Research Article GU J Sci 31(3): 687-698 (2018)

Gazi University

Journal of Science

http://dergipark.gov.tr/gujs

Comparative Antimicrobial Activity of Crude Extracts of *Protoparmeliopsis muralis* and *Parmotrema perlatum* Lichens

Sinem AYDIN[1,*], Kadir KINALIOGLU[2]

[1] *Giresun University, Department of Biology, Faculty of Arts and Science, 28100, Giresun, Turkey*

[2]*Giresun University, Department of Biology, Faculty of Arts and Science, 28100, Giresun, Turkey*

Article Info

Received: 20/09/2017
Accepted: 16/04/2018

Keywords

Parmotrema perlatum
Protoparmeliopsis muralis
Lichen
Antimicrobial activity

Abstract

The inhibitory action of the extracts of *Protoparmeliopsis muralis* and *Parmotrema perlatum* lichens was evaluated by disc diffusion and macrobroth dilution methods. The obtained results revealed that extracts generally showed antimicrobial activity except for water extracts. *P. muralis* lichen possess higher antimicrobial activity when compared with *P. perlatum* lichen. Minimum Inhibition Concentration (MIC) values of fungi is comparatively lower than MIC values of bacteria. Therefore, this work confirms the inhibitory effects of *P. muralis* and *P. perlatum* lichens as natural antimicrobials and suggests the possibility of utilizing them in pharmaceutical industry for curing of infectious diseases caused by test microorganisms.

1. INTRODUCTION

Numerous medicinal plant species is utilized to extract as raw medicines and they have many medicinal features. Medicinal plants are thougt as significant source of brand synthetic substances which possess beneficial therapeutic actions. Traditional medicine have important roles in the contemporary primary healthcare system of the advanced countries. The natural medicines are more admissible to the human body, when compare to modern synthetic medicines [1].

The density of infections induced by pathogenic microorganisms has accelerated worldwide and give rise to morbidity and mortality especially in immune compromised patients. Pathogenic microorganisms have gained resistance against currently used antibiotics. Hence, it needs to explore an alternative, more efficient, and safer antimicrobial substances. Plant species still use as valuable resource to fight substantial diseases in the world [2].

Lichens are symbiotic associations between a algae, a fungi or cyanobacterium. Lichens possess significant biological action like antimicrobial, antioxidant, antiviral, enzyme inhibitory, analgesic, antipyretic, antiinflammatuar, antitumour and anti herbicide. In addition, lichens utilized as traditional remedies in many parts of world [3].

The present exploration objects to reveal antimicrobial properties of water, ethanol and chloroform extracts of *P. muralis* and *P. perlatum* lichens.

*Corresponding author, e-mail: sinem.aydin@giresun.edu.tr

2. MATERIALS and METHODS

2.1. Lichen Material

Lichens were collected from the following localities:
P. muralis: Trabzon, Araklı, Kızılkaya plateau, 2222 m, 40°40'08" N, 40°01'39 E", 19.07.2005.
P. perlatum: Giresun, Center, Boztekke village, 22 m, 40°53'59" N, 38°19'09" E, 16. 04.2015.
P. muralis and *P. perlatum* lichens were taxonomically identified with using flora book and the voucher samples are kept in the department of Botany, Giresun University.

2.2. Lichen Extraction

The lichen materials were dried and they grounded using electric blender to get powder. The specimens were preserved in bottles. The lichen material (30 g) was extracted with 300 mL ethanol, chloroform and water with a Soxhlet extractor, separately. The extracts were filtered utilizing Whatman No. 1 filter paper and then concentrated in a vacuum at 40°C using a rotary evaporator. Extracts were kept at -80 ºC before use [4].

2.3. Extraction Yield (%)

The extraction yield was calculated utilizing below formula:

$$(\frac{W2-W1}{W0})X100$$

W_2 means the weight of the lichen extract and the container, W_1 means only the weight of the container and W_0 means the weight of the preliminary dried lichen sample.

