Iranian Journal of Pharmaceutical Sciences
2020: 16 (4): 31-42
www.ijps.ir

Original Article

Antibacterial Properties and Flavonoids Content of Some Mosses Common in Armenia

Inesa Semerjyan, Gayane Semerjyan, Henrikh Semerjyan, Armen Trchounian*

Department of Biochemistry, Microbiology and Biotechnology, Yerevan State University, Yerevan, Armenia.

Abstract

The present study was aimed to evaluate the antibacterial potential of ethanol, methanol, acetone, and aqueous extracts of some bryophytes common in Armenia (*Mnium spinosum* (Voit) Schwaegr, *Brachythecium salebrosum* (Web. et Mohr) B.S.G., *Thuidiumrecognitum* (Hedw) Lindb and *Dicranum scoparium* (Hedw). Antibacterial activity was determined using agar-well diffusion method against five bacterial species (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella enterica,* and *S. typhimirium*). The results showed that the bryophytes *D. scoparium* and *B. salebrosum* possessed high antibacterial activity in methanol extracts, whereas *M. spinosum* has high antibacterial activity in acetone extract. Antibacterial activity against *S. aureus* was comparably weaker. Ethanol extract of *T. recognitum* had greater antibacterial activity than the extract of *M. spinosum*. Of all those tested for antibacterial activity bryophytes *in vitro* extracts of *D. scoparium* have yielded the most promising results. Antibacterial activity might be caused by a high content of flavonoids in bryophytes determined. Thus, the studied bryophytes might be used to develop new antibacterial agents.

Keywords: agar-well diffusion method; antibacterial activity; bryophytes; flavonoids; plant extracts; solvents.

1. Introduction

Corresponding Authors: Armen Trchounian, Department of Biochemistry, Microbiology and Biotechnology, Yerevan State University, Yerevan, Armenia.
Tel: (+374)60-710520
Email: Trchounian@ysu.am
Cite this article as: Semerjyan I, Semerjyan G, Semerjyan H, Trchounian A, Antibacterial Properties and Flavonoids Content of Some Mosses Common in Armenia, 2020, 16 (4): 31-42.

Medicinal plants are important components of both traditional and conventional medical products since ancient times. Most people prefer herbal-based medicine over chemically synthesized drugs. Safe, effective and affordable indigenous remedies have become more popular among people living in urban and rural areas. Therefore, medicinal plants have become an integral part of health care system. Ethnobotanical studies are important to identify the ancient and modern cultures of plant use in the world and to preserve the original knowledge of medicinal plants. Moreover, more attention is paid to medicinal plants due to their effectiveness, the cost of existing pharmaceutical products and cultural preferences. It has been reported that a large number of plants have antimicrobial and antioxidant potential (1-3). In addition, medicinal plants are used as food, human medicinal drugs, veterinary medicine and are important for the economy (4).

Bryophytes or "mosses" are the oldest group of terrestrial plants, which include liverworts, hornworts and mosses. This group of non-vascular plants includes between 25,000 and 28,000 species, and they grow in shady places. Unlike vascular plants, they are herbaceous and absorb water and minerals mainly through leaves (5). Bryophytes are used as indicator species for erosion control, heavy metal pollution, detection and monitoring of radioactivity, as aquatic bioindicators, radioactivity indicators, as material for beds of seed, fuel, drugs and food sources, pesticides, for nitrogen fixation, landscaping with mosses, waste treatment, construction, clothing, furnishing, packaging, genetic engineering and for tillage, etc. (6). The active components of bryophytes, found in most of them are used as antibacterial, antifungal, cytotoxic, antitumor and insecticidal agents in medical and agricultural fields. The phytochemical agents of bryophytes include a wide range of biologically active compounds, such as carbohydrates, lipids, proteins, steroids, polyphenols, terpenoids, organic acids, alcohols, fatty acids, aliphatic compounds, acetogenins, phenylquinones, aromatic and phenolic substances (7, 8).

