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The Evaluation of Some Biological Activity of *Mentha longifolia* (L.) Huds Growing Wild in Iran

[1]Seyed Mehdi Razavi, [2]Gholamreza Zarrini and [1]Ghader Molavi
[1]Department of Biology, Faculty of Science, University of Mohaghegh Ardabili, Ardabil, Iran
[2]Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran

Abstract: *Mentha longifolia* (L.) Huds. is a perennial rhizomatous herb distributed from Southern Africa, Europe, the Mediterranean region and eastwards into Asia. *Mentha* species are also generally known under name of Puneh in Iran where they have been used for centuries as tonics, carminative, digestive, stomachic, antispasmodic and anti-inflammatory agent in folk medicine. In the present work, we study antimicrobial and cytotoxic activity of the plant extracts. The antibacterial and antifungal activity of the plant extracts were evaluated using disk diffusion method. We evaluated cytotoxicity of *M. longifolia* with MTT assay, as well as. Present finding revealed that the methanol extract of the plant leaves were active against all tested bacteria and fungi. The highest inhibitory effect was observed against *Erwinia carotovora*, a common plant pathogen bacteria, with MIC value of 128 μg mL^{-1} and inhibition zone of 41 mm. The extract also exhibited high antibacterial activity with MIC value rang of 192-512 μg mL^{-1} and inhibition zone of 34-40 mm against *S. aureus*, *Enterococcus faecalis*, *Streptococcus agalactiae* and *Escherichia coli*. The methanol extract of the plant displayed modest to strong antifungal activity against *Candida kefyr*, *Candida albicans*, *Sclerotinia sclerotiorum* and *Aspergillus niger* with MIC value range of 576-800 μg mL^{-1} and inhibition zone of 26-33 mm. Our finding showed that *M. longifolia* methanol extract has cytotoxic activity. The extract reduced the viability of McCoy cells with RC_{50} value of 1.92 mg mL^{-1}. It was be concluded that *M. longifolia* extract cab be used as an antiseptic agent and may be also a good candidate to construction of a new plant biopesticide.

Key words: Mentha longifolia, antiseptic, biopesticide

INTRODUCTION

The Labiatae family comprised of 220 genera of aromatic plants which are widely used for various purposes world wide. The genus *Mentha* is one of the important members of the family represented by species in the Flora of Iran (Mozaffarian, 1996). *Mentha* species are generally known under name of Puneh in Iran where they have been used for centuries as tonics, carminative, digestive, stomachic, antispasmodic and anti-inflammatory agent in folk medicine (Amin, 2005).

Mentha longifolia (L.) Huds. is the most widespread species of the genus in Iran. It is a perennial rhizomatous herb with erects to straggling stems reach 120 cm in height. The plant is an extremely variable species with a widespread distribution in Southern Africa, Europe, the Mediterranean region and eastwards into Asia (Rechinger, 1982).

There is many reports on bioactivity of *M. longifolia* in the literature. It was previously shown that the plant posses antioxidant (Nickavar *et al*., 2008), antimicrobial (Al-Bayati, 2009) and hepatoprotective activity (Nimica-Dukic *et al*., 1999).

Phytochemical studies revealed the presence of different flavonoides (Ghoulami *et al*., 2001), monoterpene ketones (Heganauer, 1953), tannins and saponins (Do Nascimento *et al*., 2009) in the plants of the genus *Mentha*. These chemicals are responsible for different pharmacological and biological activity of these plants. In the recent work, we focus on cytotoxic activity and antimicrobial properties of *M. longifolia* extracts on some human and plant pathogen microorganisms.

MATERIALS AND METHODS

Plant materials: The leaves of *M. longifolia* were collected from around of Ardabil, Iran during June 2009. A voucher specimen was deposited at the herbarium of faculty of sciences, University of Mohaghegh Ardabili, Ardabil, Iran (No. 1389-3).

Corresponding Author: Gholamreza Zarrini, Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran Tel: +98 0411 3392707

Preparation of the extracts: Air-dried plant leaves were Soxhlet extracted with n-hexane, dichloromethane and methanol, respectively. The extracts were dried in vacuum (Razavi *et al*., 2009).

Antibacterial assay: The antibacterial activities of the plant extracts were determined against *E. coli* (PTCC 1047), *S. aureus* (PTCC 1112), *E. faecalis* (PTCC 1394), *S. agalactiae* (PTCC 1321), *Erwinia carotovora* (PTCC, 1675) and *S. aureus* (E_{38}) by the disc diffusion method (Razavi and Nejad-Ebrahimi, 2009). Muller-Hinton Agar (MHA) (oxoid) was used for preparation of the media for bacteria. The filter paper discs (6 mm in diameter) were individually impregnated with 15 μL of stock solution of the plant extracts (100 mg mL^{-1}) and then placed onto the agar plates which had previously been inoculated with the tested microorganisms. The plates were inoculated at 37°C for 24 h. The diameters of inhibition zones were measured in millimeters. All the tests were performed in duplicate. Gentamicin (10 μg) and Erythromycin (15 μg) served as positive control. The MICs of the extracts against the test microorganisms were determined by the Agar dilution method (Razavi and Zarrini, 2010).

