Health Biotechnology and Biopharma (2017), 1(3): 65-76

Original Research Article

Phytoconstituents and biological activities of *Artemisia kopetdaghensis*

Elham Ezzatzadeh [1]*

[1]Department of Chemistry, Ardabil Branch, Islamic Azad University, Ardabil, Iran

***Corresponding author:** Elham Ezzatzadeh, Department of Chemistry, Ardabil Branch, Islamic Azad University, Ardabil, Iran. Email: dr.ezzatzadeh@yahoo.com*

DOI: 10.22034/HBB.2017.19

Received: December 24, 2017; **Accepted:** December 30, 2017

ABSTRACT

Phytochemical investigation of the aerial parts of *Artemisia kopetdaghensis* led to isolation and characterization of 5 compounds including 9-Oxo-tournefortiolide (1), 2,6,10-Trimethyl-2,5-epidioxy-7,10-epoxydodeca-3,11-dien-5-acetoxy (2), 6-Demethoxy-5,7-dihydroxy-4'-O-methylcapillarisin (3), Eupatilin (4) and 5, 7, 5'-trihydroxy-6, 2′, 4′-trimethoxyflavone (**5**). The chemical structures of these compounds were identified by spectroscopic analysis. The antioxidant activities of the pure compounds were evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays and also the antimicrobial capacity were tested against gram positive and gram negative bacteria and fungi using minimum inhibitory concentration (MIC) method.

Keywords: *Artemisia kopetdaghensis*; DPPH; MIC

INTRODUCTION

Plant secondary metabolites generally display remarkable biological activities such as antioxidant and antimicrobial properties which are useful for preserving foods from decay and contamination or preventing living tissues from various diseases [1]. Due to the continuous emergence of antibiotic-resistant strains, there is a continuous demand for new antibiotics [2]. Many plants have shown considerable cytotoxic activities and also many antitumoural agents are derived from plant origin [3-5]. The genus

Artemisia was known as "teretkh" in Persian is well known aromatic perennial herb originated from northern regions. Among 500 species of this genus, grown in the world, thirty four species are distributed in Iranian flora [6]. *Artemisia* species are well known as medicinal plants because of their biological and pharmacological properties. In traditional medicine, *Artemisia* species have been used as folk remedies for some treatment purposes [7]. Previous phytochemical investigations have been shown the presence of various phytochemical constituents such as terpenoids [8-12], flavonoids [13,14] and coumarins [15] in the different *Artemisia* species. Cytotoxic, antihepatotoxic, antimicrobial, anti-inflammatory, insecticides and antimalarials effects [16-18] have been confirmed for some *Artemisia* species. *Artemisia kopetdaghensis*, aromatic shrubs belonging to the Asteraceae family, are traditionally used in Iran for anti-inflammatory, antimicrobial, antifungal and sedative activities [19]. Furthermore, in other studies, cytotoxic effects of the methanol and hydro-ethanol extracts of *A. kopetdaghensis* as well as its essential oil have been demonstrated [20,21].

There is already no report on phytochemical constituents and biological activity of the extract of *A. kopetdaghensis.* The present research reports the chemical structure characterization of the five compounds from the (Et_2O/MeOH/Petrol) extract of plant by ^{1}H and ^{13}C NMR and Mass spectroscopy. The antioxidant activities of the five compounds were evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays. Finally, antimicrobial effects of these compounds were evaluated against some pathogenic strains of bacteria and fungi.

MATERIALS AND METHODS

Reagents, chemicals and microorganisms

Trolox (water soluble equivalent of vitamin E) was obtained from Acros Organics (Geel). Dimethyl sulphoxide, hexane, methanol, sodium acetate was purchased from Merck (Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH), and hydrochloric acid 32% were obtained from Sigma Aldrich (USA). The bacteria that were used in this study were *Bacillus pumilus* (PTCC 1319), *Escherichia coli* (PTCC 1533), *Kocuria varians* (PTCC 1484), *Pseudomonas aeruginosa* (PTCC 1310), *Salmonella typhi* (PTCC 1609) and *Listeria monocytogenes* (PTCC 1298). The fungal strains that were used in this study were *Aspergillus niger* (PTCC 5154), *Aspergillus flavus* (PTCC 5006) and *Candida glabrata* (PTCC 5297). All microorganisms were obtained from the Persian type culture collection (PTCC) in Tehran.

