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Research Full Papers

Evaluation of antioxidant potential and antimicrobial activity of Mocheh (*Lepidium draba*) extract "*in vitro*"

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Abstract

Usage of natural products like herbals, provide unlimited opportunities for novel and suitable additives. Mocheh can be used in fresh form or as an ingredient in soup and salad. This study was aimed to determine the antimicrobial and antioxidant activities of Mocheh (*Lepidium draba*) extract. The antimicrobial activity of Mocheh extract was tested against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Aspergillus niger*. The extract showed a strong antimicrobial activity with a concentration dependence and a broad antimicrobial spectrum for all tested microorganism species. The results showed that MIC of leaf extract of *Lepidium draba* on *Aspergillus niger*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* was 128, 128, 128 and 128 mg/mL respectively. The results showed that MBC/MFC of leaf extract of Mocheh on the examined microorganisms was 256, 256, 256 and 256 mg/mL respectively. The values obtained for total flavonoid content and total phenolic content were 22.13 µg/mL and 18.88 mg/mL, respectively. Measured value in the radical scavenging activity was IC_{50}= 168/21 µL/mL. The results showed that Mocheh leaf aqueous extract as a novel source of natural antimicrobial and antioxidant agents for the food and pharmaceutical industries.

Keywords: Mocheh, Extract, Gram-positive and Gram-negative bacteria, Antioxidant activity.

Introduction

Foodborne disease and food poisoning are still a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety researchers and regulatory agencies are continuously concerned with the high and growing number of illness outbreaks caused by some pathogenic and toxic microorganisms in foods (Shan *et al.*, 2007). Antimicrobial agents are defined as chemical compounds that presented or added to foods for retarding microbial growth or inducing microbial death. In the last decades, there has been particular interest in the use of abundant naturally occurring antimicrobials (herbs, spices and plants) (Klančnik *et al.*, 2010).

On the other hand, antibiotic resistance has become a global concern. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Tchinda *et al.*, 2017). This has driven scientists to search for new antimicrobial substances from various sources like the medicinal plants (Al-Marzoqi *et al.*, 2015). Natural products, either pure compounds or standardized plant extracts, provide unlimited opportunities for novel and suitable additives and drug treatments due to their unmatched range of chemical diversity and may offer a new source of antibacterial agents (Brantner *et al.*, 1994; Klančnik *et al.*, 2010).

The medicinal plants are those which contain substances in their organs that can be used for therapeutic purpose or as a precursor

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for the synthesis of other useful medicines. It was well established that the plants which are naturally rich in a wide variety of secondary metabolites, such as alkaloids, glycosides, tannins, volatiles oils and contain minerals and vitamins, possess medicinal properties (Chyad, 2017).

Several antimicrobial compounds are extracted from easily available sources, such as agricultural and horticultural crops (e.g. grapevine, citrus, hops, berries, tea leaves etc.), or medicinal plants such as pine, sage, rosemary, and many others (Klančnik *et al.*, 2010). The Mocheh (*Lepidium draba*) was named as a class have a place under the family Brassicaceae, privately known as white top or hoary cress. The plant is local to western Asia including Iran and Eastern Europe and also as an intrusive species in North America (Chyad, 2017; Radonić *et al.*, 2011). It is a perennial herb with initial spoon like leaves and rectangular stem leaves, white flowers and grips like flat egg like or heart like fruit. It could be used in rice as cooked plant. Infusion of its leaves and seeds has purgative and expectorant effects. In the local dialect is called Mocheh (Haghighi *et al.*, 2011). Brassicaceae plant kingdom harbors an inexhaustible source of active ingredients valuable in the management of many intractable diseases. Numerous studies have identified compounds within herbal plants, which are effective antibiotics and antioxidants (Hussein, 2016).

The explore of biologically active components from plants has always been great interest to scientists looking for new promising sources of practical for herb- based medicines, food supplements, pharmaceuticals and health products (Hussein, 2016).

The aim of this study was assessing the *in vitro* the possible effects of antimicrobial activity of aqueous extract of Mocheh upon food born pathogenic microorganisms including *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Aspergillus niger* and to evaluate its antioxidant properties.