Antifungal assay: The antifungal activities of the plant extracts were determined against *C. kefyr* (ATCC 38296), *C. albicans* (ATCC 14053), *A. niger* (PLM 1140), *Penicillium* sp. and *S. sclerotiorum* by the disc diffusion method. Sabouraud Dextrose Agar (SDA) was used for preparation of the media for the fungal strains. The filter paper discs (6 mm in diameter) were individually impregnated with 15 μL of stock solution of the extracts (100 mg mL^{-1}) and then placed onto the agar plates which had previously been inoculated with the tested microorganisms. Amphotericin B 10 μg disc was applied as positive control. The plates were inoculated with the fungi incubated at 30°C for 48 h. The diameters of inhibition zones were measured in millimeters. All the tests were performed in duplicate. The MICs of the extracts against the test microorganisms were determined by the Agar dilution method (Razavi and Zarrini, 2010).

Cytotoxic assay: MacCoy cell lines (Pasteur, C_{123}) were grown in RPMI 1640 (Gibco, No. 51800-019) medium. Each 500 mL of the medium consisted of 5.2 g RPMI powder, 1 g of sodium bicarbonate, 1% w/v of penicillin/streptomycin and supplemented with 10% heat-inactivated Fetal Calf Serum (FCS) in demonized water (Razavi *et al*., 2010a). Completed medium was sterilized by filtering through 0.22 μm microbiological filters (Art No. 11107-25). Cell line was maintained in a humidified atmosphere of 5% CO_2 at 37°C in incubator. The stock solutions of methanol extracts of *Malva sylvestris* flowers and leaves were prepared by dissolving the compound in DMSO (100 μL). The final concentration of the extract was 0.70, 0.50, 0.30, 0.3, 0.10 and 0.05 mg mL^{-1}. Cells were plated in the appropriate media on 24-well microplates in a 500 μL total volume at a density of 6×10^5 cell mL^{-1}. Triplicate wells were treated with media containing different concentration of the extract. The plates were incubated at 37°C in 5% CO2 for time course of 16 h. For evaluating of cell viability, each well was supplemented with 50 μL of a 5 mg mL^{-1}) solution of MTT in uncompleted media and treated for 3 h at 37°C in 5% CO_2. The media was carefully removed from each well and 1 mL of DMSO and placed in room temperature for 20 min. The plates were gently agitated until the color reaction was uniform and the OD_{570} was determined using a spectrophotometer. The amount of MTT converted to formazan is a sign of the number of viable cells. Media-only treated cells served as the indicator of 100% cell viability. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the control in the MTT assay. Viability percentage was evaluated as $OD_{treatment}/OD_{control}$ (Razavi *et al*., 2010b).

RESULTS AND DISCUSSION

Table 1 presents the results of antibacterial and antifungal assays. This finding revealed that *M. longifolia* extracts indicated considerable antimicrobial activity. The methanolic extract of the plant leaves were active against all tested bacteria and fungi. The highest inhibitory effect was observed against *E. carotovora*, a common plant pathogen bacterium, with MIC value of 128 μg mL^{-1} and inhibition zone of 41 mm. The extract also exhibited high antibacterial activity with MIC value rang of 192-512 μg mL^{-1} and inhibition zone of 34-40 mm against *S. aureus*, *E. faecalis*, *S. agalactiae* and *E. coli*. The methanol extract of *M. longifolia* was found to have inhibitory effect against meticillin resistant strain of *S. aureus* (E38), as well as.

Moreover, the methanolic extract of the plant displayed modest to strong antifungal activity against *C. kefyr*, *C. albicans*, *S. sclerotiorum* and *A. niger* with MIC value range of 576-800 μg mL^{-1} and inhibition zone of 26-33 mm (Table 2).

Table 1: Antibacterial effects of *M. longifolia* extracts

Treatment microorganism	Methanolic extract (1.5 mg mL^{-1}) Inhibition zone MIC (mm/µg/mL)	Dichloromethane extract (1.5 mg mL^{-1}) Inhibition zone MIC (mm/µg/mL)	Erythromycin (30 µg) Inhibition zone (mm)	Gentamicin (30 µg) Inhibition zone (mm)
Escherichia coli	34.512	14>1600	16	20
Staphylococcus aureus	40.192	13>1600	19	20
Enterococcus faecalis	39.192	13>1600	21	16
Streptococcus agalactiae	39.192	14>1600	21	16
Erwinia carotovora	41.128	13>1600	21	19
(E_{38}) *Staphylococcus aureus*	29.640	-	-	-

Table 2: Antifungal effects of *M. longifolia* extracts

Treatment microorganism	Methanolic extract (1.5 mg mL^{-1}) Inhibition zone MIC (mm/µg/mL)	Dichloromethane extract (1.5 mg mL^{-1}) Inhibition zone MIC (mm/µg/mL)	Amphotericin (µg 10) Inhibition zone (mm)
Candida kefyr	33.576	14>1600	34
Candida albicans	30.576	12>1600	29
Aspergillus niger	24.800	-	24
Penicillium sp.	25.800	-	23
Sclerotinia sclerotiorum	26.800	-	28

The finding of present study has also showed that dichloromethanic and hexanic extract have no considerable antimicrobial effect against tested microorganisms.

