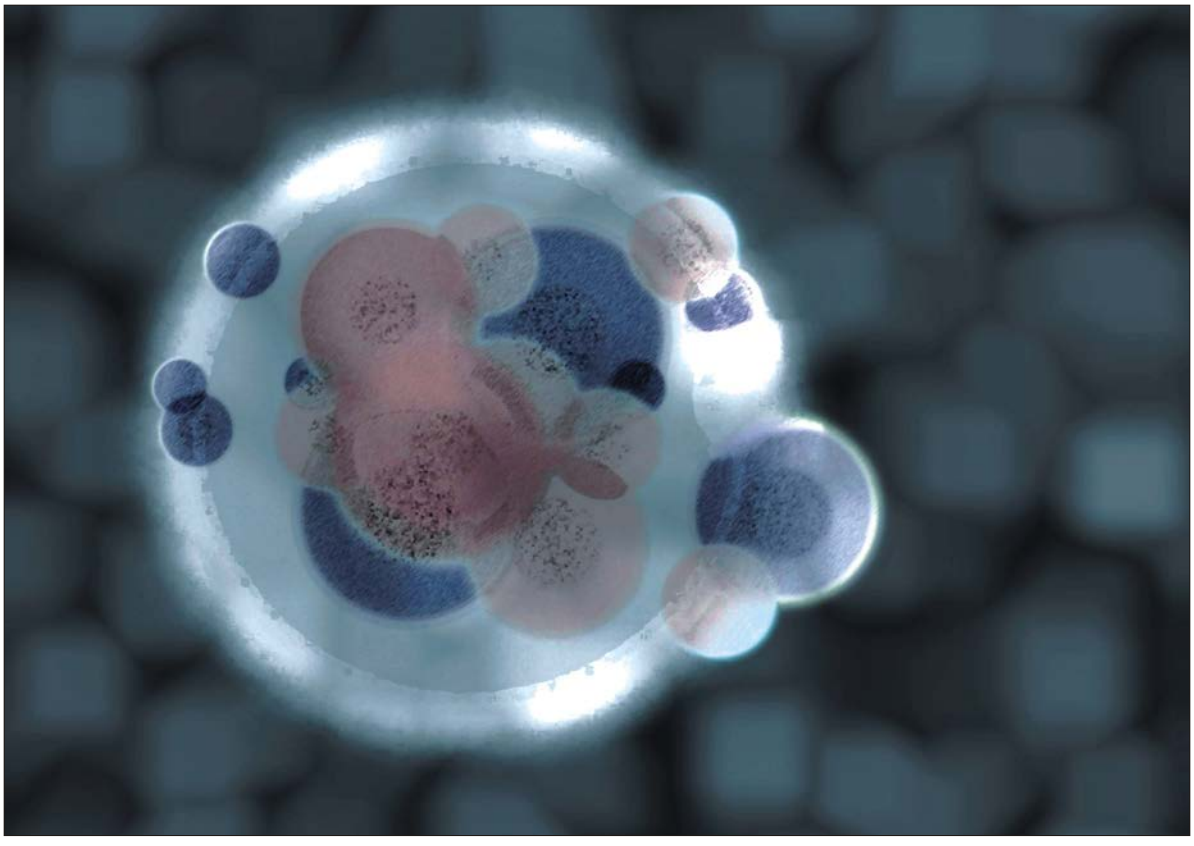

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 9 (3): 276-279, 2009
ISSN 1727-3048

In vitro Anti-Bacterial Activity of Sweet Basil Fractions Against *Helicobacter pylori*

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Abstract: In tihis study, the effect of sweet basil fractions on growth of *H. pylori* was studied by filter paper Disc Diffusion Method (DDM) on egg yolk emulsion agar. Aqueous fraction had no activity against nine tested clinical isolates. Methanol, n-hexane and butanol fractions showed *in vitro* anti *Helicobacter pylori* effects. There are no differences ($p>0.05$) among anti *Helicobacter pylori* activity of methanol, n-hexane and butanol fractions at concentration of 1 mg, but in lower concentrations, methanol and n-hexane fractions had more antibacterial activity than butanol fraction based on Duncan test. Minimum Inhibitory Concentrations (MICs) of methanol, n-hexane and butanol fractions from leaves of sweet basil were 39.1, 41 and 117.2 μg $disc^{-1}$, respectively. This study demonstrated that methanol, n-hexane and butanol fractions of sweet basil inhibited the growth of *H. pylori* strains *in vitro*.