A number of bryophytes has been used in traditional medicine to treat various diseases. They have an interesting feature – they are not attacked by bacteria, fungi or pests. They are a potential source for medicine because they contain secondary metabolites. Some active biomolecules, such as terpenoids and phenolic bibenzyls, have been studied for cytotoxicity against various human cancer cell lines, their antibiotic, antioxidant, antithrombin, antiplatelet and neuroprotective activity, and their ability to inhibit a number of biochemically important enzymes (7). Crude extracts or various bioactive compounds were isolated from mosses for anticancer efficacy on cancer cell lines.

The cytotoxic efficacy of bryophytes was reflected in several biochemical markers of the induction of apoptosis and necrosis, such as DNA fragmentation, nuclear condensation, proteolysis of poly (ADP-ribose) polymerase (PARP), activation of caspases (a family of cysteine aspartic proteases), inhibition of antiapoptotic nuclear transcriptional factor B, activation of p38 (mitogen-activated protein kinase), etc. Some of these mechanisms actually play a decisive role in the induction of apoptosis (9). Despite numerous reports on the biological activity of bryophytes, including their antibacterial activity, it is necessary to clarify the effects of various extracts and to determine their chemical compositions. In addition, various species growing in different regions may have a special chemical composition and specific properties, that should be studied.

Bryophytes are common in Armenia, but they have been little studied. About 350 species of mosses were identified in Armenia

(10), most of them in the forest zone (10, 11), and some of them are highly specialized and have a specific ultrastructure (11). For some species grown in Armenia, especially *Brachythecium campestre, Pelliaepiphilla, Tortularuralis* and others, the chemical composition and activity of some enzymes involved in metabolism of purine bases have been reported (12). This activity depends on heavy metal ions (Cu^{2+}, Cd^{2+}), polluting the environment, therefore it is assumed that these mosses serve as bioindicators for monitoring the state of the environment. In addition, there are some species with a high content of secondary metabolites that may have antimicrobial activity (7), therefore, mosses with high antibacterial activity can be used to develop antimicrobial drugs. Such a study is of considerable interest due to the problem of bacterial resistance to antibiotics (3, 13, 14) and may be a useful tool to combat this problem. Plant derived antimicrobials are also considered safer than their synthetic counterparts due to their natural origin (12).

In this study, the species of bryophytes common in Armenia were screened for their antibacterial activity and main phytochemical components. This study will enable the use of bryophytes to develop new antibacterial drugs.

2. Materials and Methods

2.1. Plant Materials

The bryophytes *Mnium spinosum* Schwaegr*, Brachythecium salebrosum* B.S.G.*, Thuidium recognitum* Lindb and *Dicranum scoparium* were collected in Armenia (at an altitude of ~1450 m). The plants were identified by Dr. A. Poghosyan (Department of Botany and Mycology, Yerevan State University (YSU), Armenia) and deposited in the Takhtadjyan Herbarium of the Department of Botany and Mycology, YSU (Vouchers no. 13450, 13451, 13452 and 13453, respectively).

2.2. Plant Extraction

Plant material was carefully collected from the soil and washed thoroughly with distilled water to remove the adhering soil or extraneous particles of dust. For microbiological studies, green parts of mosses (without rhizoids) were used, which were washed with liquid soap and running distilled water. Mosses were dried at room temperature. After that, the mosses were placed in a flask and extracted with methanol, ethanol, acetone or water (1 g moss per 20 mL solvent). The extraction was carried out on a magnetic stirrer at 18-20 ^{0}C for 48 h. After that, the samples were centrifuged at 1500 rpm for 10 min. The supernatants obtained were dried at 37 ^{0}C. The resulting powder was dissolved in 580 μL of dimethyl sulfoxide (DMSO). Samples were sterilized, as described (14). It should be noted that the solvents used are usually applied with various mosses (7, 16-18) and other plant extracts (2, 3, 13), and they are also used in folk medicine.

2.3. Test Microorganisms

Antibacterial activity was tested *in vitro* against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella enterica* and *S. typhimirium*. The strains *E. coli* VKPM-M17 (Russian National Collection of

Industrial Microorganisms, Institute of Genetics and Selection of Industrial Microorganisms, Moscow, Russia), *B. subtilis* WT-A1 (isolated from a soil sample), *St. aureus* MDC 5233 (Microbial Depository Center, National Academy of Sciences (NAS), Yerevan, Armenia), *S. typhimirium* MDC 1759 (wild type), *S. enterica* K3, isolated from infected pigs and provided by Dr. M. Mkrtchyan (Institute of Molecular Biology, NAS, Yerevan, Armenia) were used.

