Science Arena Publications
Specialty Journal of Biological Sciences
Available online at www.sciarena.com
2017, Vol, 3 (4): 38-43

Antifungal Potential of *Mentha piperita* Leaves and Stem Extracts against Phytopathogenic Fungi

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Abstract: The antifungal potential of six extracts (methanol, ethanol, water, acetone, chloroform and Hexane) of leaf and stem of *Mentha piperita* against phytopathogenic fungi such as *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus oryzae* was carried out by agar well diffusion method. The methanol extract of *Mentha piperita* leaf demonstrated marked inhibition compared to hexane, ethanol, water, chloroform and acetone. The stem of all extracts showed no activity against all the tested fungi. This study indicates that the leaves extracts have the potential in the management of diseases caused by plant pathogenic fungi.

Keywords: *Aspergillus*, solvent extract, plant diseases, aerial parts, pesticide, environment

INTRODUCTION

The main pathogenic agents in plants are phytopathogenic fungi, which cause changes during early as well as post harvest stages. In vegetables and fruits, there are wide ranging types of fungi genera which are responsible for quality problems concern to limited shelf life, organoleptic characteristics and nutritional values (Agrios.2004). Furthermore, in a few circumstances fungi are indirectly blamable for toxic or allergic disorders among customers due to the synthesis of allergens and toxic compounds (Paola,et al.2011). In developing counties, 12% pre harvest losses were due to fungal disorders in world crop production (Lee, S.E,et al.2001). Numerous third world countries haphazardly practice different kinds of toxic and hazardous chemicals in their crops to control field infections. Amongst these, important infections are caused by fungi. Huge amounts of fungicides are used by growers in their fields to control fungal diseases (Kuri SK,et al.2011). The majority of fungal diseases are come from soil, seeds, crop refuses and other sources. Fungi harbor or attack in the seeds at the time of or after sowing, storing and fruiting stage. Seeds are damaged by fungi during storage and crops at seedling stages. Fungus is inactive in seed and transfers to seedlings, adult plant and develops symptoms (Zeringue HJ,et al.1990). Seedling death and germination failure are mainly caused by fungi (Goldblatt, L. A.1971). Therefore, the farmers need to pay extra attention for excellent health of seedlings and seeds. As a

result, they face extra burden of labor, cost, toxic chemicals, as well as more issues and problems (Kuri SK,et al.2011).

Synthetic fungicides are commonly used to control fungal diseases. But these fungicides create many problems and they are toxic to humans and environment (Harris,et al.2001). The use of pesticides and pathogens resistance increase day by day due to increase in production and regulation demand, so it is need of the day to explore new and safe pesticides (Paola,et al.2011).

M*entha piperita* is grown in many countries both as cultivated and wild. Generally, it is used as tea, extraction of oil and tincture externally applied as ointment or massage. Researchers believe, it has anti-aging, stimulant, rubificient, antimicrobial, astringent, antipyretic, antispasmodic and antiseptic properties (Ali MA,et al.2002). The objective of the study was to assess the antifungal properties of the locally grown mint plant leaves and stem.

Materials and Methods

Plant Collection and Preparation

Healthy plants (*Mentha piperita* L.) were collected from Medicinal Botanical Garden of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Peshawar Khyber Pakhtunkhwa-Pakistan, washed thoroughly with clean tap water and then put for drying under shade for ten days.

Extraction procedure and preparation of plant extracts

Fifty grams' powder of *Mentha piperita* (leaves and stem) were extracted with 500 ml solvents of water, ethanol, chloroform, methanol, acetone and *n*-hexane separately for 48 hrs. Extracts filtration was done through Whatman filter paper (repeated three times). After the extracts were made concentrated in rotary evaporator, the same were shifted to a microbial free beaker for heating in a water bath (45°C) to get a dehydrated filtrate. Sealed bottles were used for the storage of crude extracts and preserved at 4°C until use.

Standardization of fungal strains

Fungal strains were arranged by transferring a loop-full of fungal cells from Sabourauds dextrose (SDA) agar slants into test tubes having Sabouraud dextrose broth followed by the incubation of the test tubes without agitation for 72 h at 28°C. The turbidity of suspension was adjusted to a 0.5 McFarland turbidity standard (1.0×10^6 CFU/mL).

Antifungal Activity

The leaf and stem extracts were tested for their antifungal activity in comparison with standard antifungal agent Amphotericin B in vitro by well diffusion method. Lawn culture was made using the test fungi on SDA. The cultured plates were place aside for few minutes using well cutter, 04 wells were prepared in those plates at appropriate distance. Plant extract volume (0.1ml=500 mg/ml) was then pouring into the wells. The plates with fungi were incubated for 48 hours at room temperature. The antifungal potential of the extract was calculated by quantifying the zone of inhibition diameters (Senthil,et al.2012).

