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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 14 (4): 305-307, 2011
ISSN 1028-8880 / DOI: 10.3923/pjbs.2011.305.307

Antimicrobial Activity of *Hedeoma drummondii* against Opportunistic Pathogens

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Abstract: *Hedeoma drummondii* is a medicinal plant with diverse properties; however, validations of its medicinal uses are scarce. To evaluate its antimicrobial properties *H. drummondii,* was tested against opportunistic pathogens of medical importance. Antimicrobial tests were performed by the microdilution method in order to determine the Minimal Inhibitory Concentration (MIC) for each extract. Extracts of this plant showed relevant antimicrobial activity, results revealed that the hexanic extract has stronger activity and broader spectrum compared to acetone and methanol extracts. The activity of hexanic extract may be attributed mainly to the presence of the monoterpenes pulegone and menthol. In conclusion, the hexanic extract possess relevant antibacterial properties which suggests that *H. drummondii* have bioactive principles; these new data provide scientific support for the use of this plant in traditional medicine, particularly for gastrointestinal diseases.

Key words: Lamiaceaea, *Hedeoma drummondii*, medicinal plant, MIC, organic extract, monoterpenes

INTRODUCTION

Since ancient times, many infectious diseases are known to be treated with herbal remedies. Even today, plants continue to be used as therapeutic remedies in many developing countries. The World Health Organization reported that 80% of the world population uses traditional medicine (Akerele, 1993). Medicinal plants are important for pharmacological research and drug development, not only because plant constituents are used directly as therapeutic agents but also because they are used as basic compounds for the synthesis of new drugs, or as models for pharmacologically active compounds (Dev, 2010). In Mexico, about 4,000 plant species have medicinal uses (Ruiz-Bustos *et al*., 2009), various of these homeopathic remedies include Lamiaceae which is a great family of aromatic plants with medicinal properties. *H. drummondii* L. (Mexican poleo) is a small plant (4-15 inches) which has a very strong and pleasant mint aroma. *Hedeoma* plants are found in North America and aerial parts are used by North American Amerindians to treat the flu, cold, fever and stomach disorders (Foster and Hobbs, 2002). The chemical composition of *H. drummondii* consists mainly of sideritoflavone, luteolin-7-O-glucoside, caffeic acid, chlorogenic acid, rosmarinic acid and p-hydroxybenzoic acid; associated to these components, antioxidant and antiproliferative activities have been identified in our previous reports (Viveros-Valdez *et al*., 2008, 2010).

On other hand, infections caused by Opportunist Pathogens (OP) are rare in immunologically normal people. However, in patients with varying degrees of immune dysfunction, OP can cause serious health problems and sometimes death. These pathogens are responsible for the outbreak of nosocomical infections in different countries of the world and contribute to the morbidity and mortality in hospitalized patients. The uncontrolled antibiotic prescriptions have been identified as causes for an elevated rate of drug resistance development. By this reason, in order to prevent the occurrence of resistant bacteria, biotechnology and pharmaceutical companies are doing efforts in natural products research, to develop and to test new antimicrobials (Jazani *et al*., 2007).

H. drummondii has diverse uses in Mexican traditional medicine; however, studies validating its uses are scarce. As a follow up of our previous work, the aim of this study was to assess the antimicrobial activity from this aromatic plant against opportunistic pathogens which possess clinical importance.

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MATERIALS AND METHODS

H. drummondii (Benth) (Lamiaceae) was collected in Allende, Nuevo León state in México, during May and June 2005. The plant was identified by Dr. Marcela González Alvarez and a plant specimen was deposited in the ethnobotanical collection of the herbarium FCB-UANL, San Nicolas de los Garza, NL (voucher herbarium specimen number: 024244).

The aerial parts of the plant were dried at room temperature and 150 g of the dry plant were sequentially extracted by maceration with hexane, acetone and methanol (3 times, 24 h each). The plant: solvent ratio was 1: 5 (w/v). Organic extracts were concentrated *in vacuo* to dryness; all extracts were stored at 4°C until use. The percentage yield of extracts from *H. drummondii* was: hexane (2.47), acetone (3.01) and methanol (9.5).

Clinical isolates of *Bacillus cereus, Listeria monocytogenes, Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Enterobacter aerogenes, Serratia marcescens, Staphylococcus aureus, Shigella flexnerii* and *Candida albicans* were tested. By using 24 h cultures of these microorganisms, a bacterial suspension was prepared and turbidity was adjusted to 0.5 McFarland standard which corresponded to 10^6 colony forming units cfu mL^{-1} (Molina- Salina *et al*., 2007).