2.4. Determination of Antibacterial Activity

Antibacterial activity was determined by the agar well diffusion method (19). Plant extracts were tested for antibacterial activity through an agar plate previously infected with microorganisms, 5µL of the studied extracts of moss were instilled in the wells. Dimethylsulfoxide (DMSO) was used as a control. All plates were kept at low temperature for 1-1.5 h for sample diffusion, and then incubated at 37 ^{0}C for 24 h. After incubation, bactericidal and bacteriostatic zones were visible on the agar plate.

2.5. Determination of Total Flavonoids Content

Total flavonoid content in ethanol extract of mosses was evaluated by the $AlCl_3$ method, as described by Andreeva and Kalinkina (20). 500 µL of plant extract was taken into a test tube, 100 µL of 10% $AlCl_3$ was introduced, then 100 µL of 1 M CH_3COONa was added, then 2800 µL of distilled H_2O was also added. The tubes were incubated at room temperature for 30 min to complete the reaction. The absorbance of the solutions was measured at 415 nm wavelength using spectrophotometer GENESYS 10S UV-VIS (Thermo Scientific, Germany). The total flavonoid content was calculated from the catechin calibration curve and expressed as mg catechin equivalent per g of dried plant material.

2.6. Data Processing

Data Processing was done using Excel 2013 Microsoft program. Statistical analysis was done using one-way analysis of variance (ANOVA). The validity of differences between different series of experiments (n=5) was evaluated by Student P-test: the value $P<0.05$ was considered, as valid.

3. Results and Discussion

3.1. Antibacterial Activity of Moss Extracts

The results on the antimicrobial activity of plant extracts (Figures 1-4) showed that antibacterial activity of the bryophyte M. spinosum (Figure 1) against B. subtilis and E. coli was greater in acetone extract, forming an inhibition zone of 2.2 cm, while the aqueous and methanol extracts had a zone of 2.0 cm. Antibacterial activity against St. aureus was comparatively weaker, and ethanol extract had almost no antimicrobial effect. Then, the bryophytes B. salebrosum (Figure 2) and D. scoparium (Figure 4) have high antibacterial activity in methanol extracts. Moreover, the bryophyte T. recognitum had a higher antibacterial activity in ethanol extract (Figure 3) than M. spinosum. Particulalry, methanol extracts of D. scoparium possessed high antimicrobial activity against E. coli, forming a zone of 3.4 cm, and acetone, ethanol and aqueous extracts gave zones of 2.1, 2.5 and 2.7 cm, respectively (Figure 4). In case of S. enterica, St. aureus and B. subtilis the activity of extracts of D. scoparium was low. The methanol extracts of bryophyte B. salebrosum exhibited strong antibacterial activity against E. coli forming a zone of 3.0 cm (Figure 2). No marked inhibition zones were determined with DMSO, as control (Figures 1-4, wells in the centers of plates).

Some interesting reports have been reviewed to compare data obtained for mosses grown in Armenia with the results recorded for various bryophytes from other regions, including the effectiveness of different solvents used. Specifically, there is an interesting report on the high antibacterial effects of acetone extract of *Polytrichum juniperinum* and methanol extract of *Tortella tortuosa* (16). Different antibacterial effects were obtained with extracts of *Rhynchostegium vagans A. Jaeger* moss: ethanol extract of *R. vagans* was the most potent with the lowest minimum inhibitory concentration (MIC) (3.91 to 61.25 µg mL^{-1}) and minimum bactericidal/fungicidal concentration (MB/FC) (3.91 to 500 µg mL^{-1}) (17). Importantly, ethanol extracts were found to be superior over antibiotics used (chloramphenicol and fluconazole) (17). Other results showed that bryophytes *Asterella angusta* had greater antibacterial activity in methanol extract, and in *Targionoa hyphophylla* and *Plagochasma articulate* the antibacterial activity was greater in ethanol extract (18).

