WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 3, 711-720 Research Article ISSN 2278 – 4357

# ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF INDIAN LICHEN (*USNEA SPS.* AND *PARMOTREMA SPS.*) AGAINST HUMAN PATHOGENIC BACTERIAL AND FUNGAL SPS

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Article Received on
27 December 2017,

Revised on 17 Jan. 2018,
Accepted on 07 Feb. 2018,

DOI: 10.20959/wjpps20183-11057

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## ABSTRACT

Lichens are valuable natural resources used for centuries throughout the world as medicine, food, fodder, perfume, spices and dyes, as well as for other miscellaneous purposes. This Study investigates the antimicrobial and antifungal properties of Lichen extract *(Usnea sps. & Parmotrema sps.)* against some human pathogenic bacterial and fungal sps.. In this study the lichen was extracted in acetone and methanol. The bacterial isolates examined in this study were *S. aureus*, *Staphylococcus sps* and *E.coli*. The fungal isolates used in this study were *Aspergillus niger, Aspergillus flavus*, *Candida sps.* And *Trycophyton sps.* In vitro antimicrobial activity was tested initially by Kirby-Bauer technique of disc diffusion method and was confirmed by minimum inhibitory concentration using Broth microdilution method. The methanol extract inhibited growth of *staphylococcus sps*. with a zone of 0.3 cm (0.2mg/mL conc.) while acetone extract of the lichen had the greatest effect on plates inoculated with *S. aureus* with a zone of inhibition of 1.4 cm at 0.2 mg/mL concentration. In the Antifungal activities of *Parmotrema sps*, *Candida sps* was showed maximum Antifungal Activity (0.9 cm) and *Trycophyton sps* was showed minimum Antifungal activity (0.6 cm). The present study demonstrates the relatively higher activity of this lichen against bacterial and fungal sps. This indicates that this lichen might be a rich source of effective antimicrobial agents.

**KEYWORDS:** Lichen extract, Antibacterial activity, MIC, Antifungal activity**.**

## INTRODUCTION

Lichen metabolites spur diverse biological activities, such as antimicrobial, antitumor, antimutagenic, antiherbivore, and allergenic.[1] The biological activity of an extract can be determined by the presence of phenolic compounds such as flavonoids, phenolic acids, and total phenols.[2,3] The development of multidrug resistance in pathogenic bacteria is a serious problem in current clinical chemistry, as it occurs because of the excessive use of existing antibacterial drugs.[4] 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.[5] Lichens provide warning signal before severe damages occur on ecosystem and health.[6]

Antioxidant compounds can be derived from many sources, using both natural and synthetic methods. Synthetic antioxidant compounds exert adverse effects on human and animal cells.[7] Natural antioxidant compounds are highly useful in medicinal chemistry and exert no negative effect on naturally occurring cells.[8]

Lichen metabolites exert a wide variety of biological actions including antibiotic, antimycotic, antiviral, Anti-inflammatory, analgesic, antipyretic, antiproliferative and cytotoxic effects.[9] Lichens are valuable plant resources and are used as medicines, food, fodder, dyes perfume, spice, and for miscellaneous purposes. The lichen flora is rather poor in the vicinity of industrial areas and big cities, as lichens are very sensitive to various air pollutions. Thus, these organisms are used as air pollution monitors.

Lichen metabolites exert a wide variety of biological actions including antibiotic, antimycotic, antiviral, anti-inflammatory, analgesic and antipyretic, anti-proliferative and cytotoxic effects.[10,11,12]

A large number of lichen species have been proven to be a source of these metabolites for food and pharmaceutical industries. Secondary lichen metabolites show a wide range of potentially useful biological activities.[13,14,15] Most lichen substances with antibiotic activity are phenolic metabolites (e.g. usnic acid and the anthraquinone endocrocin).[16]

Lichens are basically fungi that construct self-sustainable composite thallium symbiotically in association with alga and or cyanobacteria. They are included in the fungal Kingdom, but have members of other two kingdoms; Bacteria.[17]