Statistical Analysis

Means and standard deviations were calculated for three independent determinations for each variable using SPSS program.

Results

Both non-polar as well as polar solvents were used for extracting active crude extract from the leaves and stem of *Mentha piperita*. *Mentha piperita* activity against fungus was determined by use of agar well diffusion method by measuring growth inhibition zones diameter. The results of leaves extracts shown in (Table 1) indicate that the inhibition zone were increased in the order; methanol > hexane > ethanol > water > chloroform > acetone. No zones of inhibition were observed of stem extracts (data/table not shown).

Discussion

Genus Aspergillus Fungi are filamentous organisms; they generate spores in the air and have a wide distribution as saprophytes in nature. They may turn pathogenic to humans and may the reason of a range of lesions, out of which, pulmonary disease is the most significant (Naseem U,et al.2012). *Mentha arvensis* leaves extract (30% concentration) showed 73.69%, 66.67%, 68.82% and 50% inhibition against *A. fumigates, A. terrus, A. flavuss* and *A. niger* respectively (Manoorkar,et al.2014). *Mentha longifolia* methanol extract exhibited strong antifungal activity against *Aspergillus niger, Sclerotinia sclerotiorum,* Penicillium spp., *Candida kefyer* and *Candida albicans* with zone of inhibition value range 26-33 mm zone of inhibition and 576-800 g/mL minimum inhibitory concentration (Syed,et al.2012). *M. spicata* extract with 80% concentration the mycelia growth of *A. flavus* was 3.3 cm inhibited followed by *F. oxysporum* (6.4 cm), *P. digitatum* (6.1 cm) and *R. stolonifer* (4.3 cm) comparing to control fungi with 9.0 cm growth (Bushra,et al.2017). The *Mentha piperita* leaf extract showed a considerable enhancement in inhibition of *P. spinulosum* (28.20% to 43.58%) and *A. fumigatus* (38.46% to 48.71%), while *A. niger* showed a sharp reduction from 27.02% to 5.40% (Neeha B,et al.2017).

The extraction from leaves of *M. pulegium* Methanol, observed no antimicrobial activity (Hanene,et al.2013). These findings confirm the results of Hajlaoui *et al.* (2009)and Gulluce *et al.* (2007) that has shown that methanolic extract of aerial parts of (*M. pulegium* and *M. longifolia*) observed no antibiotic activity. In the present stem extracts showed no antifungal activity.

Plant extract antibiotic mode of action was due to phenolic substances present in these plants. As a response of microbial infection phenolic compounds were prepared in plants. So, it is concluded that phenolic compounds act as antimicrobial agents against microbes. The antimicrobial activities of herbs extracts are not solely due to the phenolic constituents, but this ability also depends on diverse types of secondary metabolites e.g. OH functional groups on the bioactive compounds. The bioactive compounds of herbs extracts are known as bactericidal agents, due to the capability of these compounds to bind with microbial adhesion and by doing so they upset the location of surface receptors (Branter,et al.1996). The principle of bioactive compounds by which they applied strong antimicrobial activities is accredited to their cellular membranes effect. Some research studies showed that active compounds attack cell membrane and cell wall, as a result demolish their permeability barrier and originate the liberate of intracellular components like Na glutamate and ribose.

Moreover, they interfere with protein, electron transport, enzyme activity, nucleic acid synthesis and nutrient uptake leading to the microbe's growth inhibition [18]. Polyphenolic substances are recognized to increase the antibiotic potential by raising the accumulation of metals like Fe, Cu, Zn and Mg (Padmini,et al.2010).

Conclusion

The results achieved from this study showed that *Mentha piperita* extracts exhibit antifungal effects against *Aspergillus* sp. So, the current study concluded that methanol extracts of *Mentha piperita* leaves would be useful to treat phytopathogenic fungi. Finally, the results suggest that the study plant can be utilized as a pesticide to manage the propagation of Aspergillus sp. and hence decrease the utilization of artificial fungicides. Additional investigations may be conducted for determining the chemical individuality of the bioactive materials accountable for the antifungal activity which was noted in the current research work.