It should also be noted that among microorganisms, Gram-negative bacteria (*E. coli, Erwinia chrysanthemi, Pseudomonas aeruginosa, S. enterica*) were more sensitive to bryophytes; but this activity also depended on solvents (16, 17).

Thus, it can be concluded that the bryophytes extracts have high antibacterial activity, and this activity of bryophyte extracts depends on solvent used.

3.2. Phytochemical Screening and Total Flavonoids Content

The search for potential plant based antimicrobials has increased dramatically due to the emergence of multidrug resistance (3, 13, 14). The identification of plant based antioxidants is another aspect that has gained tremendous importance in protecting cells / tissues from damage caused by free radicals (20). The phenolic compounds present in plants act as powerful antioxidants that can protect cellular structures and mechanisms from free radicals by acting as hydrogen donors and radical scavengers. Antioxidants act as free radical scavengers, and thus they help to mitigate the effect of oxidative stress in various diseases. Many studies have demonstrated the effectiveness of plant derived products as a good source of antioxidants against various diseases caused by reactive oxygen species (21). Several studies have reported that phenolic compounds, such as flavonoids and phenolic acids present in plants, are responsible for their antioxidant nature (18, 21).

Phytochemical screening indicated the presence of secondary metabolites, such as flavonoids, in mosses (Figure 5). The data showed that flavonoids were found in varying amounts in the investigated mosses. The largest number of flavonoids was contained in *M. spinosum*, *T. recognitum* (Figure 5). Probably, the antibacterial activity of mosses' extracts was caused by the flavonoids contained. This suggestion seems likely, since it is known that flavonoids are synthesized by plants in response to bacterial infection, which means that they can be effective antibacterial substances against various microorganisms (16).

Among the various phytochemical compounds of natural origin, flavonoids are known to play an important role in medicine and pharmacology. Flavonoids are biologically active secondary plant compounds. There are numerous studies suggesting their beneficial effects on human health. Particularly, these compounds are tested as supportive or alternative therapies for diseases such as cancer or type-II diabetes. After oral administration, they interact with digestive enzymes and sugar carriers in the small intestine. Flavonoid glycosides as well as some aglycones have been reported to reduce postprandial hyperglycemia in patients with diabetes through interactions with glucose carriers in the apical membranes of enterocytes (22). It has been also shown that many flavonoids interfere with ATP-dependent drug-efflux transporters which are involved in the resistance of cancer cells against various cytostatic drugs, and therefore may be potential candidates for the fight against multidrug-resistance (22).

The antibacterial activity of flavonoids depends on the structures, namely on the substitutions on the aromatic rings. With the plant extracts possessing antibacterial activity increasingly being found, more and more flavonoids have proven to be antibacterial agents, especially with hydrophobic substituents, such as prenyl groups. Flavones have been widely investigated for their antibacterial activity (23); two di-prenylated flavones cuvanon C and moruzin isolated from medicinal plants, were evaluated for their antimicrobial activity using broth microdilution methods. They showed strong activity against Gram-negative bacteria (*E. coli*, *S. typhimurium*) and only limited activity against Gram-positive bacteria (*S. epidermis*, *St. aureus*). Yin and coworkers (24) also isolated a mono-prenylated flavon called corylifol C from *Psoralea corylifolia* seeds and found to be ineffective for inhibiting the growth of *St. aureus* and *S. epidermidis in vitro*. These results indicate the importance of the degree of prenylation for antibacterial activity. The substitutive prenyl group sometimes reacts with the adjective hydroxyl group to form a six-member heterocycle, which probably reduces its activity (25). However, further study is required.

4. Conclusion

In this study, all mosses' extracts from bryophytes grown in Armenia demonstrated the ability to inhibit the development of bacteria (the diameters of inhibition zone varied from 0.3 to 3.4 cm)., Antibacterial activity against *E. coli* was found in all moss extracts. The highest activity was determined in extracts of *D. scoparium* (inhibition zone diameter of 3.4 cm), and the lowest activity was observed in extracts of *B. salebrosum* (inhibition zone diameter of 0.3 cm). Antibacterial activity depended on the solvents used. Then, antibacterial activity might be associated with flavonoids; among mosses *M. spinosum* and *T. recognitum* had the highest total flavonoid content.