Material and methods

Collection of plant materials and extract preparation

The leaves of *Lepidium draba* L. were collected at the beginning of the vegetative period (summer), from the rangelands of the Zagros Mountains, Shahrekord City, Chaharmahal Bakhtiari Province, Iran. A voucher example of the plant was stored to be recognized and validated at Ferdowsi University of Mashhad, Institute of Plant Sciences. The plant leaves were dried in the shade for a few days at room temperature and afterward pounded as powder and weighed (Chyad, 2017). Plants were extracted as described by Sigstedt et al., 2008 with some modifications. Fifty grams of plant powder were then macerated in 250 mL of distilled water. Extract was prepared using the maceration process for 72 h under constant shaking and filtered with Whatman No. 1 filter paper. The extract was evaporated to dryness under reduced pressure using a rotary evaporator (Heidolph laborota 400, Heidolph Instruments, Germany). The yield of the extract (w/w) was calculated according to Bazzaz et al., 2003.

Preparation of inoculum

The strains of *Bacillus subtilis* PTCC 1023, *Pseudomonas aeruginosa* PTCC 1707, *Staphylococcus aureus* PTCC 1337 and *Aspergillus niger* PTCC 5010were purchased from the laboratory of industrial microbiology, faculty of agriculture, Ferdowsi University of Mashhad (FUM). Microbial strains were cultured 24 h at 37ºC for bacteria and 72 h at 25ºC for fungal strains before the antimicrobial tests were performed. A 0.5 McFarland standard of microbial strains was used estimate their concentratin, which was equivalent to 1.5×10^8 colony forming unit (CFU)/mL (Amin Mir *et al*., 2016).

Antimicrobial activity

Determination of minimum inhibitory concentration (MIC)

Dilutions of Mocheh extract (512, 256, 128, 64, 32, 16, 8, 4, 2, 1 mg/mL) were prepared in

sterile Muller Hinton Broth (MHB) (Sigma-Aldrich), Potato Dextrose Broth (PDB) (Sigma-Aldrich) for bacterial and fungal strains, respectively.Ten µL of microbial suspensions (at 1.5× 10^8 CFU/mL) was added to each dilution at the 96-well microplates. The inoculated microplates were then incubated at 37˚C for 24 h and those inoculated with fungal strains were incubated at 25˚C for 72 h. The lowest concentration that prevented visible growth determined as MICs (Baba *et al*., 2015).

Determination of minimum bactericidal/ fungicidal concentration (MBC/MFC)

Ten µL of the culture from each well in which the red color was not observed, was streaked on MHA for bacterial strains and PDA for fungal strains. The plates were then incubated at 37°C for 24 h for bacterial strains or 25°C for 72 h for fungal strains and MBC or MFC was defined as the lowest concentration at which no colony of microorganism was observed (Wendakoon *et al*., 2012).

Determination of inhibitory zone by well diffusion agar method

A 20 mL of sterile MHA and PDA media was poured into plates and allowed to set. The plates were then seeded with 10 µL suspensions of microorganisms with concentration adjusted to approximately 1.5× 10^8 CFU/mL and were kept for drying. Wells were made on the plates with sterile whole puncture (6 mm diameter). Sixty µL of aqueous leaf extract of Mocheh (100, 200, 300 and 400 mg/mL) was poured into each respective wells. The plates were then incubated at 37°C for 24 h and those spread with fungal stains were incubated at 25°C for 72 h. The antibacterial activity of the plant extract was then assessed by an inhibition zone surrounding the well and zone of inhibition (ZOI) was measured (Sohail *et al.*, 2014).

Antioxidant activity

Free radical scavenging capability

DPPH (1,1-diphenyl-2-picryl hydrazine) free radical scavenging capability of *Mocheh* water extract was evaluated by the method of Bursal and Ekrem (2011) with a slight modification. Briefly, different concentrations (150, 200 and 250 µg/mL) of *Mocheh* water extract was prepared and the volume was then adjusted to 3 mL with ethanol. One milliliter of 0.1 mM alcoholic DPPH solution was added to each sample. These samples were vortexed and incubated in a dark at room temperature for 30 min. The absorbance was measured at 517 nm against blank samples. Decreased absorbance of the sample indicates the DPPH free radical scavenging capability (Bursal *et al*., 2011). The control samples contained all the reagents except the extract. The percentage of inhibition was calculated using the following equation:

% inhibition=100× (A of control−A of sample)/A of control (Baba *et al.*, 2015).