Plant materials

The fresh *A. kopetdaghensis* was collected in september 2015, from the Bojnourd area in khorassan province. Voucher specimens (No. 0888) are deposited in the herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran. The aerial parts of the plant were dried at room temperature in the shade for 5 days before the extraction.

Extraction and Isolation

The separation process was carried out using several chromatographic methods. Ground aerial parts (800 g) were extracted with Et_2O/MeOH/Petrol (1:1:1) (2×6L) at room temperature for 48 h. Evaporation at reduced pressure of 36 g (4.5% yield) of crude extract, which was suspended in EtOH (600 ml), diluted with H_2O (500 ml) and extracted successively with hexane (3×650 ml) and $CHCl_3$ (3×450 ml). Evaporation of the $CHCl_3$ extract at reduced pressure of 14.5 g of residue, which was a column chromatographed over silica gel (340 mg, 70-230 mesh) using hexane and increasing amounts of EtOAc (0-100 %) and EtOAc/MeOH (9:1) to afford 32 fractions. These were classified according to their TLC profiles and monitored by FT-IR spectroscopy. Fractions 7 and 8 (240 mg) were reunited and rechromatographed on silica gel (230-400 mesh) to obtain 80 mg of compound 1. Fractions 10 to 13 (250 mg) were exhibited two spots on a TLC after repeated chromatography purification, yielded 39 mg of the compound 2.

Fractions 16 to 20 (230 mg) showed a major spot on TLC was decolorized with charcoal in hexane-EtOAc. Filtration, evaporation and recrystallization from n-heptane-EtoAc (3:1) gave 90 mg of the pure compound 3. Fractions 26 to 32 (300 mg) were reunited and rechromatographed on silica gel (230–400 mesh) using hexane-EtOAc (7:1→ 3:1) to yield 50 mg of the compound 4 and 90 mg of the compound 5.

Biological activities

Assessment of antimicrobial activity by MIC agar dilution assay

The antimicrobial activity of natural samples was tested by determining the minimum inhibitory concentration (MIC) using the agar dilution method [22]. The lowest concentration of the compounds that prevented visible growth was considered to be the MIC. In antifungal activity evaluation, appropriate amounts of the natural compounds of *A. kopetdaghensis* were added to sterile molted sabouraud dextrose agar (SDA) medium containing Tween 20 (0.5%, v/v) to produce the concentration range of 8–512 µg/ml. The resulting SDA agar solutions were immediately mixed and then poured into petri plates. The plates were spot which inoculated with 5 µl (10^4 spores/ ml) of isolated fungus. At

the end of the incubation time, the plates were evaluated for the presence of growth. The antibacterial activity was carried out through the protocol. The only difference is 5 µl of suspension containing 10^8 CFU/ml of bacteria instead of isolated fungus. The MIC was defined as the lowest concentration of the oil to inhibit the growth of microorganisms. Ampicillin, tetracycline and fluconazole were used for gram-positive, gram-negative bacteria and fungus, respectively.

Antioxidant activity measured by DPPH radical scavenging activity

The radical scavenging activity of natural samples against the stable free radical DPPH was measured as described previously [23]. Briefly, 4 different concentrations of the isolated compounds were dissolved in methanol and incubated with a methanolic solution of DPPH (100 µM) in 96-well microplates. Related concentrations were carefully chosen according to the activity of this plant to obtain an appropriate dose-response curve. Plant extract concentrations were used in this study arranged from 1.6 to 100 µg/ml. After 30 min incubation at room temperature in the dark, the absorbance was measured by a microplate reader (Bio-Tek, model 680) at 517 nm. The inhibition (%I) for each concentration was calculated using the absorbance (A) values according to the following formula:

