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Antimicrobial activity of *Coccocarpia erythroxyli* (Spreng.) swinc. & krog

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Abstract
The present study investigates antimicrobial potential of extract of a corticolous foliose macrolichen *Cococarpia erythroxyli* (Spreng.) Swinc. & Krog. Extraction of powdered lichen was carried out by maceration process using methanol. Antibacterial and antifungal activity of lichen extract was determined by Agar well diffusion and Poisoned food technique respectively. Lichen extract displayed concentration dependent inhibitory activity against test bacteria with highest and least inhibitory activity against *Bacillus cereus* and *Escherichia coli* respectively. In case of fungi, highest and least susceptibility to extract was shown by *Curvularia* sp. and *Alternaria* sp. respectively. In suitable formulation, the lichen can be used to treat bacterial infections and to manage seedborne fungi.

Keywords: lichens, *Coccocarpia erythroxyli*, antimicrobial, agar well diffusion, poisoned food technique

Introduction
Lichens are one of the best examples for symbiotic associations. They are ecologically obligate, stable, self-supporting, composite organisms representing a photosynthetic partner (a photobiont which is a cyanobacterium or an alga) and a fungal partner (a mycobiont; an ascomycete or basidiomycete fungus). Lichens are ubiquitous and are known to be one of the life forms that survive in harsh environmental conditions. Lichens comprises of about 18500 species distributed worldwide in various habitats such as deserts, tropical and temperate regions, arctic region, from plains to high mountains on various substrates such as rocks (saxicolous), barks (corticolous), soil (terricolous), dead wood (lignicolous) and leaves (foliicollous). Lichens occur in any one of the three growth forms viz. crustose (closely attached to the substratum), foliose (leaf like; loosely attached to the substratum) and fruticose (shrubs like, hanging or erect growing on substratum). Lichens are used traditionally as flavoring agent, for production of colors, perfumes and alcohols and as medicine to treat several ailments. Lichens are known to produce a range of secondary metabolites which are popularly known as lichen metabolites or lichen substances. These metabolites are unique to lichens, rarely occur in other organisms and most of these metabolites are derived from fungal partner. Researches have shown that extracts and purified compounds of lichens exhibit various bioactivities such as antimicrobial, antioxidant, anthelmintic, insecticidal, cytotoxic, antipyretic, enzyme inhibitory and anti-inflammatory activity [1-10]. The lichen genus *Coccocarpia* (family Coccocarpiaceae) was first recognized and described by Person in 1826. The species of this genus have a prominent cortex and medulla. *Coccocarpia erythroxyli* (Spreng.) Swinc. & Krog is a foliose macrolichen and is pantropical in distribution. It is characterized by lobed foliose thallus and is widely distributed in India. It is reported from different states such as Kerala, Karnataka, Assam, Tamil Nadu and Uttar Pradesh [11-13]. The objective of the present study was to investigate antibacterial and antifungal activity of *C. erythroxyli*.

Materials and Methods
Collection and identification of lichens
The lichen, growing on the bark of *Syzygium* sp., was collected at Hulikal, Shivamogga district, Karnataka during August 2017 and was identified on the basis of morphological characteristics, color tests and the secondary metabolites detected [13, 14, 15]. The features of *C. erythroxyli* observed are shown in Table 1.

Table 1: Features of *C. erythroxyli*

Thallus characteristics	Color tests	Secondary metabolites
Thallus adnate, about 10cm across, lobes 1-4mm wide, upper side grey to brown-black in color with transverse concentric ridges, isidia lacking, lower side black in color, medulla colorless to white, apothecia about 3.5mm in diameter, hairs projecting from lower side, ascospores ellipsoid to fusiform.	K-, P-, C-, KC-	None detected

Extraction of Powdered Lichen Material

Maceration process was employed for extraction of powdered lichen material. The powdered material (5g) was left in 100ml methanol (HiMedia, Mumbai) in a stoppered container. The container was shaken occasionally. After 48 hours, the content was filtered through Whatman filter paper No. 1 and the filtrate was evaporated to dryness [16].

Test Bacteria

A total of 7 bacteria (reference strains) which included four Gram positive bacteria (*Staphylococcus aureus* NCIM 5345, *Staphylococcus epidermidis* NCIM 2493, *Bacillus subtilis* NCIM 2063 and *Bacillus cereus* NCIM 2016) and three Gram negative bacteria (*Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 2200 and *Salmonella typhimurium* NCIM 2501) were used. The pure cultures of test bacteria were maintained on Nutrient agar (HiMedia, Mumbai) slants under refrigeration conditions.

Antibacterial Activity of Lichen Extract

The test bacteria were seeded into sterile Nutrient broth (HiMedia, Mumbai) tubes aseptically and incubated overnight at 37°C in order to obtain broth cultures. Antibacterial activity of lichen extracts (10 and 20mg/ml of dimethyl sulfoxide [DMSO; HiMedia, Mumbai]) was evaluated by Agar well diffusion assay [10]. Chloramphenicol (1mg/ml of sterile distilled water) was used as reference antibiotic (positive control). DMSO was used as negative control. Zones of inhibition formed around the wells were recorded after 24 hours of incubation.

Test Fungi

Fungi viz. *Alternaria* sp., *Fusarium* sp. and *Curvularia* sp., isolated previously from moldy grains of sorghum, were used. The fungal cultures were maintained on Potato dextrose agar (HiMedia, Mumbai) slants under refrigeration conditions.

Antifungal Activity of Lichen Extracts

Poisoned food technique was carried out to investigate antifungal potential of extracts of selected lichens. The test fungi were allowed to grow on control (without extract) and poisoned Potato dextrose agar (0.5mg/ml and 1.0mg/ml of medium) plates for five days at room temperature. Antifungal potential of lichen extracts, assessed in terms of inhibition of mycelial growth of test fungi, was determined using the formula:

Inhibition of mycelial growth (%) = (Dc – Dt / Dc) x 100, where 'Dc' and 'Dt' denotes the colony diameter of test fungi on control and poisoned plates respectively [10].

