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In vitro Anti-*Helicobacter pylori* Effects of Sweet Basil (*Ocimum basilicum* L.) and Purple Basil (*Ocimum basilicum* var. *purpurascens*)

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Abstract: Anti-*Helicobacter pylori* effects of leaves of *Ocimum basilicum* L. (sweet basil, Lamiaceae) and *Ocimum basilicum* var. *purpurascens* (purple basil, Lamiaceae) were evaluated. Macerated aqueous and methanol extracts were tested against 45 clinical isolates of *Helicobacter pylori* using paper Disc Diffusion Method (DDM) on modified egg yolk emulsion agar (EYE agar). Although there were small differences in sensitivity among the isolates tested, but all isolates were susceptible to methanol and aqueous extracts. The Minimum Inhibitory Concentrations (MIC) of the extracts from leaves of sweet basil and purple basil were 677 and 729 μg mL^{-1}, respectively using agar dilution method. Antibacterial constituents of sweet basil extract were stable after one month of storage at 4°C but lost 31% its activity after 4 weeks at room temperature. The extract preserved its activity when heated to 80°C for 30 min and autoclaved at 121°C for 20 min. It was stable at pH 5-8.

Key words: *Ocimum basilicum*, *Ocimum basilicum* var. *purpurascens*, anti-*Helicobacter pylori*, basil

INTRODUCTION

Helicobacter pylori causes upper gastrointestinal tract disorders such as chronic gastritis, peptic ulcer disease, gastric carcinoma (Tytgat and Rauws, 1989). Although there are several drug treatment regimens for these significant infections including Colloidal Bismuth Subcitrate (CBS), together with antibiotics, such as amoxycillin and metronidazole (Edwards, 1993) but sometimes, eradication failure is seen. Increased resistance of *H. pylori* strains may develop leading to relapse (Goddard and Logan, 1996). Incomplete cure, side effects of antibiotics and resistant strains cause search for new sources of drugs. Medicinal plants seem to be a useful source of novel antibacterial compounds.

The genus *Ocimum* is included in the family Lamiaceae and is distributed worldwide (Oxenham, 2003). Basil is a popular culinary herb and in Iranian traditional medicine, the decoction of basil leaves is used as remedy for stomachache and headache caused by digestive weakness. Also, the leaves are considered to be tonic and carminative (Zargari, 1997). Although antimicrobial activity of the essential oil of basil has been reported (Oxenham *et al*., 2005; Sinha and Gulati, 1990) but the anti-*Helicobacter pylori* activity of the extract of basil has not been studied. Based on observed antimicrobial activity of basil and its use in traditional medicine in gastric disorder, in the present study, the anti-*Helicobacter* activity of sweet and purple basil was studied.

MATERIALS AND METHODS

Plant material and extraction procedure: The leaves of *O. basilicum* and *O. basilicum var purpurascens* were collected from a farm in Mashhad (Khorasan province, Iran) in July 2004. The plants were identified by Department of Pharrmacognosy, Faculty of Pharmacy (Mashhad, Iran). A voucher specimen of each plant (No. 153-1908-01 for *O. basilicum* and No.153-1502-02 for *O. basilicum* Var. *purpurascens*) was deposited in

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Herbarium of the Faculty Pharmacy, Mashhad University of Medical Sciences. Plants were dried in shade and powered. The powder of each plant was macerated in either methanol or distilled water for 24 h at room temperature. The mixtures were filtered and concentrated under reduced pressure at 45°C.

