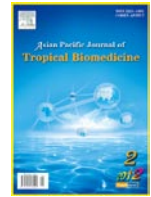




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# Antibacterial activity of *Parmelia perlata*

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### ABSTRACT

**Objective:** To test efficacy of *Parmelia perlata* (*P. perlata*), which is used in traditional medicine for rapid wound healing against test bacteria that cause wound infections. **Methods:** Different solvents such as methanol, ethyl acetate and acetone were used for extraction of *P. perlata*. The sensitivity of the test bacteria to solvent extracts of *P. perlata* was tested by measuring the zone of inhibition on growth media and by determining the minimal inhibitory concentration and minimal bactericidal concentration. **Results:** Methanol, ethyl acetate and acetone extracts of *P. perlata* have shown inhibitory activity against *Staphylococcus aureus* (*S. aureus*). **Conclusions:** The results of the present study indicate that *P. perlata* has potential antibacterial compounds against *S. aureus* that causes multitude of skin infections among human beings. Development of drugs from natural compounds can help us to combat antibiotic-resistant bacteria.

## 1. Introduction

Treatment of bacterial infections uses a wide variety of antibiotics. However, multiple drug resistance has developed in pathogens due to excessive use of existing antimicrobial drugs. Therefore, much attention is on folk medicine to develop better drugs against drug resistant pathogens[1,2]. Natural products, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drugs because of the unmatched availability of chemical diversity. Further, these products do not cause side effects and are less toxic. Lichens are used since ancient times as one of the natural drug[3].

Lichens represent a symbiotic association of a fungus with an algal partner and are important constituents of ecosystem. Lichens produce characteristic secondary metabolites such as aliphatic, aromatic, and terpenic components which have considerable biological activities

such as antiviral, antibacterial, antifungal, antitumour, antioxidant etc.[4, 5]. Until now, about 700 biologically active components were structurally identified from lichens[6]. The lichen chosen for this study was *Parmelia perlata* (*P. perlata*), which is commonly called as stone flower. *Parmelia perlata* is usually used as a spice to enhance the taste and flavor of the foods. It is also useful to treat sores, boils, inflammations, seminal weakness, and amenorrhoea and it contains compounds like tridecyl myristate, 3-ketooleanane, icosan-1-ol, usnic acid etc. [7]. It is important to note that at present, various herbal preparations to treat seminal weakness and skin creams for wound healing contain *P. perlata* as major component.

The objective of the study was to test efficacy of *P. perlata*, which is used in traditional medicine for rapid wound healing against test bacteria that cause wound infections. The bacteria selected for this study include *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. Different solvents such as methanol, ethyl acetate and acetone were used for extraction of *P. perlata*. The sensitivity of the test bacteria to solvent extracts of *P. perlata* was tested by measuring the zone of inhibition on growth media and by determining the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

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## 2. Materials and methods

The dried lichen thalli of *P. perlata* were powdered using mixer grinder. Ten grams of this powder was ground with mortar and pestle using 100 mL of respective solvents. The extracts were filtered and centrifuged at 8000 r/min for 10 min at 4 °C. The supernatants were collected and dried by evaporation at room temperature. Then the dried filtrates were re-dissolved in the solvents to obtain the concentration of 1 mg/mL, 2 mg/mL and 5 mg/mL.

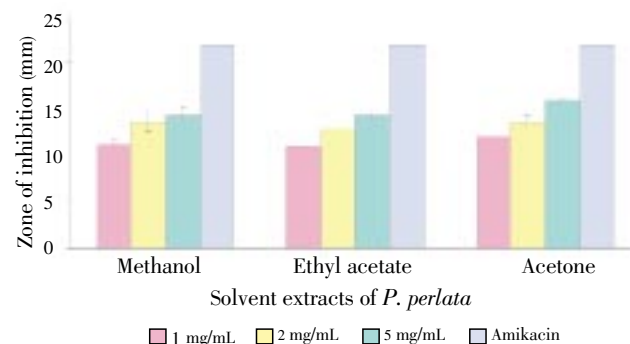
The antibacterial activity of the *P. perlata* extracts against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis* was determined by Kirby and Bauer disc diffusion method. Mueller–Hinton agar plates inoculated with 1 mL of bacterial suspension of  $1 \times 10^6$  CFU/mL according to 0.5 Mc Farland standards was used for the antibacterial assay. Sterile filter paper discs of 6 mm dia (Himedia Laboratories) were impregnated with 50  $\mu$  L of crude extracts of lichen of varying concentrations such as 1 mg/mL, 2 mg/mL and 5 mg/mL and after complete evaporation were placed on the surface of the inoculated agar plates. These plates were incubated at 37 °C for 48 h. At the end of the incubation period, the antimicrobial activities were evaluated by measuring the zone of inhibition. In this assay, negative control was the respective pure solvent and the positive control was Amikacin (Himedia). All the tests were performed in triplicates.