The first study of the antibiotic properties of lichens was carried out by antibacterial activity for several lichens and other researchers have since then studied the antibacterial activity several lichens against gram positive and gram negative bacteria.[18,19] Various workers reported antifungal activity as well as antipyretic properties of lichen.[20,21,22]

Lichens have been used in traditional medicine in ancient times. The secondary metabolites synthesized by the lichens can act as an antibiotic, pesticide, antiherbivore, insecticide, antioxidant, cytotoxic agent.[23] The lichen secondary metabolites called otherwise known as lichen acids, well known for their biological and bio-medicinal values. Lichens are also can be used as bio-indicator, as they are very sensitive to air pollution. They are also used as dyes. The present investigation was undertaken to study the antimicrobial and anti-inflammatory efficacy of usnic acid and its derivative usnic acid diacetate.[24]

## MATERIAL AND METHOD

### Isolation of Bacterial and Fungal sps

The fungal isolates used in this study were obtained on Sabouraud's dextrose agar and nutrient agar media used for isolation of *Bacterial sps.* from soil sample. The morphological and biochemical characteristics of the isolates were examined according to the Bergey's manual of determinative Bacteriology.[25]

### Lichen Material

For the present study two species of Indian Lichen i.e. *Parmotrema sps* and *Usnea sps*. Sample was collected from two regions: *Parmotrema sps*. was collected from Manipur and *Usnea sps* was collected from Indira Nagar, Lucknow.

### Extraction of Lichen Material

The lichen samples were washed to remove debris; the Lichen was dried, pulverized to powder, and stored in a sterile glass bottle in the refrigerator. 10 g portions of sieved powder was added to 100mL of solvents (acetone, ethanol) and left for three days at room temperature. The crude extract was prepared by decanting, followed by filtration through muslin cloth, and further filtered with Whatman No. 1 filter paper to obtain a clear filtrate. The filtrates were further purified by membrane filter using 0.45 $\mu$m pore size filters **[26]**. The extracts were then evaporated to dryness under reduced pressure and redissolved in respective solvents to attain the required concentrations of 0.1mg/mL and 0.2mg/mL for antibacterial screening. These extracts were kept at 4$^{o}$C till used.

**Antibacterial Assay of different Lichen extracts**

Antimicrobial susceptibility test of the selected pathogens was done by well diffusion method using Kirby-Baeur technique.[26] All the tests were performed on Mueller Hinton agar plates. Suspension of microbial cultures was inoculated on the entire surface of the Mueller Hinton agar media in a Petri plate using sterile swab sticks. Inoculated plates were incubated at 37∘C for 24 hrs. On the second day, plates were read by taking measurement of zone of inhibition around each disc. The diameter of zone of inhibition of bacteria was recorded in millimeters. Pure acetone, methanol were taken as negative control. The assay was done in triplicates and checked with the control plate. To determine the affectivity of lichen crude extracts at different volumes, two different concentrations of lichen crude extracts were taken on well diffusion method, on every Petri plate.[27,28]

**Minimum Inhibitory Concentration (MIC) Test**

Minimum inhibitory concentration was determined according to the method described earlier by adding various concentrations of essential oil (6.25–100 µg) in Sabouraud dextrose broth medium and Nutrient Broth. Further, 100 µl of inoculum was added to each tube and incubated the tubes at 28°C for 7 days. The MIC was regarded as the lowest concentration of the oil that did not permit any visible growth after 7 days of incubation.[29,30]

**Antifungal Activity of Lichen Extracts**

Antifungal activity of solvents extract of all the Lichen extracts was determined by agar well diffusion method on SDA medium.[31,32] Antifungal activities of Lichen Species were observed against fungal isolates *Aspergillus niger, Aspergillus flavus, Canida sps, Trycophyton sps.*