Bacterial isolates: Clinical isolates of *H. pylori* were obtained from the 17 Shahrivar Hospital (Mashhad, Iran). 45 clinical isolates from biopsy specimens were used. Primary isolation was performed on selective Brucella agar (Merck, Germany) supplemented with horse blood 5-7% (v v^{-1}) and starch (0.1% w v^{-1}, Merck, Germany), vancomycin 5 mg L^{-1}, trimethoprim 5 mg L^{-1}, polymyxin B 2500 μ L^{-1}, amphotricine B 10 mg L^{-1}. Bacteria were incubated for 5-7 days under microaerophilic condition (10% CO_2 and 90-100% humidity) at 37°C. Following primary isolation, *H. pylori* bacterial cells were identified according to colony morphology, Gram staining, rapid urease$^+$, catalase$^+$, oxidase$^+$, Indole$^-$, H_2S^- and nalidixic acid resistant (Goodwin and Armstrong, 1990; Boyanova *et al*., 2003).

Anti-helicobacter pylori assay: Growth inhibition was performed by the filter paper disc diffusion method (DDM) on modified egg yolk emulsion agar (EYE agar) at 37°C under microaerophilic conditions. Briefly, each 100 mL of EYE agar contained Mueller-Hinton agar (Pronadisa-Madrid) 3.8 g, egg yolk emulsion 7-10%, triphenyl tetrazolium chloride (Merck) 4 mg (Tabak *et al*., 1999).

Standard discs (d = 6mm) containing 2 mg of plant extracts were placed on egg yolk emulsion agar plate, previously inoculated with 0.1 mL bacterial suspension in sterile normal saline. The turbidity of bacterial suspension was equivalent to McFarland tube No.4 (10^8 cfu mL^{-1}). Inhibition growth assay of antibiotics versus basil extracts was performed using standard commercial discs of amoxycillin (25 μg $disc^{-1}$) and metronidazole (5 μg $disc^{-1}$). The plates were incubated for 3-4 days under microaerophilic conditions at 37°C (McNulty *et al*., 2002). The zones of inhibition were measured and reported in millimeters (average diameter by two repetitions).

The MIC of the methanol extract was determined using an agar dilution method (Shahamat *et al*., 1991) by adding various amounts (0-1250 μg mL^{-1}) of basil extracts to solid or liquid media (brain heart infusion broth+7% FCS) for 4 clinical isolates of *H. pylori* with highest sensitivity.

Various concentrations of the extracts were poured in the melted (45°C) Mueller-Hinton agar medium followed by inoculation of the plate with 0.1 mL of bacterial suspension in sterile normal saline (10^8 cfu mL^{-1}). All plates were incubated under microaerophilic condition for 4-5 days at 37°C. The MIC was expressed as the lowest concentration of the extract that inhibited visible growth. Minimum Bactericidal Concentrations (MBC) were established by lack of growth upon re-inoculation from extract-treated plates to Mueller-Hinton Agar plates.

Temperature and pH stability of basil extract and its stability during storage: 0.5 mg of methanol extract of sweet basil was dissolved in 5 mL of sterile distilled water and either was incubated at 80°C for 30 min or autoclaved at 121°C for 20 min. For the determination of stability during storage similar extracts was also stored at 4°C and room temperature for 4 weeks.

In order to determine pH stability, methanol extract of sweet basil was mixed with 0.1M Sterile Phosphate Buffer Solution (PBS) of various pH values (5, 6, 7 and 8) in separate tubes and were incubated at room temperature for 3 h (Ten Brink *et al*., 1994). Then, the discs containing extract were prepared after exposure of the extract to different thermal and pH conditions.

Phytochemical screening: The methanol extract was screened for alkaloids, flavonoids, tannins (Chhabra *et al*., 1984) and saponins (Brain and Turner, 1975).

RESULTS AND DISCUSSION

As shown in Table 1, both aqueous and methanol extracts of basil (*O. basilicum* and *O. basilicum* Var. *purpurascens)* were effective against *H. pylori*. There was no significant difference between aqueous and methanol extracts of purple basil (Table 1). No significant difference was observed between zone of inhibition of methanol extract of sweet basil and purple basil but methanol extract of sweet basil was significantly higher than that of its aqueous extract. Results shown in Table 1 indicate that amoxicilline as positive control was more effective against all strains tested whereas 44% of isolates (20 isolates) were more sensitive to extracts than metronidazole as positive control. Around one quarter of isolates (24%) were resistant to metronidazole while no isolate was found to be resistant to any of four tested extracts of *Ocimum*.