2.4. Microorganisms

Salmonella enterica and *Staphylococcus aureus* were obtained from Giresun Province Control Laboratory, *Bacillus cereus* were acquired from Rize University Department of Molecular Biology *Enterobacter aerogenes*, *Bacillus subtilis* and *Proteus vulgaris* were obtained from Fırat University Department of Biology *Morganella morganii* were obtained from Bilecik Şeyh Edebali University Department of Molecular Biology and Genetic, *Gordonia rubripertincta*, *Staphyloccocus cohnii* and *Yersinia pseudotuberculosis* were acquired from Yeditepe University Department of Genetic and Bioenginneering. *Candida albicans* and *Candida tropicalis* were obtained from Fırat University Department of Biology; *Candida parapsilosis* were obtained from Giresun University Faculty of Education, *Saccharomyces cerevisiae* was obtained from Giresun Province Control Laboratory.

2.5. Antimicrobial Activity

The antimicrobial potential of the ethanol, chloroform and water extracts of *P. muralis* and *P. perlatum* extracts were identified through utilizing disc diffusion assay. Fourteen microorganism were utilized in this research. The bacterium were sub-cultured on Mueller Hinton Agar (MHA) and incubated at 37°C for 24 h and kept at 4°C to obtain stock cultures. 20 mL of sterile MHA were poured to sterile petri plates and waited to solidified. Each lichen extract was dissolved in dimethyl sulfoxide (DMSO) at 30 mg/mL concentration except for *P. muralis* water extract. *P. muralis* water extract was dissolved in water. Dissolved extracts were sterilized through 0.45 µm pore sized filter. Standards antibiotics (tetracycline and gentamycine) were used to compare inhibition zones of lichens. The turbidity of bacterial suspensions were adjusted 0.5 Mc Farland standard, then, the bacterial suspension inoculated into MHA plates and allowed to dry.

Two petri dishes were prepared each of the bacteria strain. Discs (5 mm diameter) were put onto the inoculated agar. 25 µL *P. muralis* ethanol extract, 25 µL *P. muralis* chlororoform extract, 25 µL *P. muralis* water extract, 25 µL sterile water and 25 µL DMSO were added to discs and tetracycline and gentamycine discs were put onto the agar surface one of the petri dish, separetely. The sterile paper discs were put on inoculated petri dishes and impregnated with 25 µL *P. perlatum* ethanol extract, 25 µL *P. perlatum* chlororoform extract, 25 µL *P. perlatum* water extract and 25 µL DMSO were added to discs and tetracycline and gentamycine discs were put onto the agar surface of the other petri dish, separetely The inoculated plates were standed in refrigerator for one hour then plates were incubated at 37°C overnight. Diameter of zones were measured with a ruler. The sensitivity of the microorganisms to the studied lichens was revealed by measuring the inhibitory zones size on the agar surface around the discs [5,6].

Antifungal activity was determined by disc diffusion method with Sabaroud Dextrose Agar (SDA) and Sabaroud Dextrose Broth (SDB) [7,8]. All antimicrobial tests were done twice.

2.5.1. Determination of Values of Minimum Inhibition Concentration (MIC)

The extracts were added into Mueller-Hinton broth at concentration ranging from 0.003661-30mg/mL. Whereas the mixtures were incubated for bacteria at 37°C, for fungi the mixtures were incubated for fungi 30°C. MIC describes as the lowest concentration of the extract that did not allow and turbidity or growth of the test microorganisms [9].

2.5.2. Determination of Values of Minimum Bacteriocidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The determination of MBC/MFC of the lichens was performed by sub culturing portions of the agar from plates that showed no growth in the tests for determination of MICs. These agar were transferred respectively into plates containing freshly prepared MHA and SDA. These plates were incubated at 25-27°C for 2-3 days (for fungi) and the plates were incubated at 37 °C for 24 h (for bacteria) and were observed for growth. The value which absence of growth at the end of incubation period regarded as MFC/MBC [10]. The contents of the MIC tubes having no-growth were spread on Mueller Hinton Agar plates for colony counting. MBC was calculated by the determination of whether the activity of the extract was bacteriostatic or bacteriocidal according to the state of growth. If there was no growth, the extract was identified as bacteriocidal [11]. Moreover, absence of visual growth of fungi was thought as fungicidal concentration of the drug or extract [12].

3. RESULTS and DISCUSSION

3.1. Extraction Yield (%)

Since bioactive compound in plants possess different properties and polarities, defining the ideal solvent for extraction of bioactive compounds is crucial for maximizing extraction yields of bioactive compounds [13]. It was indicated that solvents with different polarities have different effects on percentage lichen extract amounts [14,15].