Reducing power

Two and half mL of the extract and butylated hydroxyl toluene (BHT) were mixed with 2.5 mL of 1% potassium ferricyanide and 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and were incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 200 g for 10 min. 2.5 mL of the supernatant was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. The absorbance at 700 nm was measured against sodium phosphate buffer as a blank. The increased absorbance of the reaction mixture correlates with greater reducing power (Ardestani *et al*., 2007).

Determination of total flavonoid content

Total flavonoid content in water extract of Mocheh was estimated by a colorimetric assay. At first, 1 mg water extract Mocheh was pipetted into a test tube. Then, 0.1 mL CH_3COOK (1.0 M) and 0.1 mL of 10% Al $(NO_3)_3$ in 4.3 mL ethanol solution were added. The samples were vortexed and then left to stand at room temperature for 40 min. Absorbance measurements were recorded at 415 nm. Distilled water was used as blank and also instead of sample, distilled water was used for control. A calibration curve of quercetin was plotted, and flavonoid contents were determined from regression equation of the calibration curve. The results were reported as

quercetin equivalents per milligram extract (Kossah *et al*., 2011).

Determination of total phenolic content

A calibration curve of Gallic acid in methanol was constructed in concentration range of 0.04-0.7 mg/mL. The solutions for the spectrophotometric analysis were performed as follows: in a 50 mL volumetric flask 1 mL of a standard solution of gallic acid, 6 mL of methanol, 2.5 mL of the Folin-Ciocalteau reagent, 5 mL of 7.5% Na_2CO_3 were added, reaching the final volume with purified water. The solutions were stored overnight and the spectrophotometric analysis was performed at 765 nm. The polyphenols were determined as follows: 2.5 g of extract was diluted with 50 mL methanol 80 % in falcon and was shaken at 240 rpm for 24 h. The sample was filtered twice with Whatman 0.2 µm. 500 µl of diluted extraction was added to 2.5 mL Folin-Ciocalteau reagent and 5 mL of Na_2CO_3 (7.5%). The samples were stored overnight, and the spectrophotometric analysis was performed at 765 nm (Capannesi *et al.*, 2000).

Statistical analysis

Results of the study were based on statistical package for the social sciences (SPSS) version 16.0 and differences among the means were determined for significance at $p<0.05$ by One-way ANOVA.

Results and discussion

The present work investigated the antimicrobial and antioxidant activity of Mocheh. The results of MIC and MBC/ MFC assays are presented in Table 1. The results showed that MIC of leaf extract of Mocheh on *Aspergillus niger*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* was 128, 128, 128 and 128 mg/ mL respectively. The results showed that MBC/MFC of leaf extract of Mocheh on *Aspergillus niger*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* was 256, 256, 256 and 256 mg/mL respectively. According to Kuete (2010), Kuete and Efferth (2010), the antibacterial activity of a plant extract is considered significant when MIC values are below 100 µg/mL, moderate when $100 \leq MIC \leq 625$ µg/mL and weak when $MIC > 625$ µg/mL (Victor Kuete, 2010; V. Kuete *et al.*, 2010; Tchinda *et al.*, 2017).

Table 2 shows the zone of inhibition exposed to different concentrations of aqueous extract of Mocheh by disk agar diffusion test. The results revealed that by increasing the concentration of aqueous extract of Mocheh, a greater zone of inhibition was observed. This implied that the gram-positive bacteria were more susceptible to this extract than the gram-negative bacteria. This observation is probably due to the presence of outer membrane in gram-negative bacteria that serves as a significant barrier in gram-negative species. Furthermore, the results introduced gram-positive bacteria as the most susceptible bacteria, an observation that may be attributed to the presence of single membrane of the organism which makes it more accessible to permeation by the active compounds of the examined extract (Al-Marzoqi *et al.*, 2015).