The MIC of methanol extract of sweet basil and purple basil were 677 and 729 μg mL^{-1} and the Minimum Bactericidal Concentration (MBC) were 781 and 833 μg mL^{-1}, respectively. The MIC values for five Taiwanese folk medicinal plants ranged from 1.28 to 5.12 mg mL^{-1}. Seven Greek herbal medicine extracts have been proved to be active against one standard strain and 15 clinical isolates of *H. pylori* with MICs ranging from 0.625 to 5 mg mL^{-1} (Stamatis *et al*., 2003). Cellini *et al*.

Table 1: Inhibitory effects of leave extracts of basil (*O. basilicum and O. basilicum var purpurascens)* on *H. pylori* in 45 clinical isolates

			Inhibition zone (mm)[a]			
Isolates No.	Amoxycillin (25 μg disc^{-1})	Metronidazole (5 μg disc^{-1})	Methanol extract, Ob[b]) (2 mg disc^{-1})	Methanol extract, OBP[c]) (2 mg disc^{-1})	Aqueous extract, OB) (2 mg disc^{-1})	Aqueous extract, OBP) (2 mg disc^{-1})
1	25.0	12.0	12.0	14.0	12.5	15.0
2	28.5	9.0	14.0	16.0	13.0	16.5
3	24.0	9.0	12.0	12.5	11.5	13.0
4	28.5	-[d]	16.0	13.5	12.0	18.0
5	25.0	2.0	22.5	17.0	13.0	12.5
6	40.0	-	14.0	14.0	14.0	11.0
7	33.0	10.0	16.0	16.0	16.0	13.0
8	24.0	-	14.5	15.0	14.0	13.0
9	20.0	6.0	8.0	13.0	9.0	13.0
10	13.0	41.5	16.0	13.0	14.0	12.5
11	25.0	-	13.5	17.0	13.5	13.5
12	36.0	11.0	16.0	16.5	14.5	15.0
13	20.0	50.0	18.0	15.0	18.0	17.5
14	32.0	51.0	14.0	10.5	14.5	12.0
15	50.0	6.0	18.5	15.0	16.0	18.0
16	38.0	16.5	16.0	15.5	15.5	11.5
17	35.0	6.0	20.0	13.0	19.0	15.0
18	30.0	6.0	14.5	16.5	12.5	13.5
19	54.0	9.5	14.0	17.0	13.0	15.0
20	40.0	55.0	20.0	13.5	16.0	11.5
21	35.0	9.5	21.0	20.0	13.5	16.0
22	30.0	10.0	18.0	19.0	17.0	19.0
23	40.0	29.5	18.0	20.0	16.0	21.0
24	50.0	50.5	20.0	22.0	21.5	20.5
25	34.5	-	18.0	5.0	17.5	15.0
26	42.0	46.0	11.5	20.5	12.0	16.0
27	36.0	52.0	12.5	16.0	14.0	17.5
28	45.0	10.5	21.5	20.0	8.5	16.5
29	44.5	6.0	15.0	18.0	14.0	11.5
30	50.0	28.0	14.0	19.0	13.5	13.5
31	38.0	19.0	15.5	20.0	16.0	19.0
32	60.5	47.5	14.0	14.5	1.5	12.5
33	20.0	10.0	15.5	13.5	14.5	15.0
34	37.0	9.5	20.0	17.0	17.0	16.0
35	46.0	10.0	19.5	16.5	15.0	17.0
36	48.5	-	18.5	17.5	18.0	16.0
37	44.0	9.0	22.0	18.0	13.0	11.0
38	49.0	50.5	16.0	17.0	15.5	15.0
39	22.0	13.0	14.5	14.5	12.0	11.5
40	28.0	11.0	15.0	15.0	12.5	14.5
41	35.0	51.0	15.0	13.5	14.5	14.5
42	40.5	48.0	13.0	14.5	12.0	14.5
43	28.0	1.5	18.5	16.0	14.5	11.0
44	36.5	9.0	21.0	20.0	4.0	16.0
45	37.0	46.0	12.5	15.0	12.0	14.0