MIC for each extract was determined by the microdilution method, with dilutions ranging from 15.6 to 500 μg mL^{-1}. The extract that caused complete inhibition of growth after 24 h of incubation was considered active. All assays were performed in duplicate; gentamycin and ketoconozole were used as reference for positive controls.

The extracts were subjected to phytochemical screening; each organic extract was analyzed by specific reactions, as described by Harborne (1984). The color intensity of extracts and/or the appearance of solids in them during the identification reactions, allow a semi-quantitative evaluation of the presence of secondary metabolites.

RESULTS AND DISCUSSION

MIC values for extracts against the tested microorganisms were determined (Table 1). If extracts displayed MIC values from 100 to 500 μg mL^{-1} the antimicrobial activity was considered moderate. When MIC values were below of 100 μg mL^{-1}, the antimicrobial activity was considered good (Morales *et al*., 2008). The methanolic extract of *H. drummondii* displayed moderate activity against *Staphyloccocus aureus* and *Escherichia coli* (250 μg mL^{-1}) while the hexanic extract showed good activity against *Enterobacter aerogenes* (62.5 μg mL^{-1}) but was inactive against *Klebsiella pneumoniae* and *Serratia marcescens* (>500 μg mL^{-1}). With regard to present results, diverse authors have found that antimicrobial activity relies on the organic solvents used. For example, Gatsing *et al*. (2010) and Belboukhari and Cheriti (2005) demonstrated antimicrobial activity in the polar extracts while Ara *et al*. (2009) and Osadebe *et al*. (2008) reported activity with intermediate polarity and non-polar extracts, respectively. The observed activity for the hexanic extract is acceptable, considering that a crude extract was used and the active compound could be diluted. It is possible that isolating the active compound, or compounds, will provide lower MIC values. The above result suggests that *H. drummondii* could be an important source of non-polar compounds with antimicrobial activity.

As it can be observed in Table 2, the most of the secondary metabolites were identified in the polar (MeOH) extract. The concentration of polar metabolites is higher than non-polar metabolites in aerial parts of this Lamiaceae (Viveros-Valdez *et al*., 2008, 2010). On the other hand, the volatile compounds present in hexanic extract (extract with the broadest spectrum of activity) were analyzed using a GC-MS Perkin-Elmer AutoSystem GC equipment with a Quadrex 007.5MS column (30 m×0.25 mm, film thickness 0.25 μm); the pulegone (Rt 19.23 min, 76% relative abundance) and menthol (Rt 16.92 min, 9% relative abundance), were identified as the major monoterpenes constituents, both compounds have previously been reported to possess antimicrobial activity, including against yeasts (Duru *et al*., 2004; Al-Bayati, 2009).

Table 1: Antimicrobial activity of *H. drummondii*

		MIC (μg mL^{-1})			
		Organic extract			
Micro-organisms	Gram	MeOH	Acetone	Hexane	Control*
Bacillus cereus	G+	-	-	125	16
Staphyloccocus aureus	G+	250	-	125	16
Listeria monocytogenes	G+	-	-	500	16
Escherichia coli	G-	250	-	250	8
Klebsiella pneumoniae	G-	-	-	-	16
Enterobacter aerogenes	G-	-	-	62.5	8
Proteus vulgaris	G-	-	-	500	16
Serratia marcesceus	G-	-	-	-	32
Shigella flexnerii	G-	-	-	250	16
Candida albicans	-	-	-	125	32

*Gentamycin was used as positive control for bacteria and Ketoconozole for *Candida albicans*

Table 2: Phytochemical screening of *H. drummondii* extracts

Extracts	Alk	Flav	Coum	Sap	Phenolics	Sesq	RS	Terp
Hexane	-	+	+	-	+	+	-	++
Acetone	-	+	-	-	+	-	-	+
MeOH	-	++	+	-	++	+	+	++

Alk: Alkaloid, Flav: Flavonoids, Coum: Coumarins, Sap: Saponins, Sesq: Sesquiterpene lactones, RS: Reducing sugars. Terp: Terpenoids, (++): abundant, (+): Present, (-): Absent

Present results suggest that the hexanic extract of *H. drummondii* possess activity against bacteria and the yeast like fungus *Candida albicans*. The activity of hexanic extract may be attributed to the presence of the monoterpenes pulegone and menthol and other compounds which appear to be concentrated in this non-polar extract. These results provide scientific support for the use of *H. drummondii* in traditional medicine, particularly for gastrointestinal diseases.

In conclusion, the hexanic extract of *H. drummondii* could be a good alternative in the search for new antimicrobial agents, especially because of the multidrug resistance observed with certain Gram-negative bacteria.

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