© 2017
Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 16 (6): 570 - 577
ISSN 0717 7917

Artículo Original | Original Article

Bioactive sesquiterpene lactone from *Artemisia santolina*

[Lactona sesquiterpénica bioactiva de *Artemisia santolina*]

Soodabeh Saeidnia[1], Javad Asili[2], Azadeh Manayi[1], Ahmad R. Gohari[1], Azizollah Nezhadali[3], Jalil Lari[3] & Mahdieh Kurepaz-Mahmoodabadi[1]

[1]*Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
[2]*Department of Pharmacognosy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*
[3]*Department of Chemistry, Payame Noor University (PNU), Mashhad, Iran*
Contactos | Contacts: **Mahdieh KUREPAZ-MAHMOODABADI** - ***E-mail address:*** **m-mahmoodabadi@farabi.tums.ac.ir**

Abstract: Most species of genus *Artemisia* L. (Compositae) are medicinal herbswith several uses in the folk medicine worldwide. In the present study, methanol extract of *Artemisia santolina* has been subjected for isolation of its metabolites along with evaluation of cytotoxic activity against *Artemia salina* larvae. The structures of the compounds determined by 1H-and 13C-NMR, HMQC, HMBC, 1H-1H COSY and Mass spectral analysis. Two sesquiterpenes, 1,5-dihydroxy- 4(15)eudesman-12,6-olid (artemin) (**1**), 2-hidroxy-2,6,10-trimethyl-7,10-oxide-3,11-dodecadien-5-one (**2**) and one flavonoid, 5,7,4'-trihydroxy-6,3'-dimethoxyflavone (jaceosidin) (**3**) have been successfully characterized. Cytotoxicity of the sesquiterpene lactone (**1**), was assessed on *Artemia salina* larvae and resulted in IC50 value of 6.44 µg/mL, which was more potent compared to the positive standard berberine hydrochloride (IC_{50} = 26 µg/mL). In this study, the separation and identification of two sesquiterpenes and one flavone from the aerial parts of *A. santolina* is described. Among them the compound artemin (**1**) showed a toxicity effect against *A. salina* nauplii.

Keywords: *Artemia salina*, *Artemisia santolina*, flavonoid, sesquiterpenes, 2D NMR.

Resumen: La mayoría de las especies del género *Artemisia* L. (Compositae) son hierbas medicinales con varios usos en la medicina popular en todo el mundo. En el presente estudio, el extracto metanólico de *Artemisia santolina* ha sido sometido al aislamiento de sus metabolitos junto con la evaluación de la actividad citotóxica contra las larvas de *Artemia salina*. Las estructuras de los compuestos se determinaron mediante RMN ^{1}H y ^{13C}, HMQC, HMBC, ^{1}H-1H COZY y análisis espectral de masas. Dos sesquiterpenos, 1,5-dihidroxi-4 (15) eudesman-12,6-olid (artemin) (**1**), 2-hidroxi-2,6,10-trimetil-7,10-óxido-3,11-dodecadien-5-ona (**2**) y un flavonoide, 5,7,4'-trihidroxi-6,3'-dimetoxiflavona (jaceosidina) (**3**). Se evaluó la citotoxicidad de la lactona sesquiterpénica (1) en larvas de Artemia salina y resultó en un valor de CI_{50} de 6,44 µg/ml, que era más potente en comparación con el clorhidrato de berberina estándar positivo (CI_{50} = 26 µg/ml). En este estudio se describe la separación e identificación de dos sesquiterpenos y una flavona de las partes aéreas de *A. santolina*. Entre ellos, el compuesto artemin (**1**) mostró un efecto de toxicidad contra los nauplios de *A. salina*.

Palabras clave: *Artemia salina*, *Artemisia santolina*, flavonoides, sesquiterpenos, RMN 2D

Recibido | Received: July 25, 2017
Aceptado | Accepted: August 13, 2017
Aceptado en versión corregida | Accepted in revised form: September 28, 2017
Publicado en línea | Published online: November 30, 2017
Este artículo puede ser citado como / This article must be cited as: S Saeidnia, J Asili, A Manayi, AR Gohari, A Nezhadali, J Lari, M Kurepaz-Mahmoodabadi. 2017. Bioactive sesquiterpene lactone from *Artemisia santolina*. **Bol Latinoam Caribe Plant Med Aromat** 16 (6): 570 – 577.

