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Antibacterial Potential of *Mentha piperita* Leaves and Stem Extracts against Food Borne Bacterial Pathogens

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Abstract: The current study was carried out to evaluate the antibacterial activities of *Mentha piperita* stem and leaves extracts in (*n*-hexane, methanol, ethanol, acetone, chloroform and water). Agar disc diffusion techniques were employed for determination of antimicrobial activities. Methanol leaves extract showed the highest zone of inhibition against *E. coli* (18±2 mm) and *S. aureus* (17±1 mm) as compared to other bacterial strains, while it showed the lowest activity against *P. aeruginosa* (11±0 mm). *Mentha piperita* leaves extracts should be utilized as good quality inhibition means to reduce some pathogenic bacteria.

Keywords: Solvent Extracts, Diseases, Antibacterial Activities, Aerial parts, Mint

INTRODUCTION

M*entha piperita* (*M. piperita*) is found in different parts of the world both as wild and cultivated. M*entha piperita* is utilized as extract, oil, tincture and tea. Plant experts consider it as an anti-aging, stimulant, emmenagogue, rubefacient, antimicrobial, astringent, anticatarrhal, antipyretic, antispasmodic and antiseptic properties (Ali M et al, 2002).

Utilization of herbs as medicine to cure different diseases is still in practice in many budding countries. The history to utilize herbal drug might be as old as the mankind history (Srinivas P et al, 2012). The antimicrobial activities of medicinal plants were due to presence of many phytochemicals like flavonoids, anthocyanins and isoflavones (Sharma A et al, 2013).

Food borne diseases and food poisoning caused by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and Salmonella spp., have been documented as threatening health problems because it deteriorates food products quality in many developing countries (Costa E et al, 2010; Lund BM et al, 2011). To solve food borne diseases issue, the use of these plant source products as antibiotics is environment friendly, low cost and good substitute regarding bacterial resistant issues (Schelz Z et al, 2010). Food borne ailments epidemiology is altering and research finding outcomes from different countries of the world concluded that

resistant strains of food borne microbes are a problem of community health. From the last twenty years it is seen that many food borne pathogens were resistant to the already marketed antibiotics (Slutsker L et al, 1998). So, it is need of the day to search new plant based antibiotics, which are essential to solve the antibiotics resistance problem of bacterial pathogens. Therefore, the main objective of the present research work was to determine and measure antibiotics potential of locally grown *Mentha piperita* leaves and stem against food borne pathogenic bacteria.

Materials and Methods

Plant Collection and Preparation

Healthy plants of *M. piperita* L. were collected from Medicinal Botanical Garden of Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Peshawar Khyber Pakhtunkhwa-Pakistan. The plants were washed thoroughly in tap water and shade dried for two weeks. The leaves and stem were separated. The leaves and stem were powdered with the help of cutting mill. The powder was packed in sealed plastic bags and stored in cold incubator (Gallenkamp, England) at 4°C until used.

Preparation of plant extracts

The powdered material (stem and leaves) were extracted in 500 ml aqueous, ethanolic, acetonic, methanolic, chloroform and *n*-hexane solutions for 48 hrs. The filtration of extracts was performed through Whatman filter paper (repeated three times). The extracts were concentrated in a rotary evaporator (Buchi Heating Bath B-490, Buchi Rotavapor R-200 Switzerland) after which transferring these to a 50 ml beaker (previously sterilized). These were heated in a water bath (at 50°C) to get dehydrated filtrate. These crude extracts were shifted to sealed bottles which were preserved at 4°C in cold incubator (Gallenkamp, England) until used.

Maintenance of Bacterial Strains

The bacterial strains (shown in table 1) were procured from Food Microbiology Section of PCSIR Laboratories Complex Peshawar, Khyber Pakhtunkhwa-Pakistan. These stock cultures were stored in Nutrient agar medium at 37°C.

Culture Standardization

Active cultures of bacterial pathogens were prepared by transferring a loop-full of bacterial cells from nutrient agar slants into test tubes containing Mueller-Hinton broth. After that, the test tubes were incubated without agitation for 24 h at 37 °C. The bacterial suspension turbidity was adjusted to a 0.5 McFarland turbidity standard (1.2×10^8 CFU/mL).

Antibacterial test of plant extracts

Agar disc diffusion techniques were employed for antibacterial activity of *M. piperita* leaves and stem extracts (Bauer AW et al, 1966). The test samples were prepared by dissolving measured amounts of samples in calculated solvent volumes dissolved in 5% sterile dimethyl sulfoxide (DMSO) for each extracts. Dried and sterilized Whatman filter paper discs (6 mm diameter) were then impregnated with 50 µL (200 mg/mL concentration) of the test extracts. These discs were allowed to dry and placed onto tested plates for thirty minutes for incubation. These plates were then, kept at 4°C for one hour to diffuse the test material before

incubation with the test microbial agents. Then the discs with material for testing were placed on medium (nutrient agar in our case) uniformly seeded with the pathogenic test microorganisms. For this purposes hundred micro liter of microbial suspension was spread onto the nutrient agar plates with the help of sterilized glass spreader. Then the Petri plates were incubated for 24 hours at 37 °C. At the end of incubation period, the antibiotic activity was monitored through a strong inhibition zone and calculated with Vernier Caliper scale.