Table 1. *Percentage yields of the lichen extracts*

Lichen	Solvent	Extract name and abbreviation	Yield (%)
P. muralis	Water	Water extract (SE)	5.40
	Ethanol	Ethanol extract (EE)	15.09
	Chloroform	Chloroform extract (KE)	11.23
P. perlatum	Water	Water extract (SE)	3.19
	Ethanol	Ethanol extract (EE)	16.99
	Chloroform	Chloroform extract (KE)	6.16

Table 1 demonstrates the percentage yield of extracts of *P. muralis* and *P. perlatum* in water, ethanol and chloroform solvents. Ethanol extract of *P. perlatum* gave the maximum yield (16.99%) while water extract of *P. perlatum* gave the minimum yield (3.19%). Extraction yield was increased in the following solvents in both lichens: ethanol > chloroform > water.

3.2. Antimicrobial Activity

Water, ethanol and chloroform extracts of *P. muralis* and *P. perlatum* lichens were researched to screen their antimicrobial effect against bacteria and fungi including five strains of Gram positive bacteria, five strains of Gram negative bacteria and four yeast strains using disc diffusion and macrobroth dilution methods. Results of antimicrobial activity of these lichens was presented in Table 2.

The results showed that the tested lichen extracts were effective in inhibiting microbial growth of test microorganisms with different potency. Tested lichens were inactive against *S. enterica*, *M. morganii* and *Y. pseudotuberculosis* (except chloroform extract of *P. perlatum* lichen). Lichen extracts were more effective against gram positive bacteria than gram negative bacteria. *P. muralis* lichen possess higher antimicrobial activity when compared with *P. perlatum* lichen. Inhibition zones of *P. muralis* lichen is in the range of 9-34 mm whereas inhibion zones of *P. perlatum* is in the range of 6-27 mm. Water extract of *P. muralis* didn't show inhibitory activity against tested bacteria while water extract of *P. perlatum* displayed weak activity against *B. cereus*, *B. subtilis* and *S. cohnii*. In both lichens extract antimicrobial activity were increased in following order: chloroform extract> ethanol extract> water extract. The highest antibacterial activity was obtained from ethanol extract of *P. muralis* against *P. vulgaris* (16.5 mm) and the lowest antibacterial activity was obtained from chloroform extract of *P. perlatum* against *G. rubripertincta* (6 mm) and *Y. pseudotuberculosis* (6 mm).

It wasn't observed any activity in DMSO and water which were used negative controls. Tetracycline and gentamycine have higher activity than lichen extracts.

P. perlatum and *P. muralis* lichens showed higher antifungal activity than antibacterial activity. The tested extracts showed antifungal activity against yeasts except for *C. parapsilosis*. The maximum antifungal activity was found in chloroform extract of *P. muralis* against *C. albicans* (34mm) while the minimum antifungal activity was found in chloroform extract of *P. perlatum* against *S. cerevisiae* (6 mm).

Compounds with antimicrobial activity exhibit effects that kill microorganisms or temporarily reduce their reproduction. Performed the disc diffusion method is insufficient to determine the lethal and instrinsic suppression. In addition to the disc diffusion test, MIC and MBC/MFC tests are also more effective in determining how an effect is exhibited [16].

MIC values of the *P. muralis* and *P. perlatum* lichens were given in Table 3. Higher MIC values shows limited antimicrobial activity. Among bacteria strains, the lowest MIC value was detected in chloroform extract of *P. muralis* against *B. cereus* (0.46875 mg/mL); the highest value was detected in ethanol extracts of *P. muralis* and *P. perlatum* lichens against *G. rubripertincta* and *S. cohnii* (15 mg/mL). MIC values of extracts against yeasts ranges from 0,07322 mg/mL and 15 mg/mL. MIC values of fungi is comparatively lower than MIC values of bacteria. It means that the tested lichen extracts are more effective fungi than bacteria.

The MBC is the lowest concentration that don't produce bacterial growth. MFC describes as the lowest extract concentration that don't cause any fungal growth on the solid medium used [17]. MBC and MFC values of the lichens exhibited in Table 4. MBC values are higher than MFC values.