**RESULT AND DISCUSSION**

**Isolation of Bacterial and Fungal sps.**

The bacterial isolates examined in this study, which were obtained on Nutrient Agar Media (NAM) were *S. aureus, Staphylococcus sps* and *E.coli*. The fungal isolates used in this study were obtained on Sabouraud's dextrose agar (SDA) are *Aspergillus niger, Aspergillus flavus, Candida sps.* and *Trycophyton sps.*

**Antibacterial Assay of different Lichen extracts**

Antibacterial Activity of *Parmotrema sps.* and *Usnea sps.* against human pathogenic bacterial isolates *Staphylococcus sps., Staphylococcus aureus* and other *E. coli as shown in* ***Figure.1*** .

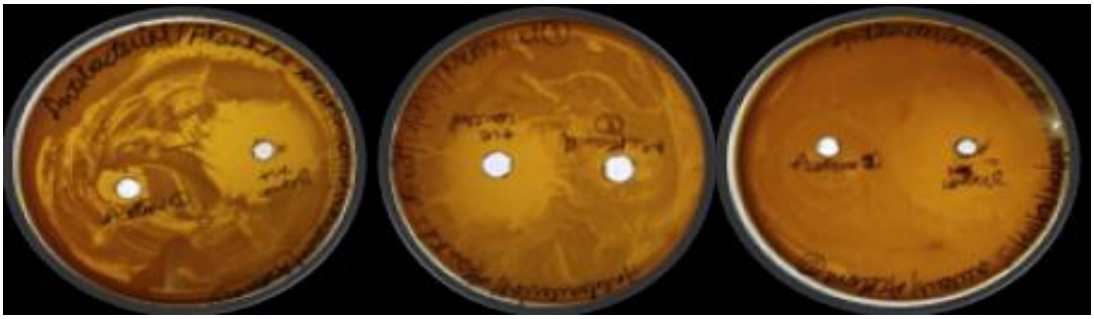
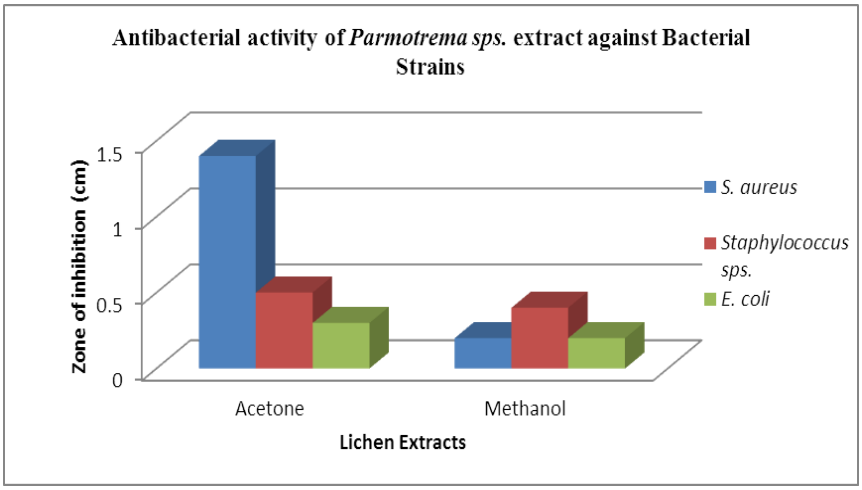


