Journal of Medicinal Plants Research Vol. 6(2), pp. 331-335, 16 January, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1232
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Phytochemical analysis and antimicrobial potential of methanolic leaf extract of peppermint (*Mentha piperita*: Lamiaceae)

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Accepted 15 November, 2011

The present investigation aims to assess the phytochemical content, antioxdative activity and antimicrobial activity of the methanolic leaf extract of locally available *Mentha piperita*, the mint plant. The methanolic leaf extract of mint leaves was analyzed qualitatively for the phytochemical contents as previously described. The 1,1-diphenyl-2-picrylhydrazyl–hydrate radical (DPPH) free radical scavenging assay was carried out with reference to butylated hydroxy toluene (BHT). The antimicrobial susceptibility test, including minimum inhibitory concentration and minimum bactericidal concentration (MIC and MBC) were determined. The functional chemical groups were determined by Fourier transform infrared spectroscopy (FTIR). The methanolic extract was found to contain tannins and flavanoids, with considerable free radical scavenging activity. The plant extract showed antimicrobial activity against clinical isolates of *Escherichia coli, Acinetobacter, Staphylococcus aureus* and two fungi such as *Candida albicans, Candida glabrata*. The FTIR results indicated the molecular configuration of different functional groups in the plant extract. The mint leaf methanolic extract showed considerable antibacterial and antifungal activity against selected bacteria and fungi. Regular intake of mint leaves presumed to ward-off the initial colonization of selected pathogenic microbes.

Key words: *Mentha piperita*, antimicrobial properties, Fourier transform infrared spectroscopy (FTIR).

INTRODUCTION

Plants have been a potential source of medicine; though in a crude form, have been used from time immemorial to heal various ailments. A variety of bioactive compounds that are present in different parts of a plant has spurred a renewed interest in developing an alternate therapy. The traditional herbal medical system has been practiced globally from ancient times; consequently, a great volume of literature is available on the antimicrobial activity of a variety of plant species. Multidrug resistance among the microbial pathogens has been a great concern world over. Phytochemicals from plants have shown great promise in the treatment of intractable infectious human diseases including viral infections (Cowan, 1999). The aqueous extracts of *Camelia sinensis* and *Trachyspermum ammi* were found to be effective against *Salmonella* isolated from curry samples (Thanes et al., 2011). Methanolic leaf extract of *Coleus amboinicus* leaves showed remarkable antibacterial activity against methicillin resistant *Staphylococcus aureus* (MRSA) (Sahgal et al., 2009). It is presumed that drugs developed from plant sources may have minimal and very slow to induce drug resistance among the pathogens. From this perspective, it is imperative to screen a variety of plants

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with potential antimicrobial activity for periodical introduction to manage the drug resistance among the human pathogens.

Mentha piperita (Lamiaceae), the peppermint (mint) plant is an aromatic perennial herb cultivated in most part of the world, have traditionally been used in folk medicine. Leaves of mint plant are frequently used in herbal tea and for culinary purpose to add flavour and aroma. The distinctive smell and flavour, a characteristic feature of *Mentha* spp. is due to the naturally occurring cyclic terpene alcohol called menthol. Menthol is prescribed as a medication for gastrointestinal disorders, common cold and musculoskeletal pain (Patil et al., 2007). The mint plants are rich sources of iron and magnesium, which play important role in human nutrition (Arzani et al., 2007). A large volume of literature is available on the medicinal properties of essential oils present in *Mentha* spp. (Gulluce et al., 2007; Rasooli, 2008). However, no much study has been directed toward the antioxidant and antimicrobial properties of the mint leaves which are locally available. Hence, the objective of the study was to assess the phytochemical contents, antioxidant and antimicrobial properties of the locally grown mint plant leaves.