Table 2. *Inhibition zones of the lichen extracts (mm)*

Microorganisms		***P. muralis* (SE)**	***P. muralis* (EE)**	***P. muralis* (KE)**	***P. perlatum* (SE)**	***P. perlatum* (EE)**	***P. perlatum* (KE)**	**Gen**	**Tet**	**Nys**	**DMSO**	**Water**
B. subtilis	Gram Positive Bacteria	NA	9±0.00	12±0.00	7±1.41	7±1.41	8±1.41	18±1.41	16±1.41	NA	NA	NA
S. aureus		NA	11±1.41	14.5±0.70	NA	NA	8.5±0.70	19±1.41	25±1.41	NA	NA	NA
B. cereus		NA	11.5±0.70	12±1.41	8±1.41	9.5±0.70	10±0.00	21.5±0.70	24±1.41	NA	NA	NA
G. rubripertincta		NA	12±0.00	12.5±0.70	NA	NA	6±0.00	14±1.41	25±1.41	NA	NA	NA
S. cohnii		NA	9±1.41	7±1.41	8±1.41	NA	10±1.41	19±1.41	21±1.41	NA	NA	NA
M. morganii	Gram Negative Bacteria	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
S. enterica		NA	NA	NA	NA	NA	NA	18±1.41	20±1.41	NA	NA	NA
E. aerogenes		NA	16±0.00	13±1.41	NA	8.5±0.70	13.5±0.70	20±1.41	16±1.41	NA	NA	NA
P. vulgaris		NA	16.5±0.70	12.5±0.70	NA	9±1.41	10±1.41	16±1.41	17±1.41	NA	NA	NA
Y. pseudotuberculosis		NA	NA	NA	NA	NA	6±1.41	18±1.41	NA	NA	NA	NA
S. cerevisiae	Fungi	NA	NA	7±1.41	NA	10±1.41	6±0.00	NA	NA	17±0.00	NA	NA
C. tropicalis		NA	27±1.41	30±1.41	NA	27±1.41	27±0.00	NA	NA	30±1.41	NA	NA
C. albicans		NA	33±1.41	34±1.41	NA	22±0.00	18±1.41	NA	NA	30±1.41	NA	NA
C. parapsilosis		NA	NA	NA	NA	NA	NA	NA	NA	25±1.41	NA	NA

NA: No Activity; Gen: Gentamycine (10µg/disc); Tet: Tetracycline (10µg/disc); Nys: Nystatin (100 µg/disc)

Table 3. *MIC values of the extracts (µg/mL)*

Microorganisms		***P. muralis* (EE)**	***P. muralis* (KE)**	***P. perlatum* (SE)**	***P. perlatum* (EE)**	***P. perlatum* (KE)**
B. subtilis	Gram Positive Bacteria	7.5±0.00	3.75±0.00	15±0.00	7.5±0.00	7.5±0.00
S. aureus		7.5±0.00	7.5±0.00	NT	NT	7.5±0.00
B. cereus		0.9375±0.00	0.46875±0.00	15±0.00	7.5±0.00	3.75±0.00
G. rubripertincta		15±0.00	7.5±0.00	NT	15±0.00	15±0.00
S. cohnii		15±0.00	7.5±0.00	15±0.00	7.5±0.00	7.5±0.00
M. morganii	Gram Negative Bacteria	NT	NT	NT	NT	NT
S. enterica		NT	NT	NT	NT	NT
E. aerogenes		NT	0.9375±0.00	1.875±0.00	NT	7.5±0.00
P. vulgaris		NT	7.5±0.00	3.75±0.00	NT	7.5±0.00
Y. pseudotuberculosis		NT	NT	NT	7.5±0.00	7.5±0.00
S. cerevisiae	Fungi	NT	15±0.00	NT	0.9346±0.00	15±0.00
C. tropicalis		0.014645±0.00	0.234375±0.00	NT	0.46875±0.00	0.46875±0.00
C. albicans		0.007322±0.00	0.234375±0.00	NT	0.46875±0.00	7.5±0.00
C. parapsilosis		NT	NT	NT	NT	NT