**Figure 1: Antibacterial Assay of different Lichen Extracts.**

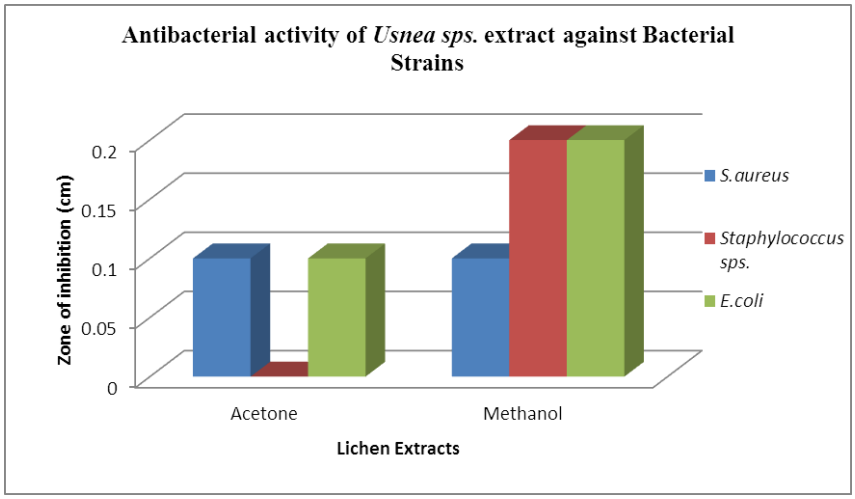
After the treatment had been applied and the inoculated plates were allowed to grow for 24 hours, the acetone extract and ethanol extract of *Parmotrema sps.* were showing activity against *S. aureus*, *Staphulococus sps*., and *E. coli*. Both concentrations of methanol extract (0.1mg/mL and 0.2mg/mL) were showing activity against all the gram positive bacteria and gram-negative bacteria. The methanol extract inhibited growth of *staphylococcus sps*. with a zone of 0.3 cm (0.2mg/mL conc.).

While Acetone extract of the lichen had the maximum effect on plates inoculated with *S. aureus* with a zone of inhibition of 1.4 cm at 0.2 mg/mL concentration. The acetone and methanol extract were showing equal inhibitory effect on *E. coli* with a zone of inhibition 0.3 cm and 0.2 cm at 0.2 mg/mL concentration, respectively as shown in Graph 1.

The methanol extract of *Usnea sps*. showed poor activity against *Staphylococcus sps.* with a no zone of inhibition at a concentration of 0.1mg/mL . Ethanol extract showed maximum effect on *Staphylococcus sps*. with a zone of inhibition of 0.2 cm at a concentration of 0.2mg/mL (as shown in Graph 2).