Table 1- Quantities of MICs and MBCs were obtained under the influence of various concentrations of aqueous extract of Mocheh leaves

Microorganisms	MIC (mg/mL)	MBC/MFC (mg/mL)
Aspergillus niger	128	256
Bacillus subtilis	128	256
Pseudomonas aeruginosa	128	256
Staphylococcus aureus	128	256

Results obtained from study of Al-Marzoqi et al. (2015) showed that active compounds of *Cardaria draba* (*Lepidium draba* L.) had wide spectrum antibacterial activity. The results revealed that both Gram-positive *Staphylococcus aureus* and *Staphylococcus saprophyticus* were susceptible to alkaloid and terpenoid compounds, while *Staphylococcus*

epidermidis was susceptible to alkaloid compounds only. In addition, all Gram-negative bacteria were resistant to active compounds except *Serratia* was susceptible to phenolic, alkaloid and terpenoid compounds, while *Proteus* and *Pseudomonas* were susceptible to terpenoid compounds only (Al-Marzoqi *et al.*, 2015).

About 20% of all investigated plants with antibacterial activity are rich in polyphenolic substances, tannins, catechins and polyphenolic acids. It is known that polyphenols can form heavy soluble complexes with proteins. Polyphenols may bind to bacterial adhesions and by doing so they disturb the availability of receptors on the cell surface (Brantner *et al.*, 1994).

In the study of phytochemicals compounds of Mocheh amounts of total phenolic content (TPC) and total flavonoid content (TFC), were 22.13 µg/mL and 18.88 mg/mL, respectively. The ability of aqueous extract of the Mocheh to quench DPPH free radical was measured. The extracts and BHT demonstrated a dose-dependent scavenging activity by reducing DPPH radical (Fig. 1). By plotting the graph of extract concentrations against the scavenging activity, a specific concentration of the sample that needed to provide 50% inhibition (IC_{50}) was calculated. IC_{50} for aqueous extract of the Mocheh was 168.21 µl/mL.

Table 2- Average inhibition zone (mm) of Mocheh leaves extract concentrations on some pathogenic microorganism's thorough well diffusion agar method

Microorganisms	Well diffusion agar			
	Concentrations (mg/mL)			
	100	200	300	400
Aspergillus niger	-[a]	-[a]	15.20± 0.42[b]	19.00± 0.58[c]
Pseudomonas aeruginosa	11.10± 0.33[a]	19.00± 0.25[b]	21.20± 0.40[c]	24.00± 0.50[d]
Bacillus subtilis	12.12± 0.36[a]	17.30± 0.44[b]	20.20± 0.59[c]	22.10± 0.57[d]
Staphylococcus aureus	13.30± 0.78[a]	18.00± 0.36[b]	20.50± 0.72[c]	22.30± 0.66[d]

Values are expressed as mean±standard deviations, $n = 3$; different letters (a, b, c and d) in each row show significant difference at $p \leq 0.05$.