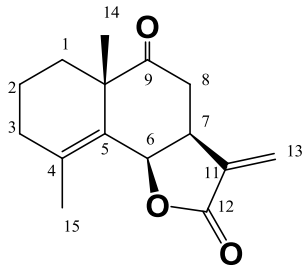
$$\%I = [(A_{DPPH} - A_{PS}) / A_{DPPH}] \times 100$$

Where A_{DPPH} and A_{PS} are the absorbance of the DPPH solutions containing methanol and plant samples, respectively. The dose-response curve was plotted using the software Sigma Plot for windows version 8.0 and then related IC_{50} values of extract was calculated. These values were divided by the extraction yield (Y) to calculate the IC_{50} value for the dry plant.

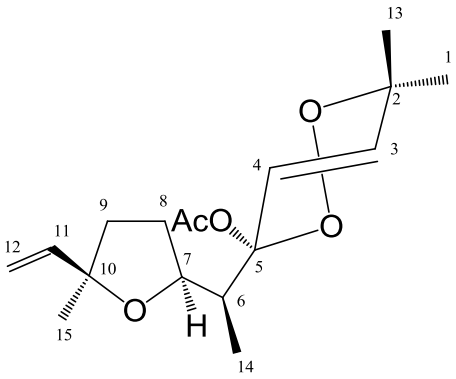
RESULTS

Spectral data of isolated compounds

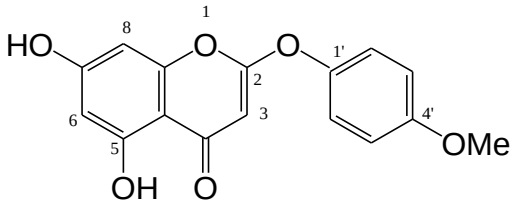
Purification processes on (Et_2O/MeOH/Petrol) extract obtained from aerial parts of *A. kopetdaghensis* by chromatography on silica gel columns as well as recrystallization for isolation and identification of the five compounds. Structure elucidation was accomplished by NMR and mass spectrometric (MS) analysis as well as comparisons with data reported in the literature [24-29]. The structures of all compounds isolated are shown in Figure 1.



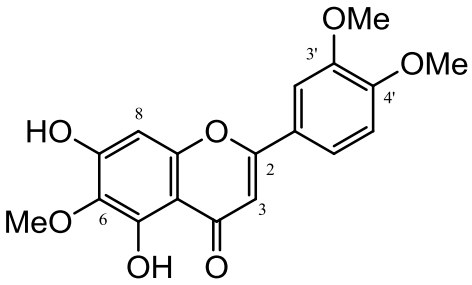
9-Oxo-tournefortiolide (**1**)



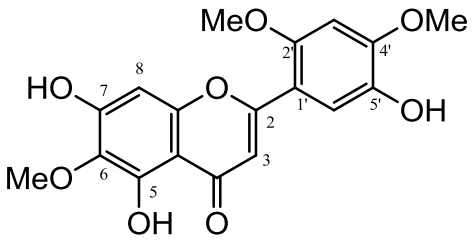
2,6,10-trimethyl-2,5-epidioxy-7,10-epoxydodeca-3,11-dien-5-acetoxy (**2**)



6-Demethoxy-4'-O-methylcapillarisin (**3**)



Eupatilin (**4**)



5,7,5'-trihydroxy-6,2',4'-trimethoxyflavone (**5**)

Fig. 1. Structures of compounds **1** to **5** obtained from *Artemisia kopetdaghensis.*