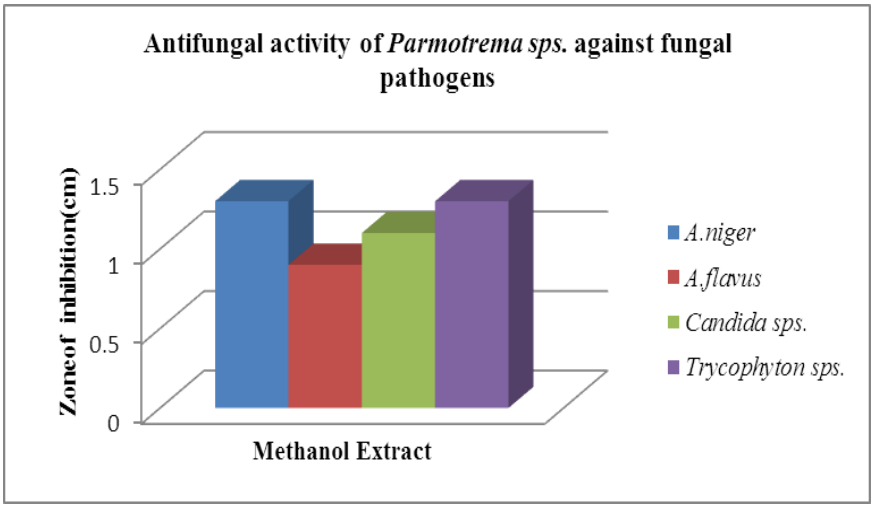

**Graph 1: Antibacterial activity of Parmotrema sps. Extract against Bacterial Strains.**




**Graph 2: Antibacterial activity of Usnea sps. extract against Bacterial Strains.**

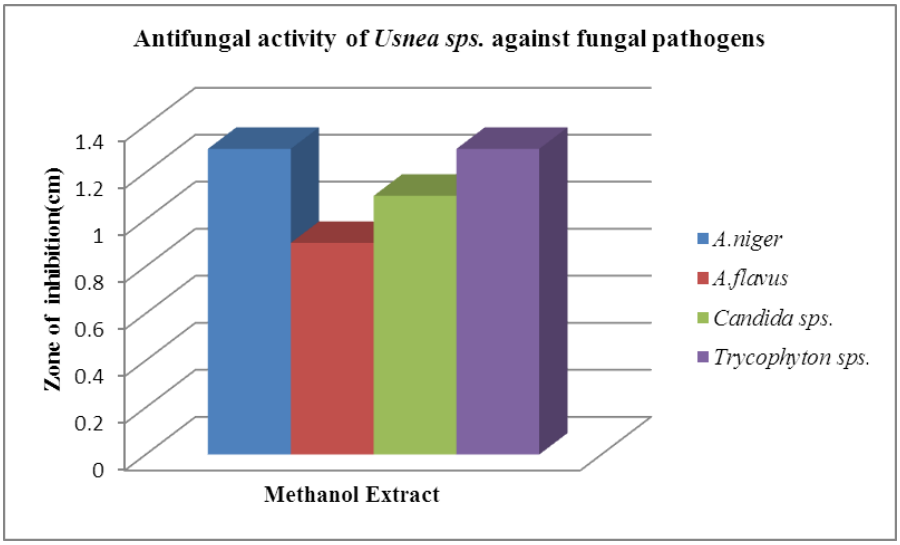
### Antifungal Activity of Lichen Extracts

Antifungal activities of *Parmotrema sps.* were observed against fungal isolates *Aspergillus niger, Aspergillus flavus, Candida sps, Trycophyton sps* as shown in Figure 2. The *Candida sps.* was showed maximum Antifungal Activity (0.9 cm) and *Trycophyton sps* was showed minimum Antifungal activity (0.6 cm) as shown in Graph 4.




**Graph 4: Antifungal activity of Usnea sps. against fungal pathogens.**

The extracts of *Usnea sps.* showed antifungal activity against fungal sps. The methanol extracts of *Usnea sps.* showed higher antifungal activity against *Aspergillus flavus* with inhibition zone diameters of 1.1 cm/15µL. The methanol extracts of *Usnea sps*. showed the Minnimum antifungal activity against *Candida sps.* with inhibition zone diameters of 0.4 mm/15µL(as shown in Graph 5).




**Graph 5: Antifungal activity of Usnea sps. against fungal pathogens**

**Minimum Inhibitory Concentration (MIC) Test**

The MIC values of the extract related to the tested bacterial strains varied between 25 and 3.125 µg/mL in case of gram-positive bacteria. The MIC value against E. coli was found to be 2.25 µg/mL. *Streptococcus sps.* was also showing 25 µg/mL MIC value while the measured MIC value for the extract against *Staphylococcus aureus* was 6.15 µg/mL which showed similarity with Srivastava *et al* .2013[33] work in which MIC values of the extract against *Staphylococcus aureus* was 6.25 µg/Ml.

## CONCLUSION

In this study, two different species of Indian Lichen (*Parmotrema sps.*and *Usnea sps.*) were used for determination of antibacterial activity against Human pathogenic bacteria (*Staphylococcus sps, S. aureus and E.coli)* and determination of antifungal activity of these lichen species against fungal pathogens *(A. flavus, A. niger, Candida sps., Trycophyton sps.)* The acetone and methanol extracts of *Parmotrema sps.* had a potential towards antibacterial activity more as compared to *Usnea sps.* Methanol extracts of *Parmotrema sps* shows better effect against *Candida sps, A.flavus, Tricophyton sps.* fungal pathogens showing minimum concentration of Inhibition(MIC). The tested lichen extracts showed significant antimicrobial activity relative to the tested bacteria, which could have of significance in human therapy, animal and plant diseases.

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