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INVESTIGATION OF ANTIMICROBIAL POTENTIAL OF A FRUITICOSE LICHEN - *ROCCELLA MONTAGNEI* BEL.

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ABSTRACT
Lichen is in a separate division in plant kingdom because of its symbiotic association and complex nature of an algae and fungi. Several lichen species are reported to be used tradiationally in many theraupatic practices. Many lichen species are reported as sources of several bioactive natural compounds. In this article, *Roccella montagnei is* evaluated for its potential antimicrobial activity. The crude extract obtained from the lichen thalli was subjected to preliminary phytochemical analysis and was exhibited the presence of various phytoconstituents. Antibacterial activity was investigated by disc diffusion method against selected bacterial pathogens and the extract exhibited significant activity which was revealed by valid inhibition zone formation.
Key Words: Antimicrobial, Lichen and *Roccella montagnei.*

INTRODUCTION

The challenge for today's pharmaceutical industry lies in the discovery and development of new pharmaceutical active molecules due to microbial resistance to available antibiotics (Bahera et al., 2005). Since long back, plants have provided a source of inspiration for novel drug compounds, as plant derived medicine have made large contributions to human health and wellbeing by becoming the natural blue print for drug discovery and the development of phytomedicines to cure disease (Iwu, 1993). Similar to higher plants, lichens were used since antiquity as natural drugs (Barner, 2000).

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Lichens represent a unique division in the plant kingdom.They colonize some of the most inhospitable habitats on earth. They can survive in extremely cold areas such as on high mountains and in regions such as the arctic. They may be virtually the only plant form surviving in some of these areas and can be vitally important sources of food for animals. They have used in traditional system of medicine including Traditional Indian medicine (TIM),Traditional chinese medicine (TCM), Homeopathic and Western medical Herbals. Lichens have been used in treatment of diverse disease like arthritis, alopecia, constipation, kidney disease, leprosy, pharyngitis, rabies, infection, warm and infestation. The medicinal utility of Lichen is regarded to presence of secondary compounds like of usnic acid and atranorin. One of the reasons for exploring biological compounds in lichens is the potential for medical use. However, much work remains to link medical effects with specific lichen species. Information on the edible and medicinal uses of the Lichens is scattered (Cherallier, 1996). Many Lichens are known to have potent antibiotic properties and many are edible. However,some lichens do not contain toxic substance. Lichen forming fungi produce antimicrobial secondary metabolites that protect many animals from pathogenic microorganisms. The first study of antibiotic properties of Lichen was carried out by (Burkholder, P.B. and Evans, A.W, (1945). Varita (1973) reported antimicrobial properties of several Lichen and other researchers have since then studied the antimicrobial activity of several Lichens against gram positive and negative bacteria as well as several fungi.

Roccella montagnei Bel is probably the only fruticose lichen that is commonly found in mangrove forests with their abundance in south India. Its presence as an epiphyte along the coromandel coast and Pichavaram mangrove forests of Tamil Nadu were also reported (Aswathi, 1988, Mohan and Hariharan, 1999). Further, India possesses a long coast line but littoral rocky areas are few, however coconut trees (*Cocos nucifera*) are the most common substratum for such lichens. The presence of pure crystalline compounds like Montagnetol (1.8%), Erythritol (1.6%, Roccellic acid (0.8%) and Orcinol (0.1%) were reported in this species (Subba Rao and Seshadri, 1940). Apart, it was also reported to possess a wide array of secondary compounds like Lecanoric acid, Methylorsellinate, Meso-erythritol, β-carotene and β-sitosterol (Neelakantan and Seshadri, 1952; Bambuwala, 2000).In this study, we aimed to detect a possible inhibitory effect of different extracts of *Roccella montagnei* on the growth of various selected human pathogens. tested by using agar disc diffusion method.

MATERIALS AND METHODS

Material Collection and Identification:

The thalli of *Roccella montagnei* Bel. was collected from the boles of *Cocus nucifera* in Thengapattinam coastal area (8°14'25"N & 77°10'20"E) of Kanyakumari district, Tamil Nadu, India, and identified by Mr. Siljo Joseph, Botanical Survey of India, Central Regional centre, Allahabad. Voucher specimens were deposited at the Herbarium of Department of Botany, Scott Christian College (Autonomous), Nagercoil, Tamil Nadu. The collected samples were then dried at room temperature for 48 h, powdered with a blender and stored for further use.

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Extraction of collected material:

30g of powdered sample was weighed and taken separately. This sample was extracted with a series of selected solvents viz. Petroleum ether, Acetone, Benzene, Methanol, Chloroform, and Distilled water individually using Soxhlet apparatus. The organic extracts obtained were evaporated to dryness by kept open in room temperature. However, in case of aqueous extraction, the extract was evaporated to dryness by heating in a water bath to obtain a semisolid mass. The final filtrate obtained in each case was later subjected to preliminary phytochemical analysis and bacterial susceptibility test.

