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EVALUATION THE ANTIMICROBIAL ACTIVITY OF FOUR FRACTIONS OF SUDANESE *PARMELIA PERLATA* LICHEN

***Dr. Asma Abdullah Mohamed Hassabo**

*Alsheik Albedri University, Faculty of Health Sciences, Department of Pharmacy, Berber, Sudan.

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***Corresponding Author**
Dr. Asma Abdullah Mohamed Hassabo
Alsheik Albedri University, Faculty of Health Sciences, Department of Pharmacy, Berber, Sudan.

ABSTRACT

Parmelia perlata (Parmeliaceae) is a lichen (i.e. symbiotic combination of algae and fungi), which is traditionally used for its medicinal properties. The present study was focused to investigate and validate the traditional uses of Sudanese *Parmelia perlata* (Shaibah) as antimicrobial and qualitatively analyzed its phytoconstituents. Methanol extract was obtained by maceration, then fractionated to: petroleum ether, ethyl acetate and methanol fractions respectively. The cup-plate agar diffusion method was adopted to assess the antimicrobial activity of the fractions against four strains of bacteria (*Staphylococcus aurous*, *Bacillus subtilus*, *Escherichia coli*, *Pseudomonas aeruginosa*) and one fungal strain(*Candida albicans*). All fractions showed good antimicrobial activity against all test strains. The phytochemical screening results was shown the presence of flavonoids and terpenoids which may be responsiple of antimicrobial activity. The obtained results was supported the traditional use of this lichen and Sudanese Parmelia perlata can be beneficial in the solving of drugs resistant pathogens.

KEYWORDS: Antimicrobial – lichens - parmelia perlata – fractions.

INTRODUCTION

Ever since the birth of mankind human being have been dependent on the plants to fulfill their basic needs of life and even for the maintenance and restoration of health.[1] In the last century, synthetic chemistry have offered alternatives to natural sources, thus had a negative impact on the study of natural products. Nevertheless the past few decades have witnessed a

renewed interest in this field.[2] This can be easily understood in the light of questions concerning the safety and side effect of synthetic compounds.[3]

Worldwide infection diseases remain some of the major causes of human mortality and morbidity, even after the arrival of modern antimicrobial chemotherapy.[4] The resistance of various pathogens become increasing, therefore much attention is on folk medicine to develop new drugs to solve the problem of resistance.[5]

Lichens are represent a symbiotic association of fungus with an algal partner. Thus, they develop with a unique morphological form, separate chemical and physiological properties.[6] Lichens have been used by human for centuries as food, dye and natural drugs as a source of biologically active compounds.[7] Antibiotic properties of the lichens are of special interest to the scientists According to one estimate, 50% of all lichens have antibiotic properties.[8]

Parmelia perlata (Huds). Ach. (*Parmeliaceae*) is a lichen with common name (Charilla - stone flower), grown in old trees and walls. It is a thallus, foloaceous, membranous, leaf like horizontally spreading lobes. The thallus is dirty white or grayish brown with bitter or saline taste.[9] *Parmelia perlata* is traditionally used as diuretic, liniment for headache, powder to help wounds heal, for Tinea like disease, treatment of toothache and sore throat.[8] It also used as light brown dye for wool, bio-indicator of air pollution of heavy metals.[6]

In Sudan *Parmelia perlata* called (shaibah, shaibat elagouz) and use traditionally to treat wound healing and for respiratory tract infection. The aim of this study is to explore the antimicrobial effect of four fractions of this lichen against bacteria and fungi and to qualitatively analyzed it for determine their phytoconstituents.

MATERIAL AND METHOD

Chemicals, solvents, glassware, standard microorganisms were obtained according to standard requirement.

Plant material

The lichen material was collected from the Airquait mountain, Eastern Sudan and dried under shade. The botanical identification was made and authenticated by Dr. Yahiya Suleiman at the herbarium of Medicinal and Aromatic plants & Traditional Medicine Research Institute, National Center For research, Khartoum, Sudan and voucher specimens were deposited there for further reference.

Test microorganisms

Bacterial microorganisms

Bacillus subtilis NCTC 823

Staphylococcus aureus ATCC25923

Escherichia coli ATCC 25922

Pseudomonas aeruginosa ATCC 27853

National Collection of Type Culture (NCTC), Colindale, England .

American Type Culture Collection (ATCC) Rockville, Maryland, USA .

Fungal microorganisms

Candida albicans ATCC 7596

Plant extraction

Hundred gm of the dried *parmelia perlata* was extracted by cold maceration (as it prepared traditionally), with 1000ml methanol (95%) for 48h at room temperature, with shaking. The extract was filtered, evaporated of methanol by rotary evaporator, concentrated, air dried and undergo fractioning to obtain petroleum ether, ethyl acetate and methanol fractions respectively. Each fraction was dissolved in methanol and conducted the antimicrobial test.