MATERIALS AND METHODS

Plant materials and extraction

Indigenously grown mint plants were collected from local village markets, in Sungai Petani, Kedah Darul Aman, Malaysia. The identification of plant material was confirmed by a botanist in the Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Malaysia. The plant material was thoroughly washed with clean water to remove soil and other dirt. Then the leaves were separated, air dried for complete drying. The dried plant material was powdered using a heavy duty blender. The powder was extracted with methanol according to the maceration method and the extract was filtered by Whatman no.1 filter paper. The filtrate was concentrated in a rotary evaporator at 40°C. The concentrated extract was oven dried at 40°C for 3 days and freeze dried for 48 h. The freeze dried extracts was stored at -20°C until use.

Phytochemical analyses

Methanolic mint leaf extract was used for preliminary qualitative screening of phytochemicals as per standard biochemical procedures as previously described by Ga Ayoola et al. (2008). The crude extract was diluted with methanol to the concentration of 1 mg/ml. The qualitative phytochemical analysis of crude methanolic mint leaf was conducted to determine the presence of reducing sugars (glycosides) saponins, tannins, anthraquinone derivatives, flavanoids and alkaloids.

DPPH radical scavenging activity

The antioxidant activity of the extract was measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl–hydrate radical (DPPH) to diphenylpicryl hydrazine by the plant extract. The absorbance values were measured spectrophotometrically at 517 nm and converted into percentage of antioxidant activity. Butylated hydroxy toluene (BHT) was used as standard. Each assay was repeated thrice and the results recorded as mean of the triplicated experiments were graphically illustrated.

The DPPH free radical scavenging activity of the extract was calculated using the following equation:

DPPH scavenging effect (%)

[(Absorbance of control - Absorbance of test sample) / Absorbance of control] × 100

Microbial cultures and growth conditions

Human oral pathogens such as *Escherichia coli, Acinatobacter* sp., *Staphylococcus* sp. isolated from a dental patient and known strains of *Candida albicans* and *Candida glabrata* were used as test organisms. Cultures of bacteria were grown for 10 h in nutrient broth (Oxoid, UK) at 37°C and were maintained on nutrient agar slants at 4°C. Cultures of *Candida* spp. were initially grown in potato dextrose broth at 28°C and were maintained at 4°C onto potato dextrose agar slants.

Antimicrobial activity assay by disc diffusion method

The dried plant extract was dissolved in methanol to a final concentration of 1 mg/ml. The leaf extracts in methanol was filter sterilized using membrane filter (pore size 0.47 µm). The bacterial strains were grown on nutrient agar (NA) then later in Mueller Hilton Broth (MHB) and the fungi were grown on potato dextrose agar (PDA) then in Mueller Hilton Broth (MHB). The final bacterial concentration was adjusted to 0.5 Mc Farland standard turbidity. This bacterial culture was used for plating onto Muller Hinton Agar (MHA) plates. Sterile Whatman filter paper (no.1) discs of 6 mm diameter were impregnated with 10 µl of crude extract at 1 mg/ml prepared using methanol. The discs were evaporated at 37°C for 24 h. The plates were air-dried under a sterile hood and the impregnated discs were placed at equidistant points on top of the agar medium. A disc impregnated with methanol was used a negative control. The plates were incubated at 37°C for 24 h. Chloramphenicol (30 ug/disc) and micanazole nitrate salt was used as a positive reference standard to determine the sensitivity of the bacterial and fungal strains respectively. Antimicrobial activity was evaluated by measuring the diameter zone of inhibition around the disc.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimal inhibitory concentration (MIC) is defined as the lowest concentration of the compounds which inhibits the growth of the microorganisms. Minimal bactericidal concentration (MBC) is defined as the lowest concentration of the compounds that kills and shows no growth of the microorganisms on an agar plate. The test microbes were grown in nutrient broth and potato dextrose broth for bacteria and fungus respectively as previously described and the cultures were adjusted to 0.5 McFarland standard turbidity. MIC and MBC values of the plant extract against bacterial strains and *C. albicans* and *C. glabrata* were determined based on a micro-well dilution method. The 24-well microtiter plates were prepared by dispensing into each well 2 ml of MH broth and 3 µl of the bacterial and fungal inoculum. The plant extract was dissolved in methanol to obtain the concentration of 1 mg/ml and was serially diluted and mixed thoroughly. Then the plates were incubated at 37°C for 24 h.