NT: Not Tested

Table 4. *MBC or MFC values of the lichen extracts (mg/mL)*

Microorganisms		***P. muralis* (EE)**	***P. muralis* (KE)**	***P. perlatum* (SE)**	***P. perlatum* (EE)**	***P. perlatum* (KE)**
B. subtilis	Gram Positive Bacteria	>30±0.00	>30±0.00	>30±0.00	>30±0.00	>30±0.00
S. aureus		7.5±0.00	30±0.00	NT	NT	7.5±0.00
B. cereus		>30±0.00	0.46875±0.00	>30±0.00	>30±0.00	>30±0.00
G. rubripertincta		NT	NT	NT	30±0.00	30±0.00
S. cohnii		30±0.00	30±0.00	>30±0.00	15±0.00	15±0.00
M. morganii	Gram Negative Bacteria	NT	NT	NT	NT	NT
S. enterica		NT	NT	NT	NT	NT
E. aerogenes		1.875±0.00	7.5±0.00	NT	>30±0.00	>30±0.00
P. vulgaris		>30±0.00	>30±0.00	NT	>30±0.00	>30±0.00
Y. pseudotuberculosis		NT	NT	NT	30±0.00	30±0.00
S. cerevisiae	Fungi	NT	15±0.00	NT	1.875±0.00	15±0.00
C. tropicalis		0.05859±0.00	0.9346±0.00	NT	1.875±0.00	1.875±0.00
C. albicans		0.05859±0.00	0.46875±0.00	NT	1.875±0.00	15±0.00
C. parapsilosis		NT	NT	NT	NT	NT

NT: Not Tested

One of the possible mechanisms by which natural agents exhibit antimicrobial activity is the inhibition of bacterial efflux pumps. While bacteria remove antibiotics from their cells, they use the efflux pumps as their self defence mechanism. Many studies have noted that phytochemicals have an important role in studies of efflux pump inhibitors of gram-positive bacteria, especially *S. aureus* and *S. epidermidis* [18].

Some researchers have suggested that the antimicrobial components of plant extracts cross the cell membranes by interacting with the proteins and enzymes of the membranes of the fungal cell, thereby causing the death of the fungal cell. Some researchers have attributed the inhibitory effect plant extracts to the hydrophobic character of plant extracts and components. Plant metabolites have shown that seperating the lipids of fungal cell membranes and mitochonria may lead to cell deaths due to cellular leaching of ions and cell contents within the cell [19].

Valadbeigi and Rashki (2015) was tested antibacterial efficiencies of methanol and water extracts of *Lecanora muralis* which is synonym of *P. muralis* against *Bacillus megaterium, Escherichia coli, S. aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Shigella sonnei*. Water extract of *L. muralis* hadn't any activity against test bacteria, however methanol extract of *L. muralis* exhibited strong antimicrobial activity. In accordance with this study, we observed no activity in water extract of *P. muralis* [20].

Rankovic et al. (2011) investigated MIC values of acetone extract of *L. muralis* against *Bacillus mycoides* IPH 197, *B. subtilis* IPH 189, *S. aureus* IPH 221, *Enterobacter cloaceae* IPH 241, *E. coli* IPH 246, *Klebsiella pneumoniae* IPH 251, *C. albicans* IPH 1316 strains. MIC values of this extract was recorded respectively 6.25 mg/mL and 3.12 mg/mL against *B. subtilis* and *S. aureus* while it wasn't found any activity against *C. albicans* [21]. In our current study, ethanol and chloroform extracts of P. muralis had higher MIC values againt *B. subtilis* and *S. aureus*. Moreover, antifungal activity had observed in ethanol and chloroform extracts of *P. perlatum*. It is thought that this differences between Rankovic and colleagues's study and our study might be arised from collecting the studied lichen species different localities and countries, using different microorganism strains and using different solvents in extracting lichens.

According to a study which was conducted by Semnain et al. (2014) water extract of *L. muralis* hadn't any activity against *S. aureus* while ethanol extract of L. muralis had activity against S. aureus and MIC value of ethanol extract of *L. muralis* was 49.81 mg/mL [22]. Similar with this work, we also detected that water extract of *P. muralis* hadn't activity against *S. aureus* but ethanol extract of *P. muralis* had activity against *S. aureus* and MIC value of ethanol extract of *P. muralis* was 7.5 mg/mL.