Key words: Sweet basil, *Helicobacter pylori*, *Ocimum basilicum*, antibacterial activity

INTRODUCTION

Helicobacter pylori strains have been associated with gastritis, peptic ulcers and gastric adenocarcinoma (Murray *et al*., 2005). There is some level of genetic/phenotypic diversity among *H. pylori* populations (Ahmed and Sechi, 2005). Multiple antibiotic regimens have been used to treat these infections, including combination of tetracycline, amoxicillin, metronidazole and bismuth (Loo *et al*., 1997). Although these regimens have led to high cure rates (Gadhi *et al*., 2001), *H. pylori* is still a difficult infection to eradicate (Loo *et al*., 1997) and eradication failure rates remain at 5-20% (Gadhi *et al*., 2001). Growing resistance to antibiotics (especially metronidazole) (Murray *et al*., 2005), side effects and eradication failures have resulted in the search for new drug agents. One of the promising remedies for this type of infection is found in nature in the form of medicinal plants, an acceptable choice by society due to the change in people's attitudes towards a more Natural living. The genus of *Ocimum* (Laminaceae family) is distributed worldwide (Oxenham, 2003). Sweet basil (*Ocimum basilicum* L.) is a culinary herb in Iran which is used fresh as vegetable or in cooked recipes. Also, it is used in traditional medicine as remedy for stomachache and headache caused by digestive disorders. The leaves are considered to be tonic and carminative (Zargari, 1997). Antimicrobial and antioxidant effects of this plant have been reported by Javanmardi *et al*. (2002) and Opalchenova and Obreshkova (2003).

In pursuance of tht earlier investigation about anti *Helicobacter pylori* effects of sweet basil leaves, in the present study we evaluated the inhibitory effects of sweet basil fractions on the growth of *H. pylori*. Based on our knowledge there is no study have done concerning the anti *Helicobacter pylori* activity of fractions of sweet basil leaves.

MATERIALS AND METHODS

Bacterial isolates: Forty five clinical isolates of *H. pylori* were included in this study to test crude methanol extract of *O. basilicum* and nine of these isolates with the highest sensitivity were used for growth inhibition tests of fractions. Bacteria had been isolated from patients' biopsies as reported earlier by Nakhaei *et al*. (2006) and were identified by colony morphology, Gram staining, rapid $urease^{+}$, $catalase^{+}$, $oxidase^{+}$, H_2S^{-} and nalidixic acid resistance (Boyanova *et al*., 2003).

Plant material and fractionation: The leaves of basil plant were collected from Mashhad (a city in Northeastern

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Khorasan Province) and dried in the shade. The plant species were identified by Herbarium of Department of Pharmacognosy, Faculty of Pharmacy (Mashhad, Iran). A voucher specimen was maintained for reference in the Herbarium of the Faculty Pharmacy.

Methanol extract from 25 g of ground leaves was prepared by percolation method. The extract was concentrated under pressure at 45°C and dried to give 5.21 g crude extract. To prepare fractions, 70 g of ground leaves was extracted by methanol 100%. The extract was suspended in 35 mL distilled water. Then the extract was fractionated three times by the same volume of ethyl acetate, after sufficient shaking. The solvent of ethyl acetate fraction was omitted under reduced pressure at 45°C. Afterwards the fraction was dissolved in 30 mL methanol and was fractionated by 30 mL hexane three times. Two resultant phases were collected separately and concentrated by rotary aperture and oven (40-45°C). 2.1 and 2.8 g of methanol and hexane fractions were obtained, respectively. Aqueous phase was fractionated by 35 mL of butanol and subsequently every fraction was dried to obtain 1.9 and 1.3 g of aqueous and butanol fractions, respectively.