Compound 1 was isolated as colorless crystals. Its molecular formula was determined as $C_{15}H_{18}O_3$ by the MS (EI 70 eV, m/z, %): 246.126 [M^+] (100) (calc. For $C_{15}H_{18}O_3$: 231 [M-Me]$^+$ (47), 203 [231-CO]$^+$ (78), 185 [203-H_2O]$^+$ (28), 157(26), 131(28), 91 (26); FT-IR (KBr, ν_{max}, Cm^{-1}): 1760 (γ-lactone), 1710 (C=O); ^{1}H -NMR (300 MHz,$CDCl_3$, δ, ppm, J/Hz); 1.70 (1H, m, H-1α), 1.54 (1H, m, H-1ß), 1.70 (2H, m, H-2), 2.12 (2H, dd, J=5, 8, H-3), 5.78 (1H, d, J=9, H-6), 3.65 (1H, m, H-7), 2.75 (1H, dd, J=7, 14, H-8α), 2.42 (1H, dd, J=3, 14, H-8ß), 6.38 (1H, d, J=3, H-

13) 5.69 (1H, d, J=2.5, H-13'), 1.20 (3H, s, H-14), 1.89 (3H, s, H-15); ^{13}C- NMR (75 MHz, $CDCl_3$, δ, ppm); 34.5(C-1), 18.2(C-2), 32.8(C-3), 128.7(C-4), 138.2(C-5), 75.5(C-6), 37.8(C-7), 41.2 (C-8), 213.7 (C-9), 47.6(C-10), 140.4 (C-11), 169.9(C-12), 124.5(C-13), 20.0 (C-14), 25.1 (C-15).

Compound 2 was obtained as colorless oil, and its molecular formula was determined as $C_{17}H_{26}O_5$ by the MS (EI, 70 eV, m/z (%): 310 $[M]^+$ (21), 278 $[M-O_2]^+$(6), 167 $[M-O_2-C_7H_{11}O]^+$(26), 139 $[M-O_2-C_7H_{11}O-C_2H_4]^+$ (34), 111 $[M-O_2-C_{10}H_{15}O_2]^+$ (100), 93 $[M-O_2-C_{10}H_{15}O_2-H_2O]^+$ (73); FT-IR (ν_{max}, cm^{-1}),1740 (carbonyl groups),1140, no band in the hydroxyl region; ^{1}H-NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz); 1.28 (3H, s , H-1), 6.90 (1H, d, J=15.7 , H-3), 6.40 (1H, d , J=15.7, H-4), 2.90 (1H, dq , J=7, 10 , H-6), 4.20 (1H, dt , J=10.0, 10.0, 5.0, H-7), 1.69 (2H, m, H-8), 1.81 (2H, m, H-9), 5.86 (1H, dd , J=17.2, 10.5, H-11), 5.16 (1H, dd, J=17.2, 1.2, H-12α), 4.96 (1H, dd, J=10.5, 1.2, H-12ß), 1.39 (6H, s, H-13 and H-15), 1.04 (3H, d, J=7, H-14), 2.18 (3H, s, OAc); ^{13}C- NMR (75 MHz, $CDCl_3$, δ, ppm); 26.9(C-1), 71.3 (C-2), 144.9 (C-3), 125.6, (C-4), 89.1 (C-5), 50.2 (C-6), 80.8 (C-7), 29.7 (C-8), 37.9 (C-9), 83.3 (C-10), 152.9 (C-11), 111.8 (C-12), 27.4 (C-13), 13.4 (C-14), 29.6 (C-15), 170.1 (carbonyl OAc), 22.2 (Me).