Thus, the mosses common in Armenia that we studied have antibacterial activity and can be used as wide range of herbal antibacterial agents. Especially, extracts of *D. scoparium* can be used in medicine, pharmaceutical and food industry (as natural alternative preventive agents for combating food poisoning diseases and preserving food products, avoiding the use of chemical antibacterial agents).

Acknowledgments

The authors would like to acknowledge Dr. A. Poghosyan from Department of Botany and Mycology, Yerevan State University (Armenia), for identification of collected plant material. Thanks to Dr. L. Hakobyan from Research Institute of Biology, Yerevan State University (Armenia), for help with improving English.

The study was done in framework of Basic support from Committee of Science, Ministry of Education, Science, Culture and Sport of Armenia, to Yerevan State University.

References

[1] Kumar V, Sharma N, Sourirajan A, Khosla P, Dev K. Comparative evaluation of antimicrobial and antioxidant potential of ethanolic extract and its fractions of bark and leaves of *Terminalia arjuna* from north-western Himalayas, India. *J. Trad. Compl. Med.* (2018) 8: 100-106.

[2] Moghrovyan A, Sahakyan N, Babayan A, Chichoyan N, Petrosyan M, Trchounian A. Essential oil and ethanol extract of Oregano (*Origanum vulgare* L.) from Armenian flora as a natural source of terpenes, flavonoids and other phytochemicals with antiradical, antioxidant, metal chelating, tyrosinase inhibitory and antibacterial activity. *Curr. Pharmaceut. Design* (2019) 25 (16): 1809-1816.

[3] Sahakyan N, Ginovyan M, Petrosyan M, Trchounian A. Antibacterial and anti-phage activity of *Plantago major* l. raw material. *Proc. Yerevan State Univ.: Chemistry & Biology* (2019) 53 (1): 59-64.

[4] Amalraj A, Gopi S, Medicinal properties of *Terminalia arjuna* (Roxb.) Wight & Arn.: A review. *J. Trad. Compl. Med.* (2017) 7: 65-78.

[5] Peters K, Treulter H, Doll S, Kidt A.S, Hankemier T, Neumann S. Chemical diversity and classification of secondary metabolites in nine bryophyte species. *Metabolites* (2019) 9: 222.

[6] Banerjee KS, Srivastava AK. Assessment of diversity and traditional uses of *Bryophytes* along some hill roads in a biodiversity hot spot region of India – A case study of mizoram. *Int. J. Nat. Resour. Ecology Manag.* (2019) 4 (3): 73-82.

[7] Klavina L, Springe G, Nikolajeva V, Martsinkevich I, Nakurte I, Dzabijeva D, Steinberga I. Chemical composition analysis, antimicrobial activity and cytotoxicity of screening of moss extracts (Moss Phytochemistry). *Molecules* (2015) 20: 17221-17243.

[8] Chandra S, Chatdra D, Barh A, Pandey K, Sharma I. Bryophytes: Hoard of remedies, an ethno-medical review. *J. Trad. Compl. Med.* (2017) 7: 94-98.

[9] Dey A, Mukherjee A. Therapeutic potential of bryophytes and derived compounds against cancer. *J. Acute Disease* (2015) 4 (3): 236-248.

[10] Manakyan VA. Results of bryological studies in Armenia. *Arstoa (1996)*5: 15-34 (in Russian).

[11] Pogosyan AV, Shahazizyan IV, Nanagulyan SG, Zakaryan NA, Eloyan IM. Ultrastructure of mosses of forest of Arailer mountain (Armenia). *Znanie (Knowledge*) (2015)12: 73-76 (in Russian).

[12] Semerjyan H., Semerjyan G. Use of purine basis exchange enzymes for pollution monitoring in environment. *Biolog. J. Armenia* (2017) 69: 63-66 (in Russian).