Preliminary phytochemical analysis:

Preliminary phytochemical screening involving chemical tests to determine the presence of Alkaloids, Phenols, Proteins, Tannins, Saponins, Flavonoids, Quinones, Resin, Xanthoprotein, Carboxylic acid, Coumarin and Carbohydrates were carried out using the methods described by Odebiyi and Sofowora (1999).

Bacterial Isolates and Bioassay:

The extracts of Petroleum ether, Acetone, Benzene, Methanol, Chloroform, and Distilled waterobtained from Lichen sample was screened against six bacterial strains. The test organisms, *Klebsiella pneumoniae, Pseudomonas aeruginosa,Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Enterococcus faecalis* were obtained from 'Scudder laboratory', Nagercoil, Kanyakumari District, Tamilnadu.

Preparation of Inoculum:

Stock cultures were maintained at 4ºC on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to the test tubes of Mueller-Hinton broth (MHB) for bacteria and were incubated without agitation for 24 hrs at 37 ºC.

Antimicrobial Susceptibility Test:

The disc diffusion method was adopted to screen the antimicrobial activity (Bauer *et al*., 1966). *In vitro* antimicrobial activity was screened by using Mueller Hinton Agar (MHA). The MHA plates were prepared by pouring 15ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The crude extracts (20µl) were loaded on 4mm sterile disc. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and then the plates were kept for incubation at 37̊ C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimetre. These studies were performed in duplicates.

RESULTS

Phytochemical analysis:

The stock crude extracts prepared from thallus extractsof *Roccella montagnei* by using petroleum ether, acetone, benzene, methanol, chloroform, and distilled water were subjected to preliminary phytochemical analysis. Acetone extract shown positive result toPhenol and tannins. Similarly,

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methanol extract shows the presence of phenol, tannin, saponin and carboxylic acid. The rest of the analysis done shown negative results which indicates that R. montagnei lacks the presence of alkaloids, flavanoids, proteins, aminoacids, quinone, resin, xanthoprotein, coumarin and carbohydrate (Table 1).

Table 1. Preliminary phytochemicals of *Roccella montagnei*

Primary metabolites	Solvents					
	Acetone	**Benzene**	**Chloroform**	**Petroleum ether**	**Methanol**	**Dis. Water**
Alkaloids	–	–	–	–	–	–
Phenols	+	–	–	–	+	–
Proteins	–	–	–	–	–	–
Tannins	+	–	–	–	+	–
Saponins	–	–	–	–	+	–
Flavanoids	–	–	–	–	–	–
Quinones	–	–	–	–	–	–
Resin	–	–	–	–	–	–
Xanthoprotein	–	–	–	–	–	–
Carboxylic acid	–	–	–	–	+	–
Coumarin	–	–	–	–	–	–
Carbohydrates	–	–	–	–	–	–

Antimicrobial assay of *Roccella montagnei*

The stock crude extracts prepared from the thallus of *Roccella montagnei* by using petroleum ether, acetone, benzene, methanol, chloroform, and distilled water were subjected to antimicrobial activity against *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Enterococcus faecalis,* and the results were recorded (Table 2).

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Table 2. Antimicrobial assay of *Roccella montagnei*

Microorganism	Petroleum ether (A)	Acetone (B)	Benzene (C)	Methanol (D)	Chloroform (E)	Distilled water (F)	control
Escherichia Coli	-	7mm	6mm	-	8mm	7mm	22mm
Klebsiella pneumoniae	-	-	8mm	-	-	8mm	23mm
Pseudomonas aeruginosa	-	-	-	-	-	8mm	26mm
Staphylococcus Aureus	-	11mm	-	11mm	-	8mm	22mm
Bacillus Cereus	-	9mm	7mm	8mm	-	9mm	20mm
Enterococcus Faecalis	-	7mm	-	-	7mm	-	20mm

Among the six different extracts used, distilled water extract exhibited maximum growth inhibition on five organisms except *Enterococcus faecalis*. Followed this, acetone extract exhibits a maximum inhibition zone formation with four organisms except *K.pneumoniae* and *P.aeruginosa*. Benzene extract have shown positive results with three species namely *E.coli, K. pneumonia* and *B.cereus*. Whereas the chloroform and methanol extracts have shown inhibition zone formation only with two species viz. *E.coli*, *Enterococcus faecalis* and *S.aureus*, *B.cereus* respectively. There was no inhibitory activity for petroleum ether extract.

DISCUSSION

The antimicrobial potential of distilled water extract of *Roccella montagnei* against five pathogens are in conformity and comparable with similar assays using methanolic extracts of *Cladonia unciali* and *Peltigera canina* which showed significant inhibition against *S.aureus* and *C.albicans* (Ingolfsottir *et al*., 1985). Similar results were also obtained from methanolic extracts of *Usnea ghattensis* against *B.megaterium, B.cereus* and *B.subtilis* (Behera *et al*., 2005).The growth of *Staphylococcus aureus* was affected maximumon Acetone extract, this result is consistent with the results reported byMie *et al.*, (2013).

