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Original Article

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Antibacterial Activity of Salvia reuterana Constituents

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Abstract

Two flavonids: 5-hydroxy-6,7,4 ´-trimethoxy flavone (salvigenin), 5-hydroxy-7,4 ´-dimethoxy flavone (thitonine) and a labdane type diterpenoid: 8,13-epoxy-14 labdene (manoyl oxide) isolated from the solvent extract of the aerial part of Salvia reuterana Boiss.The proposed structure were stablished by 1HNMR, 13CNMR, 2DNMR(C-H-Cosy, HMQC),DEPT and Mass spectroscopy and confirmed in accordance with bibiliographic data and computational chemistry results. The three components were evaluated against 7 microorganisms including gram(+/-)bacterias by measurment of growth inhibitory zone. They showed significant effect specially on gram(-) bacterias and in this way the most effective component was thitonine which was even more effective than Gentamicine as standard antibiotic.

Keywords: Salvia reuterana, salvigenine, thitonine, manoyloxide, antibacterial activity.

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INTRODUCTION

Within the Lamiaceae family, the oil rich genera are mainly in the subfamily Nepetoidea,the genera is classified in tribe Menthae of subfamily Nepetoideae[1]. Salvia is one of the members of tribe Menthae with about 700 species distributed all over the world. In Iran the genus Salvia is represented with about 60 species. 17 of which are endemic and distributed all over the country [2,3]. Some of Salvia species feature prominently in the pharmacopoeias of many countries throughout the world[4-10]. Previous chemical investigations on different species of Salvia have shown the presence of flavonoids [11-16], diterpenoids[17-25] and even the rare sesterterpens[26-29]. Salvia reuterana is one of the widespread species in Iraq and Iran, The essential oil composition and antibacterial activity of essential oil [30-32], α-Amylase inhibitory [33], free radical scavenging activity of ethanolic extract [34], Anoxiolytic and sedative effects of hydroalcoholic extract[35] and cytotoxic activity [36] of S. reuterana have been investigated before.

To the best of our knowledge S. reuterana has not been investigated for the three compounds we found, although these components have been isolated and reported from the other species. Flavonoids are characteristic constituents of green plants with the exception of algae andhornworts.They occure in virtually all plant parts

including leaves, roots, wood, bark, pollen, nectar, flower, berries and seeds. An important feature of the distribution of flavonoids in plants is the strong tendency for taxonomically related plants to produce similar types of flavonoids. Thus, useful information about the flavonoid types likely to be encountered in a plant under investigation may often be gained by reference to the literature pertaining to previous flavonoid studies of related plants, e.g. from the same genus or family [37,38]. In subfamily Nepetoidea and the genus Salvia different kinds of flavonoids have been reported [39].

There are many reports of the two isolated flavonoids (compounds 1, 2) in the literature[40-45], and biological activity of them have been investigated before [46-50]. Compound (3) is a diterpenoid with a labdane skeleton [51, 52] and different isomers of the compound have been isolated from different genus [53-55]. The biological activity have also been reported[56-58].

EXPERIMENTAL

Plant material: The aerial parts of Salvia reuterana Boiss. Was collected during the flowering stage in june 2005 from Shool-Abad, Lorestan province of Iran. Voucher specimen is deposited at the herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran.

Extraction, Fractionation and Isolation: The shade dried powdered aerial parts (500 gr) of S. reuterana was soaked in n-hexan,ethyl acetate,methanol(1:1:1) for about one week, after filtration , the solvent was evaporated in a rotavapour at 40-50° C , under reduced pressure. A semisolid green material obtained was solved in a little amount of methanol and was stored under 10° C for 24 hours, the waxy precipitate was removed by filtration and the filtrate evaporated in vacuum to afford a syrupy residue(10 gr) and then was mixed and powdered by Silica gel, particle size 0.040-0.063 mm(230-400 mesh ASTM). The green powder was fractionated by CC on silica gel eluting with n-hexan followed by a gradient of Et2O up to 100% and then MeOH of increasing polarity affording 26 fractions. After using TLC (Silica gel 60F 254) , we mixed equal fractions and the fractions reduced to 9. In fraction eluted with Et2O-MeOH (95:5), the light green cubic crystals appeared , it was washed by Et2O to purify(compound 1).