Almola et al. (2016) noted that ethanol and acetone extracts of *L. muralis* had inhibitory activity against *Bacillus* sp. Moreover, extracts of *L. muralis* exhibited higher activity against gram positive bacteria when compared with gram negative bacteria [23]. Our studies are aggrement with Almola's study. In our studies, ethanol and chloroform extracts of *P. muralis* had better activity on gram positive bacteria than gram negative bacteria.

Karagöz et al. (2009) recorded that water extract of *L. muralis* had better antimicrobial activity than ethanol extract of *L. muralis* [24]. On the contrary, it was determined that ethanol extract of *P. muralis* is more active than water extract of *P. muralis* in our study. This differences might be based on used test microorganisms, used extraction method and used lichen concentrations.

P. perlatum lichen used in our study was also investigated by various researchers in respect of antimicrobial properties. For example, antimicrobial activity of hexane extract of *P. perlatum* was screened against *E. coli, Pseudomonas* sp., *B. subtilis, Cryptococcus neofarmans, C. albicans, Aspergillus niger* and *Aspergillus fumigatus* [25].

In a study, it was researched that antimicrobial activity of silver nanoparticules which was synthesized by using ethanol extract of *P. perlatum* lichen against *Streptococcus* sp., *Enterococcus* sp., *Klebsiella* sp., *E. coli*, *Pneumoniae* sp., *Serratia* sp. and *Planomicrobium* sp [26].

Reveathy et al. (2015) found inhibition zones of ethanol extract of *P. perlatum* against *P. vulgaris*, *B. subtilis* and *S. aureus* respectively 6, 10 and 10 mm [27]. In our study inhibition zones of ethanol extract of the *P. perlatum* against *P. vulgaris*, *B. subtilis* and *S. aureus* was 9, 7 and no activity respectively. Different localities might be affect bioactivity of the same lichen species.

Kumar et al. (2014) concluded that ethanol extract of *P. perlatum* had activity on *S. aureus* strain [28]. On the other hand, we didn't detect any activity ethanol extract of *P. perlatum* against the same bacteria. This might be due to the collecting lichens from different geographies and using different extract concentrations applied to test microorganisms.

Momoh and Adikwu (2008) have compared the antibacterial activity of the ethanol extract of *P. perlata* which is synonym of *P. perlatum* on *S. aureus* versus the antimicrobial activity of colloidal silver. It was concluded that the lichen extract is effective against *S. aureus* even when colloidal silver didn't exhibit any activity against *S. aureus* [29]. On the contrary of this research, it was determined that ethanol extract of *P. perlatum* lichen could not inhibit *S. aureus* in our study.

It has been reported that 50% ethanolic and ethanolic extracts of *P. perlata* lichen is active against *S. aureus, Streptococcus mutans, Bacillus cereus, Corynebacterium xerosis* [30].

It was revealed that ethanol extract of *P. perlata* is active against *B. cereus, Mycobacterium smegmatis, Micrococcus luteus* and *S. aureus* but inactive against *E. coli, B. subtilis, P. vulgaris, P. aeruginosa, Enterococcus faecalis, Staphylococcus saprophyticus, C. albicans, Microsporum* sp., *Cypseum* sp. and *Trichophyton rubrum*. However, it was found that ethanol extract of *P. perlatum* lichen did not show activity against *S. aureus* but it had activity on *B. subtilis, P. vulgaris* and *C. albicans* [31]. This might be arised from using maceration techniques in extracting extracts from the literature study and also collecting of lichen which is mentioned in the literature from Pakistan.

4. CONCLUSION

This study has shown that *P. muralis* and *P. perlatum* lichens may be alternatives to antibiotics resistant microorganisms. Because of their antibacterial and antifungal activity, these lichens are thought to be useful in the pharmaceutical industry as agents to the treatment of infections.

While the antimicrobial activity of lichens water extracts is very limited, it is higher in ethanol and chloroform extracts. Therefore, more effective antibiotic agents can be obtained by extracting these lichen species with different organic solvents such as methanol, hexane, acetone and dichloromethane. Detailed studies on the isolation and identification of substances responsible for antimicrobial activity in lichens are needed.

ACKNOWLEDGEMENTS

This study is a part of the doctoral thesis and it was supported by Giresun University Scientific Research Projects Unit. (Project No: FEN-BAP-C- 140316-02).