Phytochemical screening

Qualitative Phytochemical analysis of crude methanol extract of the screened lichen was done for the presence or absence of phytoconstituents. Dried crude extract was reconstituted in methanol and subjected to standard phytochemical analysis following the procedures which described by.[10]

Antimicrobial test

Preparation of Test Samples

In the study of the antimicrobial activities of *Parmelia perlata* lichen, concentrations of (10% wt/v) of each fraction were used for the screening.

Preparation of the test organisms

Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37ºC for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline and finally suspended in 100ml of

normal saline to produce a suspension containing about (108- 109 C.F.U/ m)l. The suspension was stored in the refrigerator at 4°C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes (one drop) of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates.

The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.[11]

Preparation of fungal suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100ml of sterile normal saline and the suspension were stored in the refrigerator until used.

In vitro testing of extracts for antimicrobial activity

Testing for antibacterial Activity

The cup-plate agar diffusion method[12] was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts.

0.2ml of the standardized bacterial stock suspension (108 –109 C.F.U/ml) were thoroughly mixed with 20 ml of molten sterile nutrient agar which was maintained at 45ºC. the inoculated nutrient agar were distributed into sterile Petri-dishes.

The agars was left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed.

Alternate cups were filled with 0.1 ml sample of each of the extract of parmelia perlata dilutions in methanol using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours.

Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.[12]

Testing for antifungal activity

The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25ºC for two days for the Candida albicans.[12]

RESULTS

Preliminary phytochemical screening

The secondary metabolites commonly present in the tested lichen are shown in table (1).

Table (1): Phytochemical groups in methanol crude extract

Phytochemical group	Name of test	Reagents	Results
Alkaloids	Test of alkaloids	Mayer's	(+)
		Hager's	(+)
		Wagner's	(+)
		Dragendroff's	(+)
Carbohydrates	Molisch's test	Alpha naphthole	(+)
Glycosides	Modified Borntrager's test	Ferric chloride , benzene, ammonia solution	(+)
Phenols	Ferric chloride test	Ferric chloride test	(+)
Phytosterols	Salkowisk's test	Chloroform , conc. sulphuric acid	(+)
Flavonoids	a-Alkaline reagent test b-Lead acetate test	a-Sodium hydroxide b- Lead acetate test	(+) (+)
Amino acids	Ninhydrin test	Ninhydrin reagent	(+)
Diterpens	Copper acetate test	Copper acetate	(+)
Tannin		Ferric chloride	(+)
Saponin: Forth test			(-)

(+): presence of phytochemical group, (-): absence of phytochemical group.

Antimicrobial activity results

The current investigation showed that all *parmelia perlata* fractions possesses good antimicrobial activity against tested organisms. The methanol crude extract was shown activity(30.5mm) relatively equal to that of Gentamycine (30mm) against *Staphylococcus aureus* and (27 mm) against *Pseudomonas aeruginosa*, while (21mm) for Gentamycine. petroleum ether fraction was shown activity(35 mm) compare with that of Gentamycine (18) against *Bacillus subtilus* and also (33mm) against *Candida albicans* compared with that of ketoconazol drug (21mm). Ethyl acetate fraction was shown activity against *Escherichia coli* (33mm) compared with Gentamycine (25mm).

The experimental results of the antimicrobial activities are presented in Tables (2).

Table (2): Zone of diameter inhibition (mm) of samples: (*Parmelia perlata* fractions at concentration 10%), against four bacterial and one fungal strain:

Plant fractions	MDIZ (mm)				
	Bacteria				Fungi
	B.s	***S.a***	***P.s***	***E.c***	***C.a***
Pet . ether	35	25.5	21.5	23	33
Ethyl acetate	34	27.5	25	33	23.5
Methanol	27	23.5	24.5	27	26.2
Crude	29.5	30.5	27	26	27
Control					
Gentamycin40mg/ml	18	30	21	25	-
Ketoconazol 40mg/ml	-	-	-	-	21

(MDIZ: Mean diameter inhibition zones - Conc: concentration - *B.s*: *Bacillus subtilis* - *S.a*: *Staphylococcus aureus* - *E.c: Escherichia coli* - *Ps: Pseudomonas aeruginosa* - *Ca:candida albicans*).

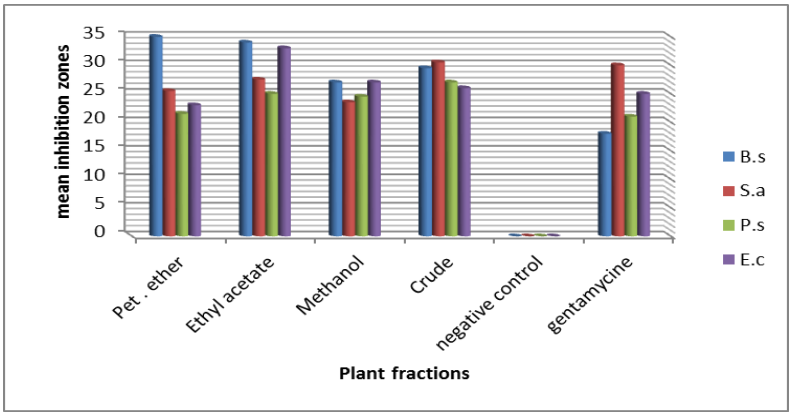

Figure (1): Antibacterial activity of fractions and standard antibacterial drug:

(*B.s: Bacillus subtilis - S.a: Staphylococcus aureus –E.c: Escherichia coli, Ps: Pseudomonas aeruginosa*).