[13] Ginovyan M, Trchounian A. Novel approach to combat antibiotic resistance: evaluation of some Armenian herb crude extracts for their antibiotic modulatory and antiviral properties. *J. App.l Microbiol.* (2019)127: 472-480.

[14] Sahakyan N, Petrosyan M, Trchounian, A. The activity of Alkanna species *in vitro* culture and intact plant extracts against antibiotic resistant bacteria. *Curr. Pharmaceut. Design* (2019) 25(16): 1861-1865.

[15] Mehmood N, Zubair M, Rızwan K, Rasool N, Shahid M, Ahmad V. Antioxidant, antimicrobial and phytochemical analysis of *Cichorium intybus* seeds extract and various organic fractions. *Iran. J. Pharm. Res.* (2012)11: 1145-1151.

[16] Savaroglu F, Ilhan S, Filik-Iscen C. An evaluation of the antimicrobial activity of some Turkish mosses. *J. Med. Plants Res.* (2011) 5: 3286-3292.

[17] Negi K, Chaturvedi P. *In vitro* antimicrobial efficacy of *Rhynchostegium vagans* A. Jaerger (moss) against commonly occurring pathogenic microbes of Indian sub-tropics. *Asian Pacific J. Trop. Disease* (2016) 6: 10-14.

[18] Pratima KS. Phytochemical screening and antibacterial activity of Bryophytes. *Int. J. Life Sci.* (2017) 5(3): 405-408.

[19] Boyanova L, Gergova G, Nikolov R, Derejian S, Lazarova E, Katsarov N, Mitov I, Krastev Z. Bulgarian propolis against 94 *Helicobacter pylori* strains *in vitro* by agar-well diffusion, agar dilution and disc diffusion method. *J. Med. Microbiol.* (2005) 54: 481-483.

[20] Andreeva V, Kalinkina GI. Development of a method for the quantitative determination of flavonoids in *Artemisia vulgaris. Khim. Rastit. Syr'ya (*Chemistry of Plant Raw Materials) (2000)1: 85-88 (in Russian).

[21] Trchounian A, Petrosyan M, Sahakyan N. Plant cell redox homeostasis and reactive oxygen species. D. Gupta (Ed.), In: *Redox State as a Central Regulator of Plant-Cell Stress Responses*. Springer, Cham. (2016)*:* 25-50.

[22] Wenzel U. Flavonoids as drugs at the small intestinal level. *Curr. Opin. Pharmacol.* (2013) 13 (6): 864-868.

[23] Farhadi F, Khameneh B, Iranshahi M, Iranshahy M. Antibacterial activity of flavonoids and their structure–activity relationship: An update review. *Phytother. Res.* (2019) 33 (1): 13-40.

[24] Yin S, Fan C.Q, Wang Y, Dong L, Yue JM. Antibacterial prenilflavone deriatives from *Prosalea corylifolia*, and their structure-activity relationship study. *Bioorg. Med. Chem.* (2004) 12 (16): 4387-4392.

[25] Xie Y, Yang W, Tang F, Chen X., Ren L. Antibacterial activities of flavonoids: structure-activity relationship and mechanism. *Curr. Med. Chem.* (2015) 22: 132-149.

Figures:

