC – 25923), Bacillus substilis (MTCC 441) were used.

Antimicrobial Assay

Disc Preparation: The 6mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C.After the sterilization the moisture discs were dried on hot air oven at 50°C. Then methanol extract discs and control discs were prepared.

Assay of Antibacterial Activity: Antibacterial activity test was carried out following the modification of the method originally described by Bauer et al., (1966) [7]. Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45ºC. The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The extract prepared discs individually were placed on the each petriplates and also placed control and standard (Nitrofurantoin - 300µg) discs. The plates were incubated at 37ºC for 24 hrs. After incubation period, the diameter of the zoneformedaround the paper disc were measured and expressed in mm.

RESULTS AND DISCUSSION

Antimicrobial activity of lichen extracts against bacterial strains were presented in the Table 1 and Fig.1. Methanol extract of lichen species revealed significant antibacterial effect against the tested organisms.

Table 1: Zone inhibition values of the methanol extracts of lichens *U.austriindica G.Aswathi , U. maculata* and *U. cf.nilgirica G. Aswathi*

S. No.	Bacteria	Zone of inhibition (mm in diameter)				
		C	**S***	***U.austriindica G.Aswathi***	***U. maculata***	***U. cf.nilgirica G. Aswathi***
1	*Bacillus subtillus*	-	20	15	19	18
3	*Staphylococcus aureus*	-	27	23	25	28
3	*Escherichia coli*	-	13	9	12	10

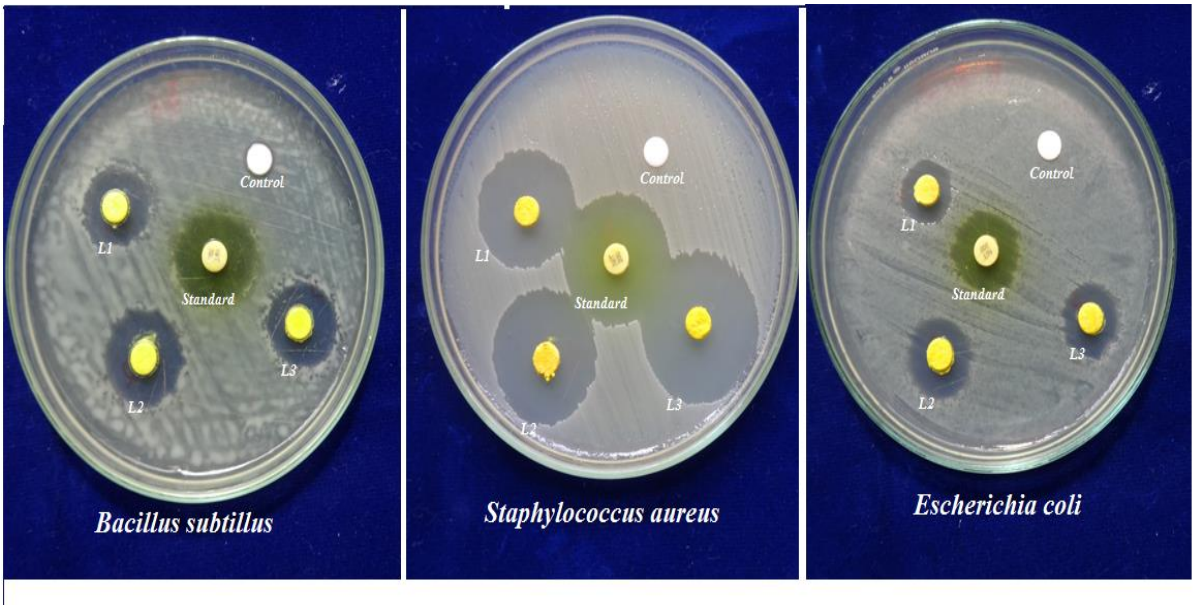

Fig.1: The antibacterial activity of the methanol extracts of lichens *U.austriindica G.Aswathi (L1) , U. maculata* (L2) and *U. cf.nilgirica G. Aswathi* (L3) against *B.substillus, S.aureus* and *E.coli*

According to Burkholder et al. [8], Rowe et al. [9], and Silva et al. [10], the lichens inhibit mostly gram-positive bacteria, but it is of great interest to note that all the extracts in this study inhibited the growth of both gram-positive bacteria and gram-negative bacteria in the present study.The antimicrobial activity of the tested lichen species was examined in several studies in various ways and with different results. Shanmugam et al., (2017) observed the dependence of the level of the antimicrobial activity of the same lichen species on the solvent used in extraction [11]. They emphasized the strongest antimicrobial activity of the methanol extracts compared to the extracts in other solvents. This is consistent with the observation of Bezivin *et al.* (2003) that polar lichen compounds were mostly found in the methanol extract [12]. Besides this, the differences in previous studies could reflect: different quantity of the same active component in lichen extracts, different components involved in antimicrobial actions, different locations of lichen sampling, and different sensitivity of tested microorganisms or different methods of testing.

In this study, the antimicrobial properties of the methanol extracts of lichens *U.austriindica G.Aswathi* , *U. maculata* and *U. cf.nilgirica G. Aswathi* , showed a different degree of antimicrobial activity depending on the tested group of microorganisms and the tested species. Generally, the tested extracts demonstrated a good antimicrobial activity. Our study confirmed the highest antimicrobial activity of *U. maculata* and *U. cf.nilgirica G. Aswathi*. Also, the tested extracts showed more potent inhibitory effects on Gram (+) bacteria,*Bacillussubtillus* and *Staphylococcus aureus*than on Gram (-) bacteria ie., *E.coli*, due to their specificity of the cell wall structure.*E.coli* showing minimum zone inhibition values compared to Gram (+) bacteria.

CONCLUSION

The methanol extracts of *U.austriindica G.Aswathi* , *U. maculata* and *U. cf.nilgirica G. Aswathi* have a potential towards antibacterial activity. The obtained results showed that the tested lichen extracts showed a significant antimicrobial activity relative to the tested bacteria, which could be of significance in human therapy,

animal, and plant diseases. Further investigations on the antibacterial activity as well as the economical and fast isolation of the metabolite from the lichena are needed.

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