Chemistry of Natural Compounds, Vol. 44, No. 6, 2008

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM *Geum kokanicum*

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UDC 547.913

Plant essential oils and their constituents from a variety of plants are known to possess antifungal and antibacterial activities [1]. Phenolic constituents present in essential oils are generally recognized as active antimicrobial compounds. Eugenol, carvacrol, and thymol are phenolic compounds in cinnamon, cloves, sage, and oregano that possess antimicrobial activity [2, 3]. The exact cause-effect relation for the mode of action of phenolic compounds has not so far been determined; however, researches indicated that they may inactivate essential enzymes, reacting with the cell membrane or disturbing material functionality [4]. In addition, the traditional medicine approaches regarding essential oils and their antimicrobial evaluation have been recognized as valuable [5].

Among the wide range of aromatic plants used in Iranian folk medicine, *Geum kokanicum* merits consideration attention in the treatment of diarrhea and other gastrointestinal disorders [6]. *Geum kokanicum* Regel et Schmath, a member of the Rosaseae family, is an endemic perennial rhizomatous plant of Iran. The plant grows in moist and high altitude regions [7]. According to our knowledge, there have been few studies conducted on the chemical compositions and pharmaceutical effects of *G. kokanicum*. However, investigations on other species of *Geum* revealed antiviral effects on CMV [8], HSV I [9] and HSV II [10].

The objective of the present study was to identify the volatile constituents of the roots and rhizomes of *G. kokanicum* and also to assess its *in vitro* antimicrobial activities.

The volatile constituents of the roots and rhizomes of *Geum kokanicum* were obtained by the hydrodistillation method and then analyzed with GC and GC/MS. The compounds identified are arranged according to their elution order in Table 1. The yield of oil was 0.05%. Seventeen compounds (representing of 92.7%) in the hydrodistillate oil were identified. The major compounds in the oil were eugenol (80.9%) and myrtenol (5.2%). The oil was then examined on 21 strains of bacteria and fungi for antimicrobial susceptibility tests. The results are reported in Table 2. As Table 2 indicates, almost all the strains were susceptible against the tested oil. *S. aureus*, *B. subtilis*, *S. dysenteriae*, *S. marcecsens*, and *E. aeroginosa* showed the largest zone diameters. The MIC values of *G. kokanicum* essential oil are listed in Table 2. Among the bacterial strains, *S. dysenteriae* and *B. subtilis* demonstrated high sensitivity to the oil with an MIC equal to 1 mg/mL. Inhibition zones for all fungal strains appeared in 1 mg per disc of essential oil. *A. flavus* showed the zone even in a dose of 0.25 mg per disc and was the most susceptible fungal strain.

Essential oils derived from many plants are known to possess antifungal and antibacterial activities. Eugenol, the major phenolic compound in cinnamon, cloves, and other spicy herbs, presents antimicrobial activity [3]. In the present study, we found eugenol as the main component in *G. kokanicum* essential oil. In addition, the susceptibility of some important microorganisms in diarrhea diseases to the oil was shown. The results contribute to the knowledge of folk medicine in which preparations of the plant have been recommended for centuries in gastrointestinal disorders.

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0009-3130/08/4406-0811

TABLE 1. Chemical Composition of *Geum kokanicum* Essential Oil

Compound	RI	Percentage	Compound	RI	Percentage
o-Cymene	1003	0.2	Verbenol	1127	0.4
Limonene	1011	0.1	*neo*-3-Thujanol	1137	1.0
(*E*)-β-Ocimene	1038	0.2	Pentyl benzene	1150	0.7
m-Cymene	1062	0.1	α-Terpineol	1164	0.7
p-Mentha-2,4(8)-diene	1077	0.3	Myrtenol	1173	5.2
6-Camphenone	1092	0.2	*iso*-Dihydrocarveol	1206	1.2
trans-Thujone	1105	0.3	Perilla aldehyde	1266	0.1
p-Mentha-1,3,8-triene	1109	0.3	Eugenol	1346	80.9
trans-Sabinol	1123	0.8			

TABLE 2. The Antimicrobial Activity of *Geum kokanicum* Essential Oil (10 mL/disk, inhibition zone mm) and the Susceptibility of the Selected Bacteria and Fungus to *G. kokanicum* Essential Oil and Control Standards (cited in brackets, μg/mL)

Test strain	Oil, 10 μL	Gentamycin, 10 μg	Erythromycin, 15 μg
Staphylococcus aureus	34 (0.4)	17 (1.2)	24 (<0.075)
Salmonella typhi	15 (4)	16 (1.2)	10 (4.8)
Escherichia coli	15(4)	10 (1.2)	- (4.8)
Klebsiella pneumoniae	11	16	- (4.8)
Bacillus cereus	23	21	-
Staphylococcus epidermilis	31	25	23
Shigella dysenteriae	24 (1)	17 (1.2)	20
Pseudomonas aeruginosa	11 (>8)	22 (1.2)	14 (1.2)
Proteus vulgaris	18	17	- (2.4)
Streptocuccus fecalis	11	-	9
Bacillus subtilis	30 (1)	30 (<0.075)	13
Citrobacter ferundi	18	15	35 (<0.075)
Enterobacter cloacae	18	18	-
Serratia marcescens	24	-	-
Enterobacter aeroginosa	24	22	13
Aspergillus niger	65 (1)	-	-
Aspergillus flavus	51 (0.25)	-	-
Aspergillus fumigatus	51 (1)	-	-
Candida albicans	34 (1)	-	-
Cryptococcus neoformans	37 (1)	-	-

Fluconazole, 25 μg. *Aspergillus niger:* - (10 μg/mL); *Aspergillus flavus*: - (40); *Aspergillus fumigatus*: - (>40); *Candida albicans*: 14 (10); *Cryptococcus neoformans*: - (40 μg/mL).

A total of 21 strains of the bacteria (15 species) and fungi (5 species) were tested, comprising *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Klebsiella pneumoniae, Bacillus cereus, Staphylococcus epidermilis, Shigella dysenteriae, Pseudomonas aeruginosa, Proteus vulgaris, Streptococcus fecalis, Bacillus subtilis, Citrobacter ferundi, Enterobacter cloacae, Serratia marcescens, Enterobacter aeroginosa, Aspergillus niger* (PIM 126)*, Aspergillus flavus* (PTCC 36006)*, Aspergillus fumigatus* (PIM 112)*, Candida albicans* (ATCC 14053), and *Cryptococcus neoformans* (Kf 33); all bacteria were isolated from clinical specimens in Shariati Hospital, Tehran, and identified using conventional methods. Also fungi were kindly obtained from the Department of Microbial Quality Control of Drugs, Faculty of pharmacy, Tehran University of Medical Sciences, Tehran, Iran. Stock cultures of bacteria and fungi were kept on Muller Hinton (Difco) and Sabouraud-4% dextrose agar (Merck,

Germany), respectively, at 4°C and renewed bimonthly. Cultures for experiments were prepared by inoculation of a loopful of culture into Muller Hinton and Sabouraud-2% dextrose broth and incubated overnight at 37 and 25°C, respectively.

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