Table 1. The phytochemical substances in the crude methanolic mint leaf extract.

Phytochemicals	Observation	Result
Benedicts's test (to determine reducing sugars)	No change in colour of the extract	Absence of glycosides
Frothing test for saponins	No frothing	Absence of saponins
Ferric chloride test for tannins	Extract change into dark blue	Presence of tannins
Borntrager's test (anthraquinone derivatives)	No change in colour of the extract	Absence of anthraquinone derivatives
Flavanoid test	Formation of brown colour	Presence of flavanoids
Test for alkaloids	No precipitation	Absence of alkaloids

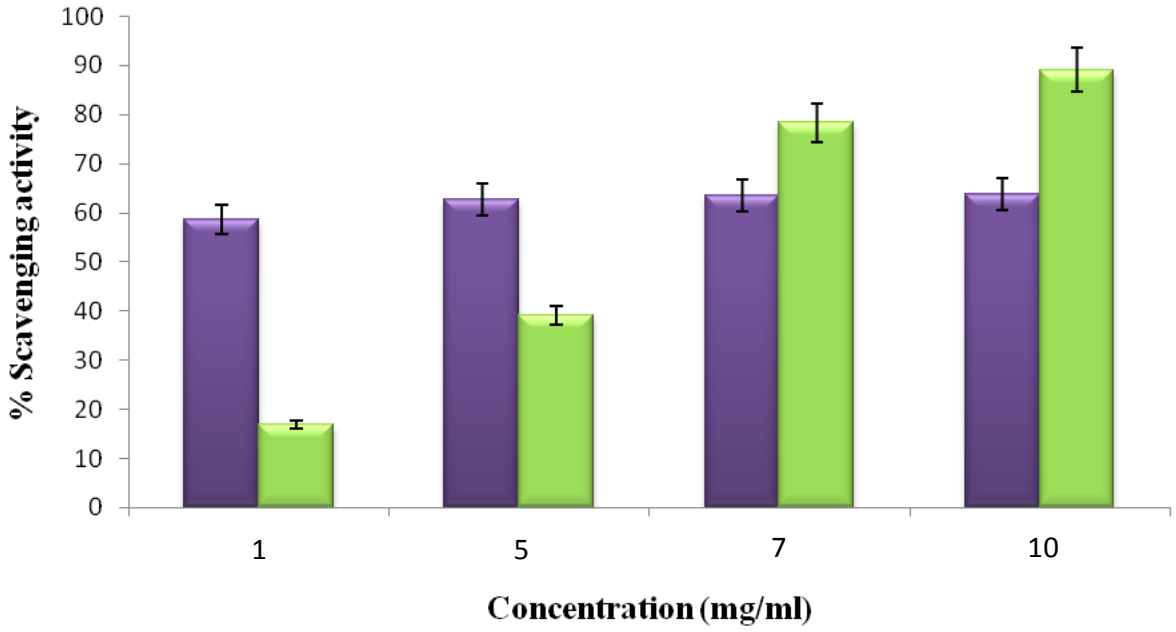

Figure 1. DPPH free radical scavenging activity of crude methanolic extract of mint leaves. (Purple bar: mint leaf extract; Green bar: BHT).

The least concentration of the extract with no visible growth after incubation was taken as the minimum bactericidal concentration.

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the extract. A small quantity (5 mg) of the extract was dispersed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Perkin Elmer 2000 infrared spectrometer. The sample was scanned from 4000 to 400 cm^{-1} for 16 times to increase the signal to noise ratio.

Statistical analysis

All the assays were carried out in triplicates. The experimental results were expressed as mean ± standard deviation. The data were analysed using one way analysis of variance (ANOVA) using SPSS version 11.