Statistical Analysis

All experiments were conducted in triplicates. Results are presented as Mean ± S.D (Standard deviation)

Results and Discussion

Antibacterial Activity of Selected Lichens

Interest in natural products with activity against pathogenic bacteria is triggered due to development of resistance in pathogens against commonly used antibiotics. Although antibiotics have revolutionized the modern medicine, however, their use is being threatened by the development of resistance in pathogens. Besides, high cost and certain adverse effects that are associated with the use of antibiotics limit their use. Lichen extracts and their metabolites are known to be effective against a myriad of pathogenic bacteria including drug resistant strains [6, 9, 10, 17, 18, 19]. The result of antibacterial activity of *C. erythroxyli* is shown in Table 2. Both concentrations of the extract exhibited inhibitory activity against all test bacteria. Overall, extract showed marked inhibition of Gram positive bacteria when compared to Gram negative bacteria. *B. cereus* and *P. aeruginosa* were inhibited to higher extent among Gram positive and Gram negative bacteria respectively. Least susceptibility to extract was recorded in case of *E. coli*. Reference antibiotic displayed higher inhibition of test bacteria while DMSO had no effect on test bacteria. The antibacterial effect of extract observed was much lesser than that of reference antibiotic. In a study by Jha *et al* [20], extract of *C. erythroxyli* failed to show inhibition of *S. aureus* and *Klebsiella pneumoniae*.

Table 2: Antibacterial activity of *C. erythroxyli*

Test bacteria	Zone of inhibition in cm (Mean±S.D)			
	Extract 10mg/ml	Extract 20mg/ml	Antibiotic	DMSO
E. coli	1.00±0.10	1.30±0.00	2.20±0.00	0.00±0.00
P. aeruginosa	1.20±0.00	1.50±0.10	2.43±0.05	0.00±0.00
S. typhimurium	1.13±0.05	1.43±0.05	2.10±0.00	0.00±0.00
S. epidermidis	1.43±0.05	1.63±0.05	3.40±0.10	0.00±0.00
S. aureus	1.40±0.10	1.60±0.00	3.10±0.10	0.00±0.00
B. subtilis	1.33±0.05	1.50±0.00	3.40±0.00	0.00±0.00
B. cereus	1.50±0.00	1.80±0.10	3.53±0.05	0.00±0.00

Antifungal Activity of *C. erythroxyli*

Fungi such as *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Alternaria*, *Curvularia*, *Fusarium*, *Drechslera*, *Helminthosporium* and *Pyricularia* are associated with the seeds of many plants. These seed-borne fungi are shown to cause seed abortion, deterioration of seed quality and yield loss. Management of seed-borne fungi using chemicals suffers from certain drawbacks such as residual effect on environment, destruction of non-target organisms and toxicity to humans. The high cost of synthetic chemicals also limits their use by many farmers. Natural products are considered to be an important alternative for these chemical agents. It is shown from the previous studies that lichen extracts exhibits potent antifungal activity against a variety of phytopathogenic fungi including seed-borne fungi [10, 18, 21-25]. In the present study, we evaluated the potential of extract of *C. erythroxyli* to inhibit seed-borne fungi by poisoned food technique. This technique is one among the most widely used antifungal assays which evaluates antifungal effect in terms of inhibition of mycelial growth of fungi in poisoned plates. Table 3 and Figure 1 show the result of antifungal potential of extract of *C. erythroxyli*. Poisoning of medium with the lichen extract resulted in considerable reduction in the mycelial growth of test fungi. The antifungal effect of extract observed was concentration dependent. Susceptibility of fungi to extract was in the order: *Curvularia* sp. > *Fusarium* sp. > *Alternaria* sp. At extract concentration of 1mg/ml, all fungi were

inhibited to >30%. Only *Curvularia* sp. was inhibited to >50% by extract (at 1mg/ml concentration).

Table 3: Colony diameter of test fungi in control and poisoned plates

Extract/control	Colony diameter in cm (Mean±S.D; n=3)		
	***Alternaria* sp.**	***Curvularia* sp.**	***Fusarium* sp.**
Control	4.20±0.10	4.90±0.00	4.60±0.00
Extract 0.5mg/ml	3.63±0.05	3.40±0.00	3.63±0.05
Extract 1.0mg/ml	2.80±0.00	2.10±0.10	3.00±0.00

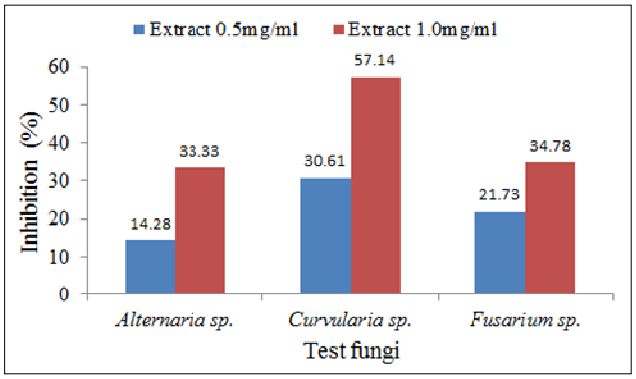

Fig 1: Extent of inhibition of test fungi by *C. erythroxyli*

Conclusions

From the results of the study, it can be concluded that the lichen *C. erythroxyli* exhibit antibacterial activity against Gram positive and Gram negative bacteria and antifungal activity against fungi isolated from sorghum seeds.

Sources of support

None

Conflicts of interest

None declared

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