Compound 3 was obtained as white-brownish crystals, and its molecular formula was established as $C_{16}H_{12}O_6$ by the MS (EI 70 eV, m/z, %), 300 $[M]^+$ (100), 27 1 $[M-CO]^+$ (14), 148 (53), 92 (59), 77 (67), 69 (92), 63 (48); FT-IR (KBr, ν_{max}, cm^{-1}): 3420 (br, ν_{OH}), 1654 (C=O), 1614, 1565, 1502, 1460 (aromatic C=C bond), 1222, 1022 (C-O-C), 822 (p-substitutent); ^{1}H-NMR (300 MHz, acetone-d6, δ, ppm, J/Hz); 12.89 (1H, s, OH), 9.85 (1H, s, OH),7.30 (2H, dd, J=9.1, 2.1, H-3' and H-5'), 7.08 (2H, dd, J=9.1, 2.1, H-2' and H- 6'), 6.39 (1H, d, J=2.0, H-8), 6.26 (1H, d, J=2.0, H-6), 5.08 (1H, s, H-3), 3.86 (3H, s, OMe-4'); ^{13}C- NMR (75 MHz, acetone-d6, δ, ppm); 183.9 (C-4),168.7(C-2),164.1 (C-7), 162.7 (C-5), 158.7 (C-4'), 155.8 (C-9), 145.2 (C-1'), 122.3 (C-2', 6'), 115.7 (C-3', 5'), 103.0 (C-10), 99.6 (C-3), 94.1 (C-6), 87.4 (C-8), 55.5 (OMe-4'). The crystallographic data at the Cambridge Crystallographic Data Centre (CCDC No. 837649) can be obtained.

Compound 4 was obtained as yellow solid, and its molecular formula was established as $C_{18}H_{16}O_7$ by the MS (EI 70 eV, m/z, %) 344 $[M]^+$ (14), 329 $[M-Me]^+$ (13), 279 (29), 167 (55), 149 (100), 69 (43), 57 (87): FT-IR (KBr ν_{max}, Cm^{-1}): 3430 (OH), 1659(C=O), 1590 (aromatic ring); ^{1}H- NMR (300 MHz, acetone-d6, δ, ppm, J/Hz); 3.81(3H, s, OMe-6),3.99 (6H,s, OMe-3',4'), 6.76 (1H, s, H-8,), 6.86 (1H, s, H-3), 7.01 (1H, d, J=8,

H-5'), 7.63 (2H, dd, J=8, 2 H-2' and H-6'), 12.98 (1H, s, OH);^{13}C –NMR (75 MHz, acetone-d6, δ, ppm); 60.0 (C_6-OMe), 56.2 (C_3'-OMe), 56.2 (C_4'-OMe), 91.4 (C-8), 103.6 (C-3), 105.9 (C-10), 110.0 (C-2'), 115.8 (C-5'), 120.8 (C-6'), 123.0 (C-1'), 132.9 (C-6), 148.3 (C-3'), 151.0 (C-4'), 153.4 (C-9), 153.5 (C-5), 159.5 (C-7), 164.7 (C-2), 183.0 (C-4).

Compound 5 was obtained as yellow amorphous solid, and its molecular formula was established as $C_{18}H_{16}O_8$ by the MS (EI, 70 eV, m/z %) 360 $[M]^+$ (22), 344 $[M-CH4]^+$ (15), 316 [M-CH4-CO] $^+$ (11), 123(100), 105(93), 77(80); FT-IR (KBr ν_{max}): 2700-3200, 1660, 1620, 1585, 1565, 1520 cm^{-1} ; ^{1}H- NMR (300 MHz DMSO-d6, δ, ppm, J/Hz); 6.89 (1H, s, H-3), 6.57(1H, s, H-8), 7.15 (2H, s, H-3' and H-6'), 3.75 (6H, s, OMe-2',4'), 3.88 (3H, s, OMe-6), 9.64 (1H, s, OH),12.96 (1H, s, OH); ^{13}C -NMR (75 MHz DMSO-d6, δ, ppm); 164.1 (C-2), 103.0 (C-3), 183.0 (C-4), 153.6 (C-5), 132.2 (C-6), 158.3(C-7), 95.1 (C-8), 153.3 (C-9), (C-10) 108.5, 126.7 (C-1'), 154.4 (C-2'), 105.0 (C-3'), 151.7 (C-4'), 140.5 (C-5'), 108.5 (C-6'), 57.0 (OMe-6), 60.8 (OMe-2'), 60.9 (OMe-4').