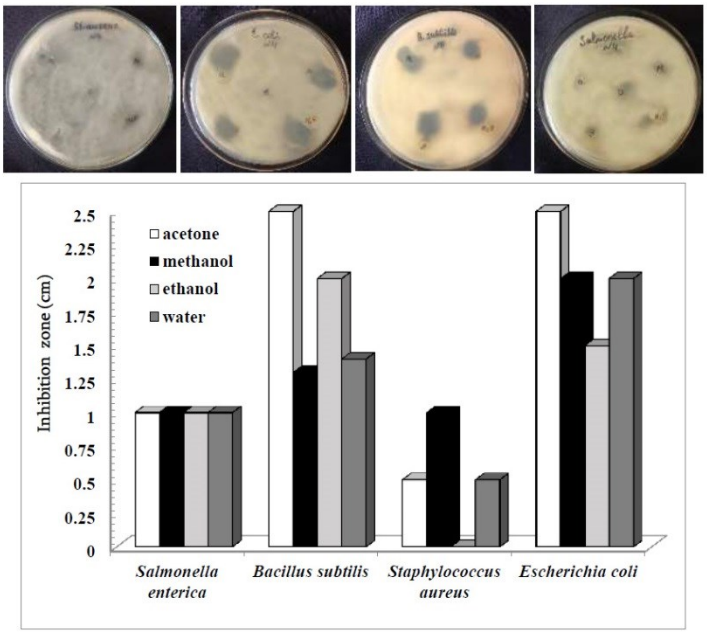

Figure 1. Antibacterial activity of acetone, methanol, ethanol and water extracts of *M. spinosum*. The zones of lack of growth of the tested microorganism under the influence of various extracts of moss *M. spinosum* are shown. All data are expressed as mean ±SEM of five independent experiments ($p< 0.05$). For the others, see "Material and methods".

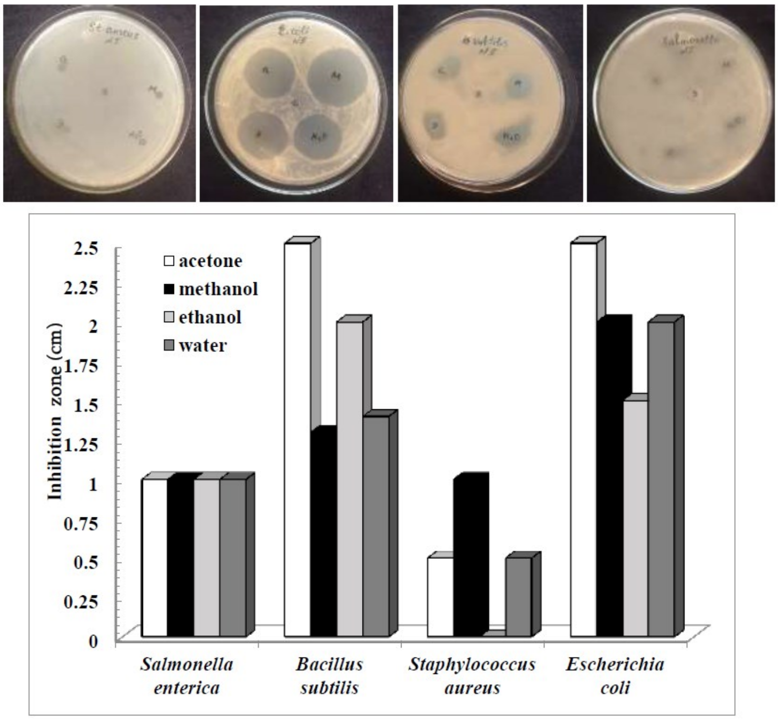

Figure 2. Antibacterial activity of acetone, methanol, ethanol, water extracts of *B. salebrosum*. For details, see the legends to Fig. 1.

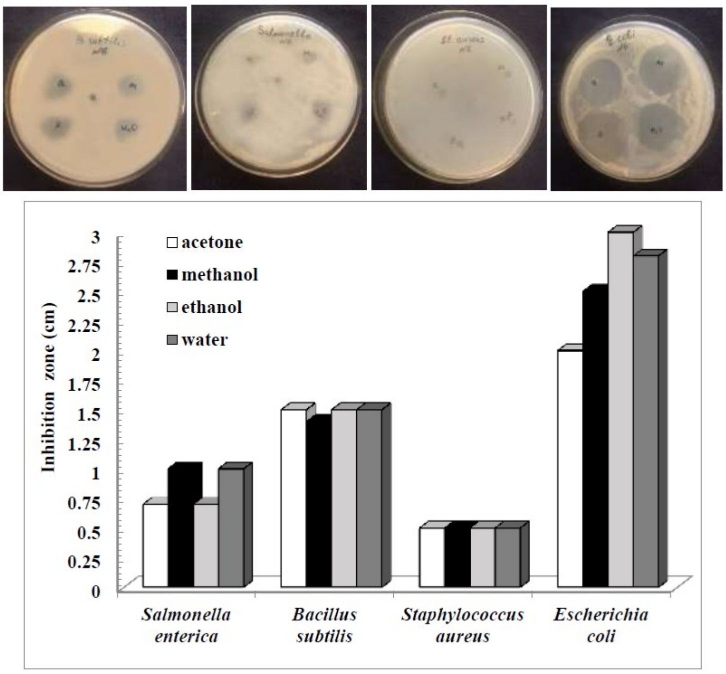

Figure 3. Antibacterial activity of acetone, methanol, ethanol and water extracts of *T. recognitum*. For details, see the legends to Fig. 1.