The antimicrobial potential of acetone and benzene extracts of *R.montagnei* are in conformity and comparable with similar assays by Dulger et al(1998), Tay et al.,(2004) and Ylmaz et al., (2004)

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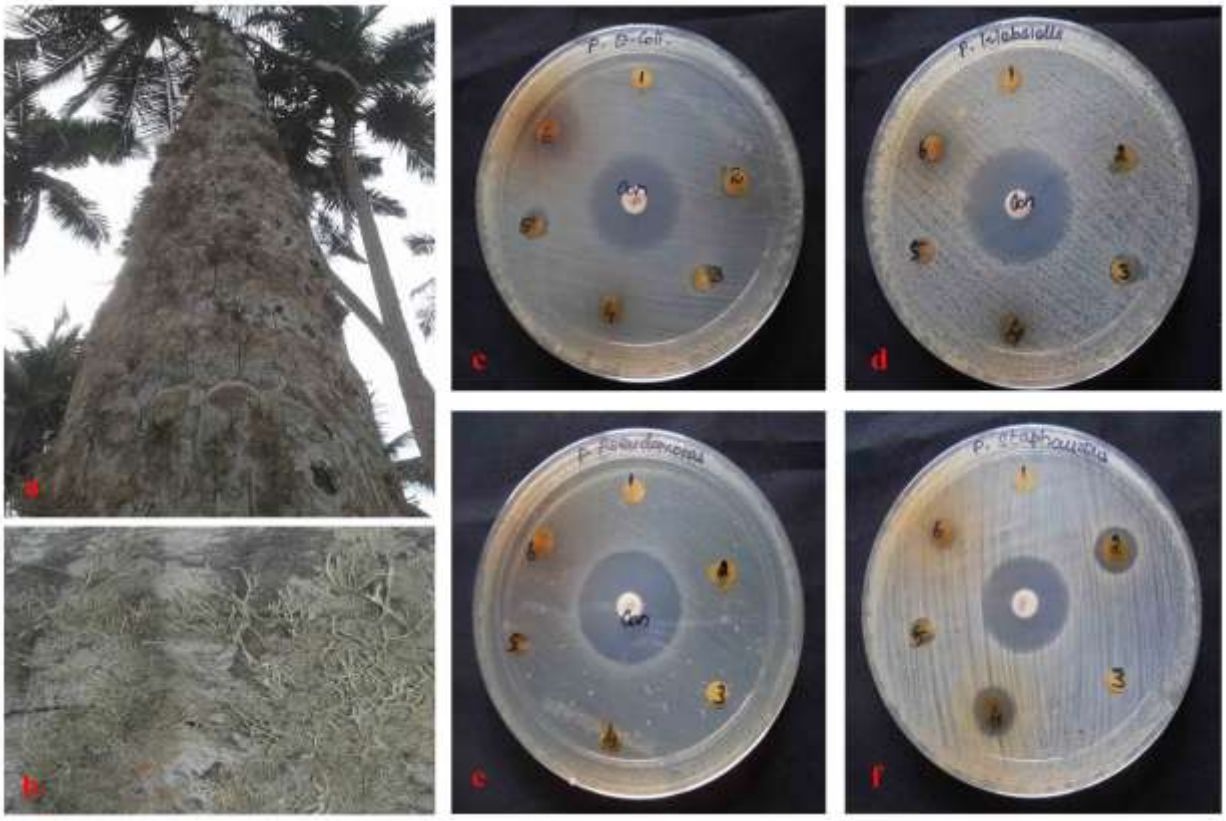
using acetone and benzene extracts of *Cetraria islandica, Ramalina farinacea* and *Cladonia foliacea* showing distinguishable activity against *P.vulgaris, S.aureus* and *C.albicans*.

The thallus of *R.montagnei* is known to contain class of Depsides and Terpenes (Rundel, 1987,Huneck and Yoshimura,1996)while acetone extract contain Terpenoids, Depsides, Polysols etc. (Bombuwala, 2000). The extracts from only threelichens, *Letharia columbiana, Letharia vulpine* and *Vulpicida Canadensis* were active against *E. coli*. The Acetone extract is more efficient than the methanolic extract were also reported by (G. Shrestha *et al.*, 2014). The bacterial pathogen *E. coli and S. aureus* has a maximum inhibition activity this result has been also indicated that the usnic acid compound actively inhibited the growth of *E. coli and S. aureus.* (Maulidiyah *et al.*, 2020).The pathogenic bacteria *Klebsiella pneumoniae* showed the minimum inhibition activity against the phytochemical which is related to the work done by (Manlapaz *et al.*, 2022).



a – Habitat of *Roccella montagnei* on the bole of *Cocus nucifera*, **b –** An enlarged view of the lichen thallus, **Antimicrobial Assay of *R. montagnei*: c -** *E.coli*, **d-** *Klebsiella pneumoniae*, **e-** *Pseudomonas aeruginosa*, **f -** *Staphylococcus aureus*

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