Antimicrobial activity

The antimicrobial activity of the five compounds that isolated from *A. kopetdaghensis* was evaluated against a set of 9 microorganisms and their potency were assessed qualitatively and quantitatively by minimum inhibitory concentration (MIC) values. The results are given in (Table 1**)** and indicate that the compound 2 and compound 1 remarkably inhibited the growth of all tested bacteria, while three compounds 3, 4 and 5 showed weak antimicrobial activity against these microorganisms. The compound **2** showed best antibacterial activities against *P. aeroginosa* with MIC of 16 μg/ml. This compound was active against *S. typhi*, *K. varians*, *E. coli L. monocytogenes* (MIC values of 32 μg/ml and 64 μg/ml, respectively). Also compound 1 exhibited good anticandidal activity against all tested gram-negative bacteria and *Bacillus pumilus,* one of the gram-positive bacteria. Best inhabitation in this compound was observed against *E. coli* (MIC values of 32 μg/ml). This is particularly interesting from a medical point of view because this microbial agent is responsible for severe infections. Our results about the antimicrobial activity of terpene type compounds 2 and 1 are similar to other reports about the analogies of these compounds [30-32]. None of the isolated components showed significant activity against fungal microorganisms.

Table. 1. Antimicrobial activity of the isolated compounds from *A. kopetdaghensis* by MIC method (µg/ml)

Microorganism	Sesquiterpene lactone 1	Peroxide sesquiterpene 2	2-Phenoxychromone 3	Flavonoide 4	Flavonoide 5	Antibiotics*
Gram-negative bacteria						
Escherichia coli	32	64	256	512	-	16
Pseudomonas aeroginosa	64	16	256	512	512	8
Salmonella typhi	64	32	512	256	512	32
Gram-positive bacteria						
Bacillus pumilus	64	128	256	256	256	64
Kocuria varians	128	64	128	512	512	16
Listeria monocytogenes	128	64	512	512	512	16
Fungi						
Aspergillus flavus	512	512	-	-	-	64
Candida glabrata	512	256	-	512	-	128
Aspergillus niger	512	512	-	-	-	64

*Ampicillin, Tetracycline and Fluconazole were used as references for Gram-positive, Gram-negative bacteria and fungus, respectively. (Range of concentration: 8–512 µg/ml)

Antioxidant activity

The isolated compounds were subjected to screening for their possible antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay methods (Table. 2). DPPH is a stable free radical which can readily experience reduction in the presence of an antioxidant. It shows a maximum ultraviolet and visible (UV–Vis) absorbance at 517 nm. The reduction in the intensity of absorption at 517 nm of methanol solutions of DPPH radical in the presence of antioxidants is usually taken as a measure of their antioxidant activity. In this study, the ability of compounds to scavenge DPPH radical was determined based on their concentrations providing 50% inhibition (IC_{50}).

Table. 2. Antioxidant activity of the isolated compounds from *A. kopetdaghensis* and Trolox in DPPH free radical scavenging activity

Sample	DPPH IC_{50} (µg/ml)
Sesquiterpen lactone 1	33.21±2.40
Peroxide sesquiterpene 2	ND[a]
2-Phenoxychromone 3	92.21 ± 1.1
Flavonoide 4	71.66 ± 0.66
Flavonoide 5	89.50 ± 0.65
Trolox	19.72 ± 0.82

[a]No inhibition for compound 2 Observed for concentrations up to 2 mg/ml, ND (Not determined)

DISCUSSION

In our study, compound 1 was shown the best radical scavenging activity with IC_{50} value of 33.21±2.40 µg/ml. Literature review shows that the compounds containing hydrogen atoms in the allylic or benzylic positions may show better activity because of relatively easy abstraction of a hydrogen atom from these functional groups by peroxy radicals formed in the test circumstances [33]. The compounds with allylic or benzylic hydrogens such as terpenoids and steroids were reported [34-37]. As expected for all phenolic compounds, 2-phenoxychromone (compound 3**)** and two flavonoids (compounds 4 and 5**)** were shown higher antioxidant activities (IC_{50}=92. 21 ± 1.1; 71.66 ± 0.66 and 89.50 ± 0.65,

respectively) in comparison with the non-phenolic compound 2.