The minimum inhibitory concentration is defined as the lowest concentration able to inhibit any visible bacterial growth[8]. To measure the MIC values, various concentrations of the stock such as 256, 128, 64, 32, 16, 8, 4, 2, 1  $\mu$ g/mL were prepared using Mueller Hinton Broth and assayed against the test bacterium. About 0.2 mL of 24 h old *Staphylococcus aureus* equivalent to 0.5 Mc Farland standards was added to 40 mL of sterile nutrient broth and was used as the inoculum. To 1 mL of various concentration of the extract, 1 mL of inoculum was added and the test tubes were incubated at 37 °C for 24 h. Three control tubes were maintained for each test batch. These include tube containing extract and nutrient broth devoid of inoculum; tube containing the growth medium and inoculum and tube containing medium alone. The MIC of each sample was determined by measuring the optical density at 620 nm using spectrophotometer and by comparing the result with those of the non-inoculated nutrient broth.

The MBC was determined by streaking the test dilution used for minimum inhibitory concentration on Mueller Hinton Agar plates. These plates were incubated for 24 h at 37 °C. The highest dilution that yielded no single bacterial colony was taken as the minimum bactericidal concentration[9].

## 3. Results

Methanol, ethyl acetate and acetone extracts of *P. perlata* have shown inhibitory activity against *Staphylococcus aureus* only. Among the different concentrations tested 5 mg/mL displayed maximum inhibition against *Staphylococcus aureus* for all the three extracts and the diameter of zone of inhibition was 16 mm for acetone extract, 15 mm for ethyl acetate extract and 14 mm for methanol extract (Figure 1).



**Figure 1.** Antibacterial activity of *P. perlata* extracts on *Staphylococcus aureus*.

The minimum inhibitory concentration of *P. perlata* against *S. aureus* was 64  $\mu$ g/mL for methanol and acetone extracts and was 128  $\mu$ g/mL for ethyl acetate extract (Table 1). The minimum bactericidal concentration of *P. perlata* was 256  $\mu$ g/mL for all the solvent extracts tested against the bacterium *Staphylococcus aureus*.

**Table 1.**

Minimum Inhibitory Concentration of extracts of *P. perlata* on the growth of *Staphylococcus aureus*.

Dilutions of the lichen extract	Concentration of lichen extract ( $\mu$ g/mL)	Effect of extracts of <i>P. perlata</i> on growth of <i>Staphylococcus aureus</i>		
		Methanol extract	Ethyl acetate extract	Acetone extract
1	256	–	–	–
2	128	–	–	–
3	64	–	+	–
4	32	+	++	+
5	16	++	++	++
6	8	++	++	++
7	4	++	++	++
8	2	+++	+++	+++
9	1	+++	+++	+++
Medium and extract	512	–	–	–
Medium and inoculum	0	+++	+++	+++
Medium alone	0	–	–	–

–: indicates absence of bacterial growth; +: indicates growth of bacteria.

## 4. Discussion

*Parmelia* is mentioned in Indian Materia Medica as useful

in treating number of ailments and is also used as a food supplement in India. *P. perlata* has potent antimicrobial activity against different bacteria namely *Clavibacter michiganensis*, *Pseudomonas solanacearum* and fungi like *Fusarium oxysporum* and *Rhizopus nigricans*[10]. In the present study the lichen *Parmelia perlata* was used to check the antimicrobial property against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis* that are capable of causing wound infections. Methanol, acetone and ethyl acetate extracts of *P. perlata* showed activity against *Staphylococcus aureus* only.

Antimicrobial activities of medicinal plants differ with different extraction system[11]. Methanol, acetone and ethyl acetate extracts of *Parmelia praesordiosum* and *Rocella belangeriana* showed antibacterial activity against *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Staphylococcus aureus*[12]. Ethanolic extract of *P. perlata* is reported to be active against *Staphylococcus aureus* and the efficiency of the extract increased in the presence of colloidal silver[13]. The solvent system used in this study for extraction of *P. perlata* has not been tried before for antibacterial activity against the test bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. Further research needs to be done to determine the compounds that are responsible for antibacterial activity against *Staphylococcus aureus*. The findings also indicate that scientific studies carried out on medicinal plants having traditional claims of effectiveness[14–18] can give fruitful results.

### Conflict of interest statement

We declare that we have no conflict of interest.

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