Fractions eluted with ether-methanol (40:60)

to ether-methanol (10:90)was submitted to repeated CC on silica gel with a gradient of n-hexane-ether, methanol mixture, in the fr. Eluted with n-hexane-ether (1:1) , the yellowish crystalline needle appeared and purified after washing by Et2O (compound 2). The Fr. eluted with Et2O-MeOH (4:1) followed by TLC(Silica gel, n-hexan-Et OAC 2:1) showed 3 spots under UV (254 nm). After spraying a (1:1) mixture of Acid Sulfuric 5% in EtOH, Vanillin 1% in EtOH and 110° C heat on hot plate ,another spot was appeared with a lower Rf . Preparative TLC was used many times to obtain the pure compound 3.

Microorganisms: A collection of 7 microorganisms was used , including gram(+/-) bacterias, Staphylococcus aureus (PTCC 1113), Staphylococcus epidermidis (PTCC 1349),Bacillus subtilis (PTCC 1023), The gram (-) bacterias, Salmonella para A(PTCC1230) , Escherichia coli (PTCC 1330) Shigella flexneri (PTCC 1234), Psudomonas aeiuginosa (PTCC 1310) identified by Research Center of Science and Industry, Tehran, Iran. Microorganisms (obtained from enrichment culture of the microorganisms 1 mL of Muller-Hinton broth, incubated at 37° C for 12 h.) were cultured on Muller-Hinton Agar medium. After drilling wells on medium oils dissolved in n- hexane and 50 μL from solutions (30 mg/L) was applied to each well.. After 24 h. of incubation at 37 ° C, diameter of inhibition zones were measured . A blank containing only n-hexane showed no inhibition in a preliminary test. Each test was carried out in triplicate and the average was calculated for inhibition zone diameter . Controls were performed with Gentamicine as standard antibiotic (10μg) general experimental procedures.

Instrumental: 1HNMR(300MHz) and 13CNMR(75MHz) spectra were recorded on a Varian GEMINI 300 and a Bruker DRX 500,in CDCl3 , with residual solvent peak as int. reference. The Mass runs were performed using a Mass Specterometer 8430, Finnigan-Mat, Magnetic Sector, 70 ev, scanning rate:0.3 scan/decade. The chemicals and solvents for this study were purchased from Merck Co. Ltd.

RESULTS AND DISCUSSION

Isolation and purification of two flavones and a diterpenoide was done. They were identified as:

Compound 1: m.p=188°C , M+ at m/z=328 corresponding to the molecular formula C18H16O6 , 1HNMR(300 MHz,CDCl3 , δ, ppm, J/Hz) : 12.78(1H,s,OH),7.83(2H,d, 8.9,H-2´,H-6´), 7.02(2H, d, 8.9, H-3´, H-5´), 6.59 (1H, s, H-3), 6.55(1H,s, H-8), 3.98(3H, s, Me-6), 3.97(3H,s, Me-7), 3.90(3H,s, Me-4´) ; 13CNMR(75MHz,CDCl3, δ, ppm): 164.43 s (C-2), 104.50 d (C-3),183.08 s (C-4),153.63 s, (C-5), 132.99 s (C-6), 159.12 s (C-7), 90.95 d (C-8), 153.44 s (C-9), 106.51 s (C-10), 123.92 s (C-1´),128.41 d (C-2´, C-6´),114.91 d (C-3´,C-5´), 163.00 s (C-4´), 55.94 q (Me-4), 56.71 q (Me-7),61.27 q (Me-6). The C-H Cosy spectra shows correlation between protons and carbons as well, given in figure 1.

Compound 2: mp= 168-172°C, M+ at m/z 298 corresponding to the molecular formula C17H14O5 ,1HNMR (300MHz,CDCl3 , δ, ppm, J/Hz) :12.83 (1H,s,OH),7.85 (2H,d,8.9, H-2, H-6 ´), 7.02(2H,d, 8.9,H-3 ´,H-5 ´), 6.59 (1H,s,H-3), 6.49(1H,d,2.16,H-6),6.37(1H,d,2.16,H-8),3.91 (3H,s,Me-7),3.89 (3H, s, Me-4´) ; 13CNMR(75 MHz, CDCl3, δ, ppm): 165.82 s (C-2),98.432 d (C-3), 182.844 s (C-4), 162.58 s (C-5), 104.74 s (C-6), 164.41 s (C-7), 93.01 d (C-8),162.98 s (C-9), 105.95 s (C-10),123.96 s (C-1´), 158.09 s (C-4´), 128.43 d (C-2´,C-6´), 114.89 d (C-3´,C-5´), 55.92 q (Me-4´), 56.18 q (Me-7).