ACKNOWLEDGEMENTS

We are grateful to Dr. V. Mozaffarian (Research Institute of Forest and Rangelands, Tehran) for helpful assistance in collecting plant material and botanical identification.

REFERENCES

[1]. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. *J Na t Prod*, 2007; 70: 461-77.

[2]. Rustaiyan A, Javidnia K, Farjam MH, Mohammadi MK, Mohammadi N. Total phenols, antioxidant potential and antimicrobial activity of the methanolic extracts of Ephedra laristanica. *J Med Plants Res*, 2011; 5: 5713-17.

[3]. Harvey AL. Natural products as a screening resource. *Curr Opin Chem Bipl*, 2007; 11: 480-84.

[4]. Rustaiyan A, Javidnia K, Farjam MH, Aboee-Mehrizi F, Ezzatzadeh E. Antimicrobial and antioxidant activity of the Ephedra sarcocarpa growing in Iran. *J Med Plants Res*, 2011; 5: 4251-55.

[5]. Ezzatzadeh E, Farjam MH, Rustaiyan A. Antioxidant and antibacterial activities of methanol extract of blepharis glomerans flowers. *Int J Agricul Sci Res*, 2012; 7(1): 431-34.

[6]. Rechinger KH. *Artemisia* In Flora Iranica. Compositae. Rechinger KH, Hedge IC (Eds), *Akademische Druk and Verlagsanstalt*, Austria, 1980; 185-216.

[7]. Rustaiyan A, Masoudi SH. *Phytochememistry Let*, 2011; 4: 440-47.

[8]. Marco JA, Sanz JF, Sansemon F, Rustaiyan A, Saberi M. Sesquiterpene lactpnes from artemisia species. *Phytochemistry*, 1993; 32: 460-62.

[9]. Rustaiyan A, Bamoniri A, Raffatrad M, Jakupovic J, Bohlman F. Eudesmane derivatives and highly oxygenated monoterpenes from Iranian Artemisia species. *Phytochemistry*, 1987; 26: 2307-10.

[10]. Rustaiyan A, Zare K, Ganji MT, Sadri HA. A melampolide and two dihydro artemorin derivatives from Artemisia gypsacea. *Phytochemistry*, 1989; 28: 1535-36.

[11]. Rustaiyan A, Ezzatazdeh E. *Asian J Chem*, 2011; 23: 1774-76.

[12]. Sanz JF, Rustaiyan A, Marco JA. A melampolide from artemisia olip'eriana. *Phytochemistry*, 1990; 29: 2919-21.

[13]. Cubukcu B, Melikoglu G. Flavonoids Of Artemisia Austriaca. *Planta Med*, 1995; 61: 488-89.

[14]. Wollenweber E, Favre-Bonvin H, Houlville M, Rustaiyan A. A Phloracetophenone derivative and flavonoid aglycones from the lipophilic

exudates of Artemisia aucheri. *Phytochemistry*, 1992; 11: 105-07.

[15]. Rybalko KS, Konovalova OA, Sheichenko VI, Zakharov PI. Armin-A new coumarin from Artemisia armeniaca. *Chem Nat Comp*, 1997; 12: 262-65.

[16]. Rustaiyan A, Nahrevanian H, kazemi M. A new antimalarial agent; effect of extracts of Artemisia diffusa against Plasmodium berghei *Pharmacogn Mag*, 2009; 4: 1-7.

[17]. Rustaiyan A, Masoudi S. *Nat Prod Commun*, 2011; 4: 440-47.

[18]. Mirdeilami SZ, Barani H, Mazandarani M, Heshmati GA. Ethnopharmacological survey of medicinal plants in Maraveh Tappe region, north of Iran. *Iran J Plant Physiol*, 2011; 2: 327-80.

[19]. Ramezani M, Behravan J, Yazdinezhad A. .*Flavour Frag J*. 2006; 21(6): 869-71.