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

[1] Chandra, M., "Antimicrobial activity of medicinal plants against human pathogenic bacteria", International Journal of Biotechnology and Bioengineering Research, 4(7): 653-658, (2013).

[2] Natarajan, D., Shivakumar, M.S., Srinivasan, R., "Antibacterial activity of leaf extracts of *Biophytum sensitivum* (L.) DC.", Journal of Pharmaceutical Science and Research, 2 (11): 717-720, (2010).

[3] Sökmen, B.B., Aydın, S., Kınalıoğlu, K., "Antioxidant and antibacterial properties of a lichen species *Diploschistes scruposus* (Schreb.) Norman," IUFS Journal of Biology, 71(1): 43-51, (2012).

[4] Kumar, S., Dhankhar, S., Arya, V.P., Yadav, S., Yadav, J.P., "Antimicrobial activity of *Salvadora oleoides* Decne. against some microorganisms", Journal of Medicinal Plants Research, 6(14): 2754-2760, (2012).

[5] Murray, P.R., Baron, E.J., Pfaller, M.A., Tenovar, F.C., Yolke, R.H., "Manual of Clinical Microbiology", ASM Press, Washington DC, 2892, (1995).

[6] Saric, C.L., Cabarkapa, S.I., Beljkas, M.B., Misan, C.A., Sakac, B.M., Plavsic, V.D., "Antimicrobial activity of plant extracts from Serbia", Food Processing Quality and Safety, 1(2): 1-5, (2009).

[7] Ertürk, Ö., "Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants", Biologia Bratislava, 61(3): 275-278, (2006).

[8] Ünal, M.Ü., Uçan, F., Şener, A., Dinçer, S., "Research on antifungal and inhibitory effects of DL-limonene on some yeasts", Turkish Journal of Agricultural Forestry, 36: 576-582, (2008).

[9] Yiğit, D., Yiğit, N., Aktaş, E., Özgen, U., "Ceviz (*Junglans regia* L.)'in antimikrobiyal aktivitesi", Türk Mikrobiyoloji Cemiyeti Dergisi, 39(1-2): 7-11, (2009).

[10] Oshim, I.O., Desmond, C.O., Nwobu, R.A.U., Ezugwu, U.M., Urama, E.U., "Kinetics of minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration of *Vernonia amygdalina* (Bitter leaf) on microorganisms isolated from wound infections", International Journal of Surgery Research, 5(1): 8-14, (2016).

[11] Benli, M., Güney, K., Bingül, Ü., Geven, F., Yiğit, N., "Antimicrobial activity of some endemic plant species from Turkey", African Journal of Biotechnology, 6(15): 1774-1778, (2007).

[12] Wankhede, S.B., Routh, M.M., Rajput, S.B., Karuppayil, S.M., "Antifungal properties of selected plants of *Apocynaceae* family against the human fungal pathogen *Candida albicans*", International Current Pharmaceutical Journal, 2(7): 12-125, (2013).

[13] Pham, H.N.T., Nguyen, V.T., Vuong, Q.V., Bowyer, M.C., Scarlett, C.J., "Effect of extraction solvents and drying methods on the physiochemical and antioxidant properties of *Helicteres hirsuta* Lour. Leaves", Technologies, 3: 285-301, (2015).

[14] Bisht, S., Sharma, S., Kumar, V., Kumar, M., Bisht, S.S., Nautiyal, B.P., "Assessment of antimicrobial efficiancy of secondary metabolites of lichen species from Uttarakhand Temperate Himalayas, India", Journal of Natural Products, 7: 168-176, (2014).

[15] Aoussar, N., Manzali, R., Nattah, I., Rhallabi, N., Vasiljevic, P., Bouksaim, M., Douira, A., Manojlovic, N., Mellouki, F., "Chemical composition and antioxidant activity of two lichen species (*Pseudevernia furfuraceae* L. and *Evernia prunastri* L.) collected from Morocco", Journal of Materials and Environmental Science, 8(6): 1968-1976, (2017).

[16] Altuner, E.M., Çetin, B., Çökmüş, C., "*Tortella tortulosa* (Hedw.) Limp. özütlerinin antimikrobiyal aktivitesi", Kastamonu Üniversitesi Orman Fakültesi Dergisi, 10(2): 111-116, (2010).