RESULTS

The results of the qualitative phytochemical analysis of the crude methanolic mint leaf extract showed the presence of tannins and flavanoids (Table 1). The free radical scavenging effect of the crude methanolic extract showed an increasing order of magnitude, still the difference in the free radical activity is marginal with reference to increasing concentrations of the crude extract tested. However, the free radical scavenging activity of BHT is in a dose-dependent manner (Figure 1).

The evaluation of antimicrobial potential by disc diffusion method indicated that all the bacterial and fungal strains tested showed growth inhibition toward the plant extract, however, with differing sensitivity. Among the bacterial pathogens, *E. coli* is more sensitive compared to *Staphylococcus* and *Acenatobacter*. With respect to the fungal pathogens, *C. albicans* showed a higher inhibition zone compared to *C. glabrata* (Table 2). The

Table 2. Antimicrobial evaluation of methanolic leaf extract of mint leaves by disc diffusion assay.

Microorganisms	Zone of inhibition (mm)	
Bacteria	**Plant extract**	**Control (Chloramphenicol (30 µg))**
Escherichia coli	1.37±0.29	9.17±0.60
Acinatobacter sp.	0.833±0.058	9.00±0.54
Staphylococcus sp.	1.10±0.1	9.20±0.70
Fungi		**Micanozole nitrate salt**
Candida albicans	1.63±0.058	2.23±0.058
Candida glabrata	0.567±0.058	0.733±0.058