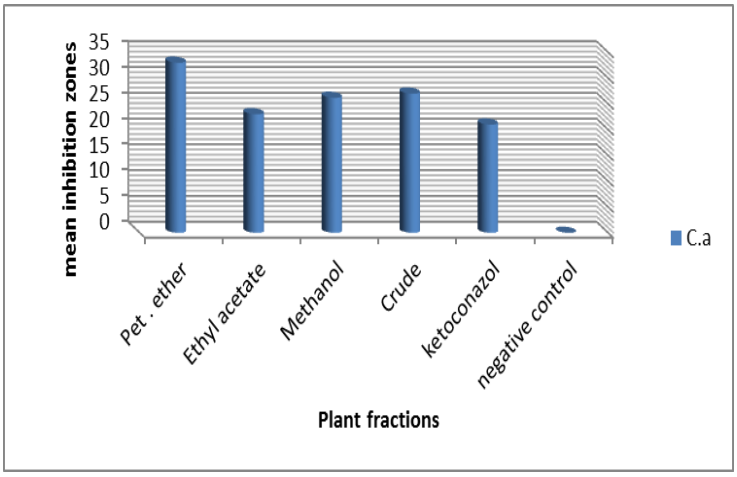

Figure (2): Antifungal activity of fractions and standard antifungal drug:

Ca: Candida albicans.

DISCUSSION

The lichen *Parmelia perlata* was mainly used for culinary and other purposes by people for long period, but many of its traditional uses are needed to be scientifically explore.[13] So the present study is mainly focus to evaluate and validate the pharmacological use of *Parmelia perlata* as antimicrobial agent against various pathogens. The overall data presented indicates that all fractions under the test of Sudanese *Parmelia perlata* have good antimicrobial activity against *Bacillus subtilis*, *E.coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. The agar cup plate method was used in this study to assess the antimicrobial activity because it have advantages to being reproducible, accurate comparison between compounds can be made and it is suitable for evaluation of antibacterial and antifungal activities of variety of compounds.[14]

(Hussain M. *etal*. 2014). reported that in vitro antimicrobial test of Parmelia perlata showed good antimicrobial activity against *Pseudomonas aeruginosa* (21), *E.coli* (21.75) and *Staphylococcus aureus* (20.25). In addition, (Thippeswamy B. *etal*. 2012). found that the antibacterial activity of *Parmelia perlata* crude extract was less in *E.coli*. In the present study methanol crude extract was shown high activity (30.5mm) relatively equal to that of Gentamycine (30mm) against *Staphylococcus aureus* and (27mm) against *Pseudomonas aeruginosa* while (21mm) for Gentamycine activity. petroleum ether fraction was shown activity(35 mm) higher than that of Gentamycine (18) against *Bacillus subtilus* and also more activity (33mm) against *Candida albicans* compared with that of ketoconazol drug

(21mm). Ethyl acetate fraction was shown high activity against *Escherichia coli*(33mm) compared with Gentamycine (25mm).

The antibacterial effectiveness of medicinal plants varies dramatically depending on the phytochemical characteristics of plant, which vary due to differences in the methods of extractions or the environment where the plant was grown. So the variation of the results of this study from the previous studies may be due to this reasons.

(Esimone *etal*. 2007) and (Momoh. *etal*. 2008) mentioned that the result of phytochemical analysis show that the lichen *Parmelia perlata* contain saponins and does not contain alkaloids, however in this study the phytochemical analysis of the crude extract show the presence of: Alkaloids, Carbohydrates, Glycosides, Phenols, Phytosterols, Flavonoids, Amino acids, Diterpens, Tannins. But it also show the absence of saponins. This result have been observed to be consistent with findings in several lichens species(1), but the differences between three studies may be related to the variation of geographical sources of lichen. The phytochemical polyphenols and terpenoids have long recognized to posses potent antimicrobial activities[16], so their presence in this lichen may responsiple of its antimicrobial effect.

The results of present study supports the traditional usage of the studied lichen as antimicrobial agent in the treatment of respiratory tract infections, wound infections (in which *Staphylococcus aureus* represent causative agent). Also *pseudomonas aeruginosa* exhibit multidrug resistance for antibiotics and the result of this study was shown high antibacterial activity toward this pathogen so this lichen can be useful to overcome the problem of multidrug resistance bacteria. Further research should be needed to determine the compounds that are responsible for antimicrobial l activity.

CONCLUSION

Microbes used in this research study were causative agents of infectious disease, diarrhea, skin infections, abscess, respiratory infections, while *Parmelia perlata* inhibit the growth of these microbes, so it can be used for the treatment of infections which caused by them. It would be advantageous to benefit from high activity of *Parmelia perlata* in the solving of multidrug resistant pathogens.

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