Statistical Analysis

Means and standard deviations were calculated for three independent determinations for each variable using Statistical Package for Social Sciences (SPSS, 21.0) program.

Results

The antibacterial activity of *M. piperita* leaves extracts in different solvents is presented in table 1. The methanolic extracts showed strong inhibition zone as compared to other extracts. The inhibition activities increase in the order of methanol > n-hexane > ethanol > acetone > chloroform > water. No zone of inhibition was observed in stem extracts in different solvents extract (data/table not shown).

Discussion

In this research work, we used polar and aqueous solvents for crude active extracts from leaves and stem of *M. piperita*. The study results revealed that leaves extracts were active against bacterial strains as compared to stem extract. M*entha piperita* dried powder generally possessed 0.3-0.4% of volatile oil. The oil comprises of menthofuran, menthone, menthol and menthyl acetate (Leung AY, 1980). Many bioactive compounds like limnone, menthyl acetate, menthol, menthone and menthofuran are present in *Mentha piperita* leaves (Fleming T, 1998). These substances have greater therapeutic properties particularly to cure impaired digestion, eruptions, dyspepsia, epigastric bloating, common cold and itching relief (Alkofahi AS et al, 1990).

Flavonoids and tannins are famous for its scavenging, antimicrobial, healing and anti-inflammatory properties (Thiago ASA et al, 2008). Methanol leaves extract of *M. piperita* showed antimicrobial activities due to the presence of flavonoids and tannins (Pramila DM et al, 2012).

The Minimum Inhibitory Concentration (MIC) of *M. piperita* leaves extracts was 10 mg/mL (*Bacillus substillis* and *Pseudomonas aerogenosa*), 0.25 mg/mL (*Streptococcus aureus*) and *Pseudomonas aureus* was inhibited at 0.35 mg/mL (Bupesh G et al, 2007). *M. piperita* leaves juice displayed maximum zone of inhibition (17.24 mm) whereas minimum zone of inhibition (15.82 mm) revealed by stem juice (Sabahat Saeed et al, 2005) *M. piperita* leaves illustrated substantial biological activity due to limnone, menthone, menthol, menthofuran and menthyl acetate (Bupesh G et al, 2007). Continued and frequent consumption in daily diet of *M. piperita* leaves could be helpful to keep the disease causing bacteria below the threshold limit (Pramila DM et al, 2012). The research work (Deans SG, 1998) showed that *M. piperita* have potent compounds which possess strong antimicrobial activities. The plants cells possess biological activities due to secondary metabolites. Quantification of these compounds differ according to the weather, plants portions, seasons and maturity stage. Leaves are extremely useful and chief source of bioactive compounds (Jain AK et al, 2012; Mensah JK et al, 2008).

Conclusion

Medicinal botanical researches are in progress throughout the global world. Therefore, the current study will be useful and will provide guideline for the isolation and preparation of new medicines, health care products and drugs. Finally, it can be concluded that the bioactive molecules present in the leaves of *M. piperita* would find a way for treating food borne diseases and this fragrant plant should be pointed out for exploration and its capability as medication of different illnesses.

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Table1. Antibacterial Activity of *M. piperita* Leaves Extracts.

Bacteria	Zone of Inhibition (mm)						C^1	C^2
	Extracts							
	Hexane	Methanol	Ethanol	Acetone	Chloroform	H_2O		
Staphylococcus aureus	16±1	17±1	15±1	12±1	11±0	07±1	22±2	NZI
Escherichia coli	17±1	18±2	16±1	10±0	09±0	11±0	20±2	NZI
Enterococcus faecalis	13±0	15±0	14±0	13±0	NZI	09±0	18±2	NZI
Klebsiella pneumoniae	14±0	16±0	10±0	11±0	11±0	NZI	17±1	NZI
Pseudomonas aeruginosa	15±0	11±0	13±0	12±0	NZI	NZI	19±1	NZI
Salmonella Typhi	10±0	12±0	11±0	NZI	13±0	NZI	16±1	NZI
Bacillus cereus	11±0	13±0	12±0	NZI	NZI	NZI	18±1	NZI

Results of the average of three replicates, ± = Standard Deviation, NZI = No zone of inhibition, C^1=Positive control (Kanamycin 30 µg/disc), C^2= Negative control (5% DMSO, 50 µL/disc).