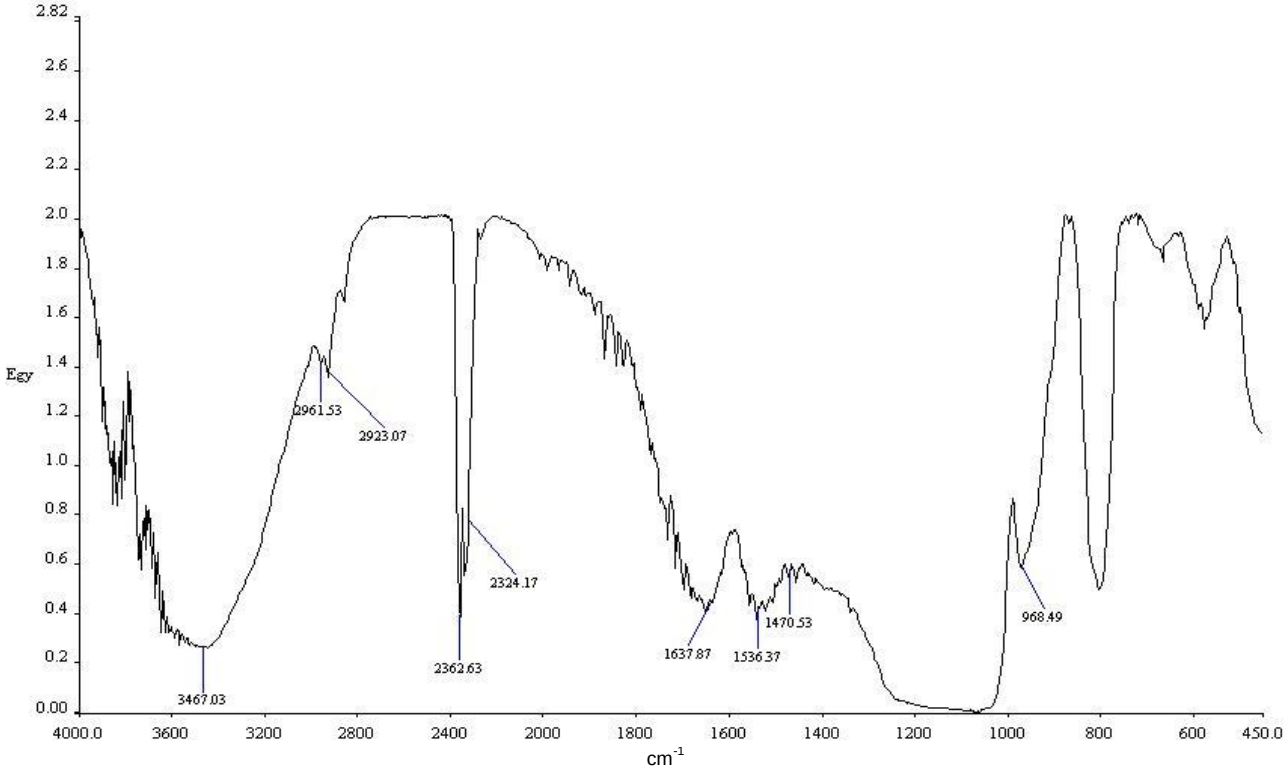

Figure 2. Fourier transform infrared spectroscopy (FTIR) analyses crude methanolic mint leaf extract.

minimum inhibitory concentration and minimum bactericidal concentrations of the plant extract against the test organisms were 3.125 and 6.25 µg/ml, respectively.

The aim of using FTIR analysis is to determine the existence of functional groups that exists on the isolate. The IR spectrum of the peppermint leaf extract in the form of KBr pallet is shown in Figure 2. The absorption at 3467 cm^{-1} is due to the stretching of hydroxyl groups that are present in the extract. The band at 2961 and 2923 cm^{-1} are due to the C-H asymmetric and symmetric stretching of saturated (sp^3) carbon, respectively. The band at 1637 cm^{-1} is assigned to the bending mode of absorbed water, since plant extracts are known to have a strong affinity for water. The band at 1536 cm^{-1} is due to C=C stretching associated with the aromatic skeletal mode of the extracts. The weak bands at 1470 and 968 cm^{-1} are assigned to C-H bending and C-O skeletal vibrations, respectively. It is also worthwhile to note that the prominent bands at 2362 and 2324 cm^{-1} are attributed to the asymmetric and symmetric stretching of CO_2.

DISCUSSION

The formation of biofilms in the form of plaques that harbours oral bacteria on teeth, causes tooth decay and has been implicated as a cause of serious infections (Zgoda and Porter, 2001). One among the methods to

manage the oral bacteria could be the use of herbal extracts. The herbal extracts offer several advantages such as unlimited availability and possibility of minimal problem of drug resistance by the oral bacteria. Mint leaves have been included in cooking as a flavouring agent in many parts of the world.

The present study showed the presence of tannins and flavanoids in the methanolic mint leaf extract. A correlative relationship has been reported between the phytochemicals such as tannins and flavanoids and the free radical scavenging activity and antibacterial activity (Kaur et al., 2010). Tannins and flavanoids have therapeutic uses due to their anti-inflammatory, anti-fungal, antioxidant and healing properties (Thiago et al., 2008). The antibacterial activity of *Quercus* sp. Bark is related to the richness of phenolic compounds such as flavanoids and tannins (Hideyuki et al., 2002; Meng et al., 2001). Thus, the antibacterial and antifungal activity of methanolic leaf extract of *M. piperita* is attributable to the presence of tannins and flavanoids. Nowadays, menthol is added in commercial tooth pastes to offer protection against oral microbial infections. The present study indicated the antibacterial activity of mint leaves against the selected oral pathogens. Frequent and continued intake of mint leaves in daily diet may prove beneficial in keeping the pathogenic microbes below the threshold level. The aqueous extract of *M. piperita* has considerable antibacterial activity against *Helicobacter pylori*, the main etiological agent of chronic gastritis and peptic ulcer disease (Castillo-Juarez et al., 2009). Further studies can be directed to evaluate the antimicrobial potential of mint leaves against a variety of gastrointestinal pathogens. Natural antioxidants and antimicrobial agents are preferred in food and cosmetic industry for their potential benefits compared to their chemical counterparts. Mint leaves could be a possible alternative to chemicals as it can be harnessed as antibacterial, antioxidant and flavouring agent.

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