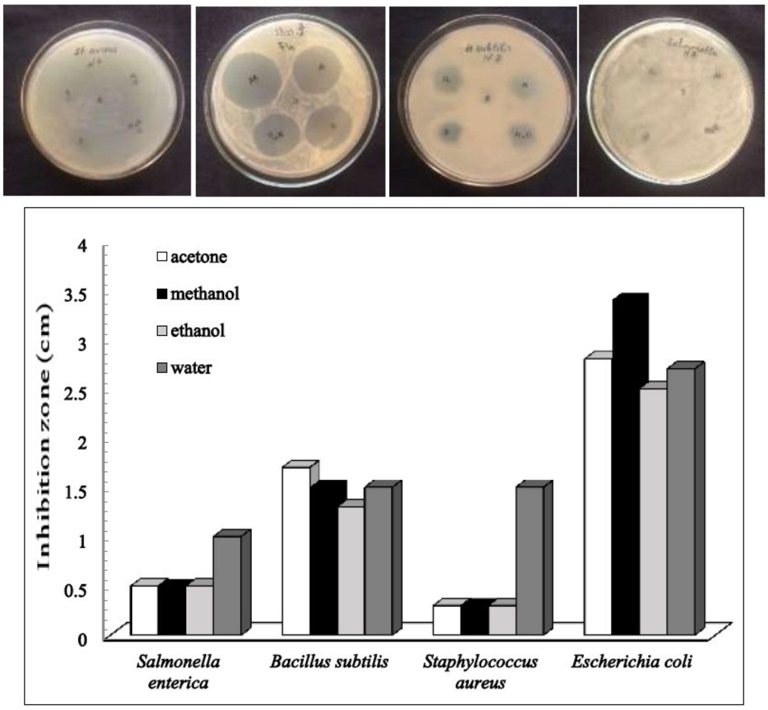

Figure 4. Antibacterial activity of acetone, methanol, ethanol and water extracts of *D. scoparium.* For details, see the legends to Fig. 1.

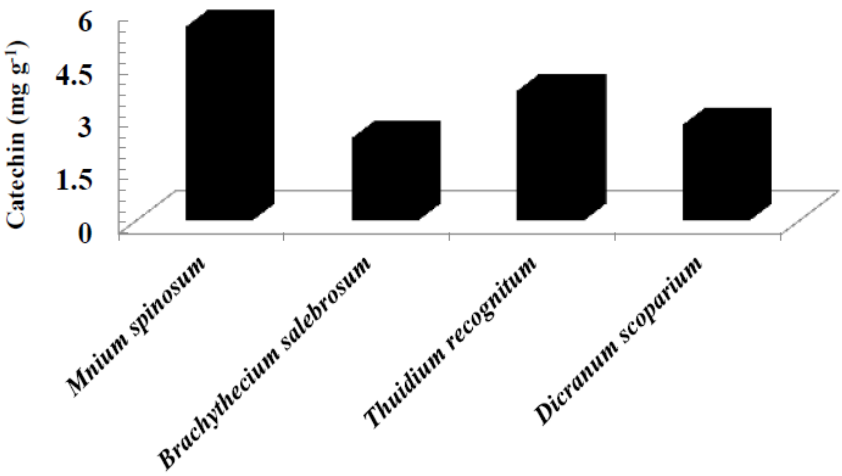

Figure 5. Total flavonoid content in ethanol extracts of different mosses studied. The total flavonoid content was quantified by the standard curve of catechin (mg g^{-1}). For details, see «Materials and methods».

ONLINE SUBMISSION

www.ijps.ir