References

1. Agrios, G.N. 2004. Losses caused by plant diseases. p. 29-45. Plant Pathology. Elsevier, Oxford, UK.
2. Paola DD, Andrea C, Diego A, Patricia L, Fernando F and Marco DR. 2011. Antifungal Activity of Medicinal Plant Extracts against Phytopathogenic Fungus *Alternaria* SPP. Chilean Journal of Agricultural Research. 71(2): 231-239.
3. Lee, S.E., J.E. Kim and H.S. Lee. 2001. Insecticide resistance in increasing interest. Agri. Chem.Biotechnol. 44: 105-112.
4. Kuri SK, Islam MR and Mondal U. 2011. Antifungal potentiality of some botanical extracts against important seedborne fungal pathogen associated with brinjal seeds, *Solanum melongena* L. Journal of Agricultural Technology. 7(4): 1139-1153.
5. Zeringue HJ and Bhatnagar D. 1990. Inhibition of aflatoxin production in Aspergillus flavus infected cotton bolls after treatment with neem (*Azadirachta indica*) leaf extracts. Journal of American Oil Chemical Society. 67: 215–216.
6. Goldblatt, L. A. (1971). Control and removal of aflatoxin. Journal of American Oil Chemistry 48(10): 605–610.
7. Harris, C.A., M.J. Renfrew, and M.W. Woolridge. 2001. Assessing the risk of pesticide residues to consumers: recent and future developments. Food Additives and Contamination 18:1124-1129.
8. Ali MA, Saleem M, Ahmad W, Parvez M and Yamdagni R. 2002. A Chlorinated Monoterpene Ketone, Acy-lated-Sitosterol Glycosides and a Flavanone Glycoside from *Mentha longifolia* (Lamiaceae). Phytochemistry. 59(8): 889-895.
9. Senthil KS, Sivamani P, Baskaran C, Jamal MM. 2012. Evaluation of Anti Microbial Activity and Phytochemical analysis of Organic Solvent extracts of *Calotropis gigantea*. IOSR Journal of Pharmacy. 2 (3):389-394.
10. Naseem U, Ali J, Khan FA, Khurram M, Hussain A, Rahman I, Rahman Z and Shafqatullah. 2012. Proximate Composition, Minerals Content, Antibacterial and antifungal Activity Evaluation of

Pomegranate (*Punica granatum* L.) Peels Powder. Middle-East Journal of Scientific Research. 11 (3): 396-401.

11. Manoorkar VB and Gachande BD. 2014. Evaluation of antifungal activity of some medicinal plant extracts against some storage seed -borne fungi of Groundnut. Science Research Reporter. 4(1): 67-70.
12. Bushra A, Sobia C, Rashida P, Muhammad A and Mirza AM. 2017. Chemical composition and antifungal potential of medicinal plants against seedborne mycofl ora of eggplant (*Solanum melongena* L.). Acta Bot. Croat. 76 (1): 72–79.
13. Neeha B, Jyoti, Garg AP. 2017. Antifungal Activities of Some Medicinal Plants and Weeds. Journal of Emerging Technologies and Innovative Research. 4(05):132-136.
14. Hanene G, Aouadhi C, Moufida W, Faten T, Sebei H, Maaroufi A and Hasnaoui B. 2013. Chemical Composition and In Vitro Antimicrobial Activities of Mentha pulegium Leaves Extrats against Foodborn Pathogens. Journal of Food Safety. 33: 239–246.
15. Hajlaoui H, Trabelsi N, Noumi E, Snousi M, Fallah H, Ksouri R and Bakhrouf A. 2009. Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine. World J. Microbiol. Biotechnol. 25:2227–2238.
16. Gulluce M, Sahin F, Sokmen M, Ozer H, Ozeer H, Daferera D, Sokmen A, Polissiou M, Adiguzel A and Ozkan H. 2007. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia L. ssp. longifolia*. Food Chem. 103: 1449–1456.
17. Branter A, Males Z. and Antolic A. 1996. Antimicrobial activity of paliurus spina-christi Mill. (christis thorn). *J. Ethnopharmacol.* 52: 119-122.
18. Zhang YM, White SW and Rock CO. 2006. Inhibiting bacterial fatty acid synthesis. *J. Biol. Chem.* 281: 17541-17544.
19. Padmini E, Valarmathi A, Usha Rani M. 2010. Comparative analysis of chemical composition and antibacterial activities of *Mentha spicata* and *Camellia sinensis.* Asian J. Exp. Biol. Sci. 1(4): 772- 781.
20. Syed MR, Gholamreza Z, Ghader M. 2012. The Evaluation of Some Biological Activity of Mentha longifolia (L.) Huds Growing Wild in Iran. Pharamcologia. 3(10):535-538.

Table 1 Antifungal Activity of *M. piperita* Leaves Extracts

Fungi	Zone of Inhibition (mm)							
	Extracts						Control	
	Water	Methanol	Ethanol	Acetone	Chloroform	Hexane	+Ve	-Ve
Aspergillus niger	08±0	19±1	10±0	03±0	05±0	11±0	17±1	NZI

Aspergillus parasiticus	10±1	16±1	12±0	04±0	07±1	13±1	16±1	NZI
Aspergillus flavus	06±0	18±2	09±1	06±0	04±0	14±1	15±0	NZI
Aspergillus fumigatus	07±0	15±1	14±0	NZI	03±0	15±0	20±2	NZI
Aspergillus oryzae	12±1	14±0	10±0	05±0	08±0	13±1	21±2	NZI

Results of the average of three replicates, ± = Standard Deviation, NZI = No zone of inhibition, +Ve= positive control (Amphotericin B), -Ve= Negative control (5%DMSO).