

ASSESSMENT OF ANTIOXIDANT AND INSECTICIDAL ACTIVITY OF SUBSTANCES ISOLATED FROM LICHENS in Mihintale sanctuary

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Lichens are the symbiotic phenotype of nutritionally specialized fungi (mycobiont) that live as ecologically obligate biotopes in symbiosis with an algal and or cyanobacterial photobiont. Many lichens are economically and ecologically important. They involve in preparing ground for vegetation succession and also used as a raw material in making dyes, perfumes and medicines. Lichen acids are used as primitive antibiotics, antioxidants and also as a pesticide for mosquito larvae^{1,3}.

Lichens produce around 600 secondary metabolites, popularly known as lichen substances. Out of 600 lichen substances, around 550 are unique and not available in any other organisms. Most of these lichen substances are used as an important character in identification of lichens⁴. Some species of lichens are used to detect air quality, as they are very sensitive to air pollutants especially SO₂ and NO₂. Therefore used as bio indicators.

Lichen species were collected from 5 randomly selected sites (25 m × 25 m) from rock surfaces, tree branches and barks, which are 1.5 meter above the ground level. Lichen material were air-dried for few hours and preserved in packets after oven drying at 60°C for six hours. Specimens were then stored in a refrigerator.

20g of powdered lichen material was added to 100 ml of acetone and methanol, separately. It was found that acetone was better than methanol for extracting the lichen compounds in all lichen species. The mixture was shaken for 30 minutes in a shaker at room temperature. The extract was filtered through Whatman No 1 filter paper and the filtrate was concentrated under reduced pressure rotor vapor to a pasty mass. Lichen extracts were stored in a refrigerator until tested.

Chemical substances in lichen extracts were identified using a thin layer chromatography (TLC) and color of the spots under UV light (365 nm)⁴. Acetone, Toluene / acetic acid (170:30), Toluene: Ethyl Acetate: Formic acid (139:83:8) and Dichloromethane / chloroform (1.5:0.5) solvent systems were used to run TLC. Toluene:Acetic acid (170:30) system was found to be ideal solvent system for TLC test of *Rocella montagnei*, *Parmotrema tinctorum* and *Dirinaria confluens*. Therefore, retention factor (R_f) of each lichen substance was calculated in that solvent system. Toluene:ethyl Acetate:formic acid (139:83:8) solvent system was used for TLC test of *R. Minuta*.

Insecticidal activity of each extract was determined against mosquito larvae. Twenty larvae were placed separately, into sterile beakers with 100 ml water. Different concentrations of Acetone extracts of lichen material (0, 2, 4, 6, 8, and 10 mg ml⁻¹) were added to each sterile beaker. The larvicidal effect of each extract was determined by counting the number of dead larvae after 24 hours. The test was repeated twice and percentage larval mortality for each concentration of extract was determined.

In order to check the antioxidant activity, TLC test was carried out for each lichen extract using following solvent system; Hexane:CH₂Cl₂ (50%:50%); CHCl₃ (100%). Developed TLC plates were soaked in 0.4 ppm DPPH (2, 2-diphenyl-1-picrylhydrazyl) in methanol.

The appearance of white color spots on purple background revealed the presence of antioxidant compounds in each extract.

Antimicrobial activity of lichen extracts was determined against *Escherichia coli*. Nutrient agar was taken as a medium to grow bacteria and disks were soaked in acetone extracts of lichen material. The disks were introduced into bacteria inoculated nutrient agar plates. Antimicrobial activity was determined by measuring the diameter of the clear zone around each disk.

The acetone extract of *Dirinaria confluens* showed dose dependent mortality of the mosquito larvae with the highest percentage mortality of 77.5 at 10 mg ml⁻¹ extract, compared with other lichen species. Acetone extracts of *Roccella montagnei*, *D. confluens* and *R. minuta* and methanol extract of *D. confluens* exhibited marked antioxidant activity by decolorizing the purple color of DPPH. Out of the above two extracts, *D. confluens* exhibited higher antioxidant activity than other lichen species especially, the non UV active compounds appeared in TLC plates.

This study revealed significant antioxidant and insecticidal activities of the acetone extracts of *Dirinaria confluens* and *Roccella minuta*. None of the extracts in the concentration range of 0.02 g ml⁻¹ to 0.10 g ml⁻¹ showed any antimicrobial function against *E. coli*.

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