[20]. Ebrahimi M, Ramezani M, Tehrani SO, Malekshah OM, Behravan J. Cytotoxic effects of methanolic extract and essential *oil* of Artemisia Kopetdaghensis. *J Essent Oil Bear Pl*, 2010; 13(6): 732-37.

[21]. Oliaee D, Boroushaki MT, Oliaee N, Ghorbani A. Evaluation of cytotoxicity and antifertility effect of. Artemisia kopetdaghensis. *Advan Pharmacol Sci*, 2014; 745760.

[22]. Gul HI, Ojanen T, Hanninen O. Antifungal evaluation of some bis Mannich bases derived from acetophenone and their corresponding piperidinols and stability studies. *Biol Pharm Bull*, 2002; 25: 1307-10.

[23]. Sarker SD, Latif Z, Gray AI. Natural Products Isolation, 2nd ed. *Humana Press Inc*, New Jersey, 2006; 10-11.

[24]. Rustaiyan A, Bamoniri A, Raffatrad M, Jakupovic J, Bohlman F. Eudesmanederivatives and highly oxygenatedmonoterpenes from Iranian Artemisia species. *Phytochemistry*,1987; 26: 2307-10.

[25]. Appendino G, Garibaldi P, Nano GM, Tetenyi P. Tetrahydrofuran-type terpenoids from Tanacetum vulgare. Phytochemistry, 1984; 23: 2545-51.

[26]. Dongyu GU, Yi Y, Rahima A, Haji Akber A. Characterization and identification of chemical compositions in the extract of Artemisia rupestris L. by liquid chromatography coupled to quadrupole time-of-flight tandem *mass spectrometry*. *Mass Spectrom*. 2012; 26: 83-100.

[27]. Leon L, Maldonado E, Cruz A, Ortega A. X-ray diffraction; Mimosa; medicinal plants; leaves; plant extracts; phytochemicals; heterocyclic compounds; spectral analysis; stereochemistry. *Planta Med*, 2004; 70(6): 536-39.

[28]. Suleimenov EM, Smagulova FM, Morozova OV, *et al*. Sesquiterpene lactones and flavonoids from Artemisia albida. *Chem Nat Comp*. 2005; 41(6): 689-91.

[29]. Deng Yan-Ru, Song Ai-Xin, Wang Han-Qing. The structures of these compounds were determined by means of spectral and chemical studies. *J Chinese Chem Soc.* 2004; 51: 629-36.

[30]. Griffin SG, Wyllie SG, Markham JL, Leach DN. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flav Frag J.* 1999; 14: 322-32.

[31]. Scortichini M, Rossi MP. The *antimicrobial activity* of *20 10*% (*v*/*v*) *solutions* in *ethanol* of *terpenes* and *terpenoids* at *several concentrations* was *tested against Erwinia amylovora NCPPB 595* in the *liquid medium 523. J App Microbiology*, 1991; 71(2): 109-112.

[32]. Souza AB, Martins CH, Souza MG, *et al.* Antimicrobial activity of terpenoids from Copaifera langsdorffii Desf. against cariogenic bacteria. *Phytother Res*, 2011; 25(2): 215-20.

[33]. Larson RA. Naturally occurring antioxidants. Boca Raton, FL, USA: *CRC Press*, 1997.

[34]. Bourgoul S, Pichette A, Lavoie S, Marzouk M, Legault J. *Flav Frag J*, 2012; 27: 69-74.

[35]. Khanavi M, Sharifzadeh M, Hadjiakhoondi A, Shafiee A. Phytochemical investigation and anti-inflammatory activity of aerial parts of Stachys byzanthina C. Koch. *J Ethnopharmacology*, 2005: 97: 463-68.

[36]. Piozzi F, Paternostro MP, Servettaz O, Arnold NA. Occurrence of (+)-6-desoxyandalusol in Stachys ionica and Stachys distans. *Biochem Syst Ecol*, 2002; 30:887-89.

[37]. Piozzi F, Savona G, Hanson JR. Kaurenoid diterpenes from Stachys lanata. *Phytochemistr*y, 1980; 19: 1237-38.