[a] = Diameter of each disc was 6 and values are mean of duplicate discs, [b] = OB: *Ocimum basilicum*, [c] = OBP: *Ocimum basilicum var purpurascesns*, [d] = -, Not determined

(1996) reported MIC values for garlic extract against 19 strains of *H. pylori* ranging from 2 to 5 mg mL^{-1} indicating that *Ocimum* extracts tested in this study exhibited more potent anti-*Helicobacter* activity. It has been shown that the efficacy of antibiotics is pH dependent. For example, amoxicillin is most effective at neutral pH and tetracycline presents greater activity at low pH (Worrel and Stoner, 1998). In the present study, the effect of the pH on the bactericidal activity of *Ocimum* extracts was investigated. The extracts were stable at pH 5, 6 and 8 with no loss of anti-*Helicobacter* activity. The effect of thermal stability on the anti-*Helicobacter pylori* activity was also studied on 3 isolates with highest sensitivity to extracts. As shown in Table 2, the methanol extract of sweet and purple basil preserved its antibacterial activity against three isolates with highest sensitivity to amoxicillin after either heating for 30 min at 80°C or autoclaving at 121°C for 20 min. These results indicate that the active components are thermally stable and it appeared that the volatile components were not responsible for the observed activity. The extract maintained full stability after storage at 4°C for 30 days while lost 31% its activity after 4 weeks at room temperature.

Preliminary phytochemical screening of the methanol extract of sweet and purple basils indicated the presence of saponins in both plants. No alkaloids, flavonoids and

Table 2: Effect of temperature treatment on the anti-*H. pylori* activity of methanol extract of sweet basil

	Inhibition zone (mm)		
Isolate number	Control	80°C	121°C
Sweet basil			
Strain 15	18.5	19.0	18.5
Strain 28	12.5	12.5	13.0
Strain 32	14.0	14.0	14.5
Purple basil			
Strain 15	15.0	15.0	15.5
Strain 28	20.0	20.0	20.0
Strain 32	14.5	14.0	15.0

tannins was detected. It is been shown that rosmarinic acid is the predominant phenolic acid present in leaf tissues and is one of the most abundant caffeic acid esters present in *Ocimum* species (Javanmardi *et al*., 2002). Rosmarinic acid and its derivatives have been reported to have anti-microbial, anti-oxidant, anti-HIV and anti-inflammatory or cyclooxygenase inhibitory activity (Javanmardi *et al*., 2002). Opalchenova and Obreshkova studied the activity of sweet basil against multi-drug resistant isolates from the genera *Staphylococcus*, *Enterococcus* and *Pseudomonas* and showed a strong inhibitory effect of basil on the test bacteria (Opalchenova and Obreshkova, 2003). The anti-*Helicobacter* activity could be partly due to rosmarinic acid but other constituents present in basil extracts could also account for the observed activity.

Considering the increased development of resistance of *H. pylori* to antibiotic, searching for new antibiotics with natural origin is valuable especially in third world countries where most people get their medication form local market based on traditional medicine. In the present study, it was shown that the aqueous and methanol extracts of sweet and purple basil had anti-*Helicobacter pylori* activity. Although the concentration of extracts in paper discs was high in comparison with the concentration of common antibiotics but basil is a natural product which is used as vegetables and has other advantages such as being tonic. It has a beneficial action on the respiratory tract and is often used in asthma and bronchitis (Zargari, 1997; Perry, 1980).

Further studies are necessary to confirm the *in vivo* activity of basil and to determine the chemical structure of the active compounds.

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