Evaluation of the anti *Helicobacter pylori* activity: Experiments were accomplished by a filter paper Disc Diffusion Method (DDM) on egg yolk emulsion agar (EYE agar) as reported by Nakhaei *et al.* (2006). Each plate was inoculated with 0.1 mL bacterial suspension in sterile normal saline, corresponding to a value of 4 McFarland optical density scale (10^8 cfu mL^{-1}) (McNulty *et al.*, 2002).

Dried extract and fractions were dissolved in proper solvent (sterile distilled water or methanol) and after addition of 0.02 mL of every solution on standard discs (d = 6 mm), discs were dried by evaporation of solvents in the oven (40°C). Filter paper discs containing 2 mg of crude methanol extract or a variable incremental amount (31.25, 62.5, 125, 250, 500 and 1000 μL $disc^{-1}$) of every fraction were placed on the surface of the agar. Standard commercial discs of amoxicillin (25 μg $disc^{-1}$) were used as positive control while hexane, methanol and butanol were used as negative control (discs were soaked at every solvent and then solvents were evaporated). Then, plates were incubated under microaerophilic condition (10% CO_2) and 90-100% humidity at 37°C. The zones of inhibition were measured in millimeters. Experiments were repeated three times and the average diameter of inhibition growth were considered.

The fractions were screened for antibacterial activity against nine clinical isolates of *H. pylori* with the highest sensitivity toward crude methanol basil extract.

MICs of the fractions were reported as the lowest concentration that inhibited the growth.

RESULTS AND DISCUSSION

Growth of all clinical isolates of *H. pylori* (n = 45) was inhibited by crude methanol extract of sweet basil and the mean of inhibition zone was 16.19 (±3.23) mm. Of all the fractions tested against *H. pylori*, aqueous fraction had no activity against tested clinical isolates. There are no differences ($p>0.05$) among anti *Helicobacter pylori* activity of 1 mg concentration of methanol (22.7±2.6 mm), n-hexane (21.1±4.0 mm) and butanol (22.1±3.8 mm) fractions. But in lower concentrations (62.5, 125, 250 and 500 μg $disc^{-1}$), methanol and n-hexane fractions had more antibacterial activity than butanol fraction in the same concentrations based on Duncan test (Table 1). The effect of amoxicillin (25 μg $disc^{-1}$) was significantly more than each of fractions of sweet basil ($p<0.01$). MICs of methanol, n-hexane and butanol fractions from leaves of sweet basil were 39.1, 41 and 117.2 μg $disc^{-1}$, respectively by DDM.

Herbal extracts have been used for healing of diseases from ancient times. Anti *Helicobacter pylori* of several plants have been studied in the past. Fukai *et al.* (2002) reported the chemical constituents of licorice that exhibited inhibitory activity against the growth of *H. pylori*. Tabak *et al.* (1999) showed that methylene chloride extract of cinnamon inhibited growth of *H. pylori*. Among the ten cinnamon components tested, cinnamaldehyde at 200 μg $disc^{-1}$ had the strongest inhibitory effect fallowed by eugenol 200 μg $disc^{-1}$ and carvacrole 2000 μg $disc^{-1}$ (Tabak *et al.*, 1999). In another study by Gadhi *et al.* (2001) methanol extract and hexane fractions of rhizome and leaves of *Aristolochia paucinervis* exhibited an inhibitory activity at a concentration of 128 μg mL^{-1}. Hexane fraction of rhizome demonstrated a higher inhibitory activity (MIC: 4 μg mL^{-1}) than hexane fraction of leaves (MIC: 16 μg mL^{-1}) (Gadhi *et al.*, 2001). Present study, after fractionation by solvents of different polarity shows that aqueous fraction had no effect on tested *H. pylori* isolates. Methanol, buthanol and n-hexane fractions of sweet basil showed anti *Helicobacter pylori* activity. MICs of these fractions were 39-117 μg $disc^{-1}$ indicating that sweet basil fractions tested in this study exhibited more potent activity against *H. pylori* isolates. Although, the effect of amoxicillin on tested bacteria was more than fractions of basil but sweet basil is a natural product that has other useful properties such as antimicrobial (Oxenham *et al.*, 2005; Chiang *et al.*, 2005), anti-oxidant and anti-inflammatory activities (Javanmardi *et al.*, 2002).