[17] Dellavalle, P.D., Cabrera, A., Alem, D., Larranaga, P., Ferreira, F., Rizza, M.D., "Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria* spp.", Chilean Journal of Agricultural Research, 71(2): 231-239, (2011).

[18] Abidi, S.H., Ahmed, K., Sherwani, S.K., Kazmi, S.U., "Synergy between antibiotics and natural agents results in increased antimicrobial activity against *Staphylococcus epidermidis*", The Journal of Infection in Developing Countries, 9(9): 925-929, (2015).

[19] Al-Rahman, A.N., Mostafa, A.A., Abdel-Megeed, A., Yakout, S.M., Husein, S.A., "Fungicidal activities of certain methanolic plant extracts against tomato phytopathogenic fungi", African Journal of Microbiology Research, 7 (6): 517-524, (2013).

[20] Valadbeigi, T., Raskhi, S., "GC-MS Analysis and anticancer effect against MCF-7 and HT-29 cell lines and antioxidant, antimicrobial and wound healing activities of plant-derived compounds", Journal of Basic Research in Medical Sciences, 2 (4): 1-11, (2015).

[21] Rankovic, B.R., Kosanic, M.M., Stnojkovic, T.P., "Antioxidant, antimicrobial and anticancer activity of the lichens *Cladonia furcata*, *Lecanora atra* and *Lecanora muralis*", BMC Complementary and Alternative Medicine, 11(97): 1-8, (2011).

[22] Semnain, S.N., Rahnema, M., Ghasempour, H., Alizadeh, H., "Evaluation of antibacterial effects of *Lecanora muralis* sp. extract on *Staphylococcus aureus* and *Salmonella typhimirium* in in-vitro in animal model", Journal of Fasa University of Medical Sciences, 4 (3): 318-326, (2014).

[23] Almola, Z.S., Al-Ni'ma, B.A., Ramadan, N.A., "Antibacterial effect of some Iraqi lichen extracts", International Journal of Science and Technology, 5 (9): 448-456, (2016).

[24] Karagöz, A., Doğruöz, N., Zeybek, Z., Aslan, A., "Antibacterial activity of some lichen extracts", Journal of Medicinal Plants Research, 3 (12): 1034-1039, (2009).

[25] Hoda, S., Vijayaraghavan, P., "Evaluation of antimicrobial prospective of *Parmotrema perlatum* hexane extract", International Journal of Pharmaceutical Research and Allied Sciences, 4 (2): 47-53, (2015).

[26] Reveathy, M., Mathiazhagan, A., Annadurai, G., "Biosynthesis, characterization, antibacterial activity of silver nanoparticles using the lichen *Parmotrema perlatum*", European Jounal of Biomedical and Pharmaceutical Sciences, 2 (4): 348-361, (2015).

[27] Reveathy, M., Sathyashree, S., Manimekala, N., Annadurai, G., Ahila, M., "Preliminary phytochemical investigation and antibacterial effects of lichen *Parmotrema perlatum* against human pathogens", European Jounal of Biomedical and Pharmaceutical Sciences, 2 (4): 336-342, (2015).

[28] Kumar, V., Naik, B., Kumar, V., Kumar, S., Singh, D., Aslam, M., "Evaluation of antibacterial and antioxidant activity of some lichens of Uttarakhand", American Journal of Current Biology, 1 (1): 1-8, (2014).

[29] Momoh, M.A., Adikwu, M.U., "Evaluation of the effect of colloidal silver on the antibacterial activity of ethanolic extract of the lichen *Parmelia perlata*", African Journal of Pharmacy and Pharmacology, 2(6): 106-109, (2008).

[30] Abdur, R., Latif, A., Sumbul, R., Afaq, S.H., "Study on extracts of *Parmelia perlata* Ach. for its antimicrobial potential against certain microorganisms", International Research Journal of Pharmacy, 4 (11): 102-106, (2013).

[31] Hussain, M., Raza, S.M., Farooq, U., Bakhsh, H., Majeed, A., Aziz, A., "In vitro antimicrobial potential of lichen (*Parmelia perlata*) against different pathogenic microbes", International Journal of Pharmaceutical Science, 4(4): 666-670, (2014).