Compound 3: M+ at m/z=290 corresponding to the molecular formula C20H34O.
1HNMR(300MHz,CDCl3,δ,ppm,J/Hz) :
5.93(1H, dd, 17.27, 10.67, H-14), 5.23(1H, d, 17.27, H-15´), 5.04(1H, d, 10.67, H-15), 1.29(3H, s, Me-17), 1.17 (3H, s, Me-19), 0.87(3H, s, Me-16), 0.80(6H, s, Me-18,

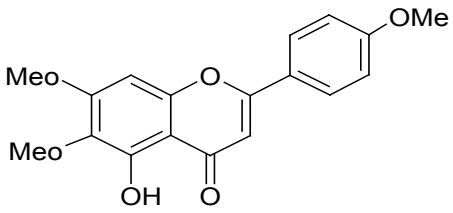
Me-20) ; 13CNMR(75 MHz, CDCl3, δ, ppm): 45.35 t (C-1), 18.82 t (C-2),44.74 t (C-3), 33.77 s (C-4), 61.90 d (C-5), 20.90 t (C-6), 42.38 t (C-7), 75.17 s (C-8), 56.47 d (C-9), 40.08 s (C-10), 19.46 t (C-11), 39.65 t (C-12), 74.02 s (C-13), 146.40 d (C-14), 111.58 t (C-15), 33.62 q (C-16), 27.62 q (C-17), 15.75 q (C-18), 24.61 q (C-19), 21.87 q(C-20); DEPT spectra (figure2) also shows 8 CH2 groups at δ=18.80, 19.48, 20.89, 40.07, 42.37, 44, 75, 45.34, 111.62 corresponding to carbones: 2, 11, 6, 12, 7, 3, 1, 15 respectively. The correlation between carbones and protons is obvious in HMQC spectra as well, specially between H-15 , H-15´ and C-15 ; H-14 and C-14, also between 5 methyl groups and corresponding carbones (figures 3,4).

Antibacterial activity of the isolated compounds were determined against 7 microorganisms including gr(+/-) bacteria by measurement of growth inhibitory zone. They showed significant effect and in this way the most effective component was compound 2 , which was even more effective than Gentamicine as standard antibiotic on bacteria, results are listed in table 1.

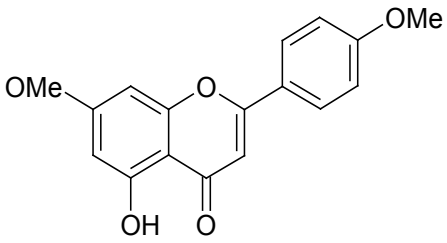
Table 1: Antibacterial activity of three components isolated from S. reuterana extract

Inhibition Zone*						
Microorganisms	(-/+)Gram	1	2	3	4	5
Staphylococcus aureus PTCC 1113	+	13	12	11	12	
Staphylococcus epidermidis PTCC 1349	+	11	10	13	20	-
Bacillus subtilis PTCC 1023	+	10	20	14	14	-
Salmonella para A PTCC 1230	-	18	23	20	14	-
Escherichia coli PTCC 1330	-	20	21	12	15	-
Shigella flexneri PTCC 1234	-	13	18	12	12	-
Psudomonas aeiuginosa PTCC 1310	-	10	13	10	15	-

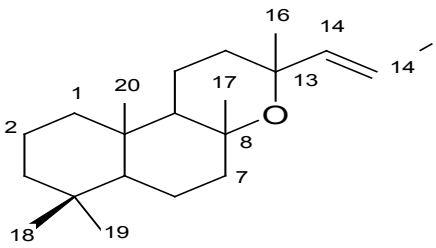
**Values are the mean diameter of inhibitory zones (mm); 50µl of solutions (30 mg/l) was applied to each well.*
1- Salvigenin , 2- Thitonin, 3- Manoyl oxide, 4- Gentamicine, 5- n-hexan.



compound 1 : 5-hydroxy-6,7,4´-trimethoxy flavone



compound 2: 5-hydroxy-7,4´-dimethoxy flavone



compound 3: 8,13-epoxy-14 labden

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