Table 1: Inhibitory effects of sweet basil fractions on tested *H. pylori* isolates

	Inhibition zone* (mm)					
	Concentration (μg disc^{-1})					
Isolates	31.25	62.5	125	250	500	1000
n-hexane						
10	6.0±0.0	7.7±1.5	9.3±2.9	13.7±2.3	17.0±2.6	19.0±2.6
15	7.7±1.5	9.7±0.6	11.0±0.0	14.3±0.6	18.7±0.6	23.7±0.6
20	9.0±2.6	15.0±1.0	16.7±0.6	18.3±0.6	20.3±0.6	23.7±2.1
21	9.3±0.6	12.3±0.6	14.3±0.6	16.3±0.6	18.0±1.7	25.0±1.0
27	6.0±0.0	6.0±0.0	8.3±0.6	10.3±0.6	12.0±0.0	14.3±0.6
28	9.3±3.1	15.0±1.0	20.3±0.6	21.3±1.2	23.3±1.2	25.3±1.2
29	6.0±0.0	8.3±0.6	9.7±0.6	13.3±1.2	17.3±2.9	21.0±1.0
37	6.0±0.0	7.0±1.7	10.7±1.5	13.7±2.1	13.7±2.3	17.0±1.0
Methanol						
10	7.3±1.2	12.7±1.2	15.0±1.0	17.7±2.1	21.3±1.5	25.7±1.5
15	7.7±1.5	9.7±0.6	14.3±0.6	18.0±1.0	18.7±1.5	22.0±1.0
20	7.3±1.2	10.7±0.6	14.3±0.6	16.7±0.6	19.0±1.0	21.0±1.0
21	6.0±0.0	8.3±0.6	7.3±5.5	15.3±0.6	16.7±0.6	19.3±1.5
27	7.3±1.2	10.3±0.6	14.2±0.3	19.3±0.6	22.3±0.6	25.7±0.6
28	7.3±1.2	10.7±0.6	12.0±1.0	15.7±1.5	19.7±3.2	21.0±2.6
29	6.7±1.2	8.7±1.2	12.3±1.5	16.3±1.5	19.7±0.6	22.0±1.0
37	6.7±1.2	8.7±1.2	12.3±1.5	16.3±1.5	19.7±0.6	22.0±1.0
Butanol						
10	6.0±0.0	6.0±0.0	9.0±1.0	17.7±1.5	22.3±1.5	28.7±1.2
15	6.0±0.0	6.0±0.0	9.7±0.6	15.0±1.0	18.3±0.6	21.3±0.6
20	6.0±0.0	6.0±0.0	9.7±0.6	12.7±1.2	17.7±0.6	20.0±1.0
21	6.0±0.0	6.0±0.0	9.3±0.6	12.0±0.0	17.7±0.6	20.7±0.6
27	6.0±0.0	6.0±0.0	6.7±1.2	9.3±1.5	16.3±0.6	20.7±0.6
28	6.0±0.0	6.0±0.0	9.7±0.6	12.3±0.6	16.7±1.5	19.7±0.6
29	6.0±0.0	6.0±0.0	7.3±1.2	10.7±0.6	14.8±0.3	18.0±0.0
37	6.0±0.0	6.7±1.2	7.7±2.9	14.3±0.6	18.3±0.6	27.7±0.6

*Diameter of every disc was 6 mm and values are Mean±SD of triplet experiments

The anti *Helicobacter pylori* activity could be due to non-polar compounds. Javanmardi *et al.* (2002) showed that rosmarinic acid is the predominant phenolic acid present in leaf tissues of Iranian basils.

In view of growing antibiotic resistance and side effects of available antibiotics against *H. pylori*, searching for new sources of drugs is valuable. In this study butanol, n-hexane and methanol fractions of sweet basil showed *in vitro* anti *Helicoacter pylori* activity. Therefore, use of sweet basil is recommended for food preparation as an alternative to synthetic compounds to improve flavor and taste. Further studies are necessary to evaluate anti *Helicobacter pylori* constituents of sweet basil and confirm their activity *in vivo*.

ACKNOWLEDGMENT

This study was supported by Islamic Azad University, Mashhad Branch, Mashhad, Iran by grant No. 6755 for the Scientific Research Project.

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