On the other hand, our finding showed that *M. longifolia* methanol extract has modest cytotoxic activity. The extract reduced the viability of McCoy cells with IC_{50} value of 1.92 mg mL^{-1} (Fig. 1).

It was previously documented that methanol, ethanol and water extracts of *M. longifolia* have a good antimicrobial activity (Ikrani and Inam-ul-haq, 1980; Jawad *et al*., 1988; Akroum *et al*., 2009). However, no activity was reported against *E. coli*, *S. aureus* and *C. albicans* in these studies. Our finding depicted that the methanolic extract of the plant is very active against the mentioned human pathogen microorganisms. Therefore, it can be concluded that Iranian sample of *M. longifolia* has a great antimicrobial potential than other samples and could be regarded as a specific chemotype of the species.

Phytochemical studies revealed the presence of flavonoides in great quantity in *M. longifolia* (Ghoulami *et al*., 2001; Akroum *et al*., 2009). Many flavonoides like quercetin, luteolin, apigenin and kaempferol glycosylated derivatives were found in the plant leaves. It was realized that this compound are responsible for high antimicrobial activity of the plant methanol, ethanol and water extracts. It can be also concluded that high antimicrobial activity of the methanolic extract than dichloromethanic or hexanic ones should be attributed to prescience of flavonoides as polar compounds. It has also been shown that different glycosylated flavonoides exert a synergism effect in antimicrobial activity. Moreover, nonpolar volatile compounds like menthol isolated from *M. longifolia* essential oil were found to have strong antimicrobial activity (Al-Bayati, 2009).

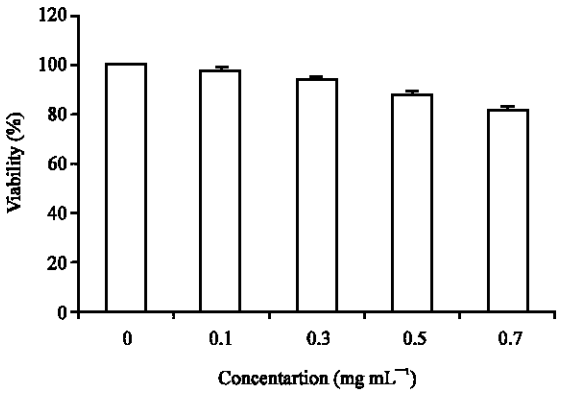

Fig. 1: Cytotoxic activity of methanolic extract of *M. longifolia* on Mc-Coy cell line using MTT test, Bars indicate standard error

The results herein reported showed that the plant methanolic extract has a great potential to suppress a meticillin resistant strain of *S. aureus* (E38). In the last decades, continuous using of antibiotics has caused some pathogen microorganism species ultimately evolved resistance to some antibiotics. The utilize of medicinal plant extracts or plant derived chemicals with antimicrobial activity could dissolve the problem.

The results of present study showed that *M. longifolia* methanol extract possess modest cytotoxic and antiproliferative activity. It is assumed that this biological effect of the plant extract is dependent to presence of phenolic compounds like flavonoides. A previous paper also demonstrated the *M. longifolia* extracts indicated high antioxidant potential (Nickavar *et al*., 2008). Antioxidants may act as free radicle scavengers which suppress the free radicle damages in biological systems and then may be associated with a

reduced risk of cancer. Therefore, *M. longifolia* can be regarded as chemopreventive agent against cancer.

On the hand, Present findings showed that the *M. longifolia* extract exhibited a good inhibitory effect against plant pathogen microorganisms, *E. carotovora* and *S. sclerotiorum*. The former microorganism is a pathogen bacterium causes soft rot in a wide range of fruits and vegetable species and is considered as a serious problem in horticulture (Strange, 2003). The later organism causes stem rot in many plants and it is one of the most prevalent plant pathogen fungi. Therefore, the extract can be used as a biopesticide. Recently, the use of synthetic pesticides is claimed to negatively affect the environment and actually it dose not represent an appropriate tool for the control of plant pathogens developing resistance. So, natural derived materials can be exploited as an alternative for synthetic pesticides (Razavi and Nejad-Ebrahimi, 2009).

CONCLUSION

It was concluded that *M. longifolia* can posses antiseptic activity. The utilizing of this plant in treatment of sore throat and throat irritation in the Iranian folk medicine is validated scientifically by the results obtained in the present study.

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