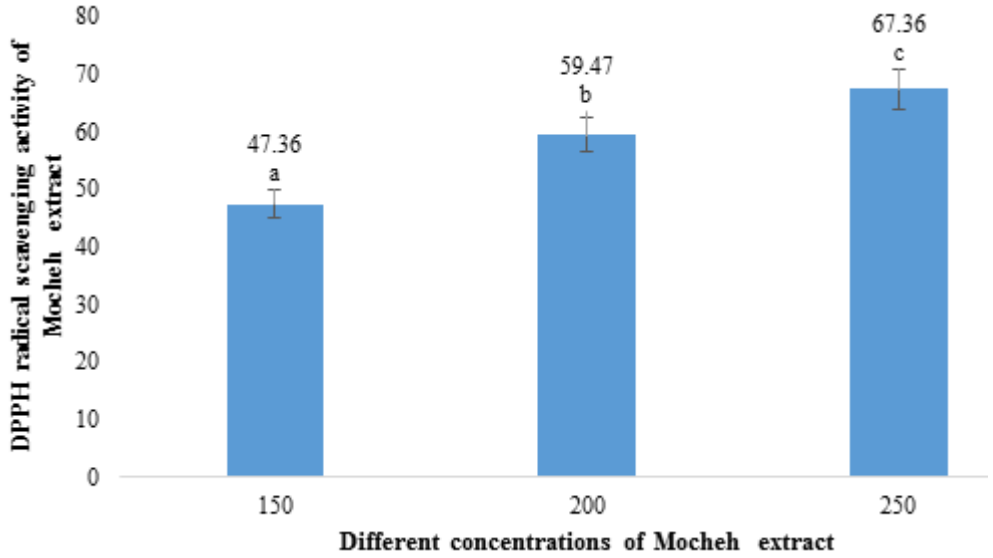

Fig. 1. DPPH radical scavenging activity of aqueous leaf extract of Mocheh.

Hussein (2016) identified the phytochemical compounds of the ethanolic extract of the leaves of *Lepidium draba* L. by gas chromatography–mass spectrometry, fourier-transform infrared spectroscopy and atomic absorption spectrophotometry techniques. The results showed that the leaves of this plant are a rich source of biologically active chemical

compounds, fibers and minerals. The author stated that the leaves of *Lepidium draba* L. can be used as a multifunctional drug source, although clinical trials are needed to prove the effectiveness (Hussein, 2016). Chyad et al. (2017) demonstrated that the extract of *Lepidium draba* had anticancer, analgesic and anti-inflammatory activities (Chyad, 2017).

Conclusion

According to the findings of this study, Mocheh extract showed more effective impact on the growth of gram-positive bacteria than the gram-negative ones. Since Mocheh is extensively distributed in Iran and its extract showed significant antimicrobial and antioxidant activities, this plant might be utilized as a raw material to produce natural antioxidants and/or preservatives for the food industry. Moreover, our findings are supporting the use of Mocheh as a traditional remedy in the treatment of gastrointestinal disorders.

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ارزیابی پتانسیل آنتی‌اکسیدانی و فعالیت ضدمیکروبی عصاره مچه (*Lepidium draba*) در شرایط برون‌تنی

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چکیده

استفاده از محصولات طبیعی مانند گیاهان دارویی، فرصت‌های نامحدودی برای معرفی مواد افزودنی جدید فراهم کرده است. از مچه برای تهیه سوپ، سالاد و غذاهای تازه استفاده می‌شود. این مطالعه با هدف سنجش فعالیت ضدمیکروبی و آنتی‌اکسیدانی گیاه مچه انجام شد. اثر ضدمیکروبی عصاره مچه در برابر *باسیلوس سوبتیلیس*، *سودوموناس آئروژینوزا*، *استافیلوکوکوس اورئوس* و *آسپرژیلوس نایجر* مورد آزمایش قرار گرفت. نتایج نشان داد که حداقل غلظت مهارکنندگی عصاره مچه برای *آسپرژیلوس نایجر*، *سودوموناس ائروژینوزا*، *باسیلوس سوبتلیس* و *استافیلوکوکوس اورئوس* به‌ترتیب ۱۲۸، ۱۲۸، ۱۲۸ و ۱۲۸ میلی‌گرم بر میلی‌لیتر بود. حداقل غلظت کشندگی عصاره نیز به‌ترتیب برای سویه‌های مذکور ۲۵۶، ۲۵۶، ۲۵۶ و ۲۵۶ میلی‌گرم بر میلی‌لیتر بود. مقادیر به‌دست آمده برای فلاونوئید کل و محتوای فنلی کل به‌ترتیب ۲۲/۱۳ (میکروگرم بر میلی‌لیتر)، ۱۸/۸۸ (میلی‌گرم در میلی‌لیتر) بود. فعالیت آنتی‌اکسیدانی برحسب IC_{50} ۱۶۸/۲۱ میکرولیتر در میلی‌لیتر بود. نتایج این مطالعه در مورد فعالیت‌های بیولوژیکی عصاره مچه احتمال استفاده از عصاره آبی برگ مچه را به‌عنوان منبع جدیدی از مواد ضدمیکروبی و آنتی‌اکسیدان طبیعی مواد غذایی و صنایع دارویی ارائه می‌دهد.

واژه‌های کلیدی: مچه، عصاره، باکتری‌های گرم مثبت و گرم منفی، فعالیت آنتی‌اکسیدانی.

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