INTRODUCTION

Artemisia L., containing large and heterogenous plants, is one of the largest and most widely genus of the Asteraceae (Compositae) family. This genus is numbering over 400 species distributed mainly in the temperate zone of Europe, Asia and North Amarica (Mozafarian, 1996). There are 34 species of this genus growing wildly in different parts of Iran, of which two species are endemic (Mirhydar, 1995; Zargari, 1997).

According to the literature, over 260 *Artemisia* species have been investigated to reveal that they contain many classes of secondary metabolites including terpenoids, flavonoids, coumarins, glycosides, sterols, and polyacetylenes (Juteau *et al.,* 2002; Yu *et al.,* 2003). Different species of *Artemisia* have a wide range of biological effects including antimalarial, cytotoxic, antibacterial, antifungal, antioxidant (Tan *et al.,* 1998; Rustaiyan & Masoudi, 2011), carminative, antipyretic, antiparasitic, anthelmintic, antiseptic, antispasmodic, antimicrobial, anti-inflammatory, appetite-stimulating, digestive, funguicidal, emmenagogue, stomachic, vermifuge, vulnerary, and hypnotic activity (Aniya *et al.,* 2000).

Literature review revealed that *Artemisia* extracts especially dichloromethane extracts of *A. santolina* Schrenk showed anti proliferative effects on malignant cell lines. *Artemisia* has been also considered as a promising chemotherapeutic agent in cancer treatment (Zamanai *et al.,* 2011).

On the other hands, ethanol extract of *A .santolina* with IC_{50}: 80 µg/ml exhibited potent antileishmanial activity after 24 hrs of incubation (Emami *et al.,* 2012).

To the best of our knowledge, there is no report on phytochemical investigation of this species except chemical constituents of the essential oil (Rustaiyan *et al.,* 2000; Nezhadali *et al.,* 2010). For this reason, we aimed to isolate and identify the main compounds of the methanolic extract of *A. Santolina* which grows widely in the Eastern parts of Iran.

MATERIAL AND METHODS

Reagents and instruments

All the chemicals and solvents were purchased from Merck Company. Column chromatography was performed on Merck silica gel 60A (particle size 230-400 mesh). Preparative TLC was carried out on Merck Silica gel 60 GF_{254} plates. Fractions were monitored by TLC (Merck pre-coated silica gel 60 F_{254} aluminum plates) and compound visualized using vanillin/sulfuric acid reagent. All solvents were pre-distillated. NMR spectra were recorded at 25º C using the instrument Bruker Avance 500 MHz spectrometer in chloroform-*d* ($CDCl_3$) and acetone, using TMS as internal standard.

The plant material

Aerial parts of the *A. Santolina* were collected from natural provenance in the Birjand, Khorasan province of Iran in September 2003. The plant was identified by Dr. Mozafarian, Valiallah. A voucher specimen was deposited in herbarium of Dr. Zargari, Mashhad Faculty of Pharmacy, Mashhad, Iran. The plant material was air-dried in a dark place and stored in a sealed plastic bag in a dry, dark and cool place until used.

Extraction of the plant

About 400 g of milled aerial parts of the plant *A. santolina* were defatted with shaking in petroleum ether at room temperature for 24 hours. The solvent was subsequently drained and the plant material was air-dried before extraction with methanol. The extraction was performed by maceration method for 24 h. Then, the extract was filtered and evaporated under reduced pressure. This procedure was repeated four times, and the extracts were collected and concentrated by rotary evaporator.

Isolation process

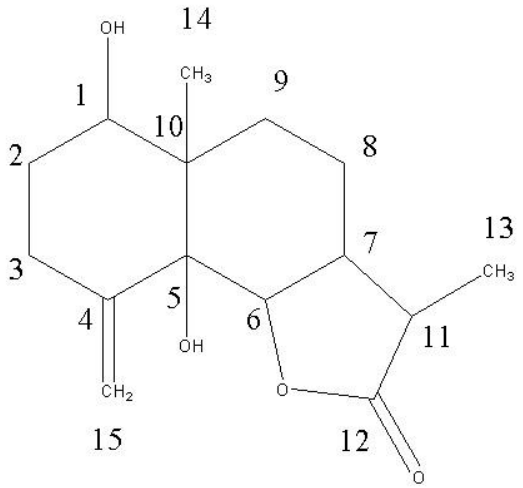
The methanolic extract (7 g) was subjected to silica gel column chromatography (CC) with petroleum ether: AcOEt (1:0, 6: 4, 7: 3, 0:1) as eluent, to give three fractions (A-C). The fraction A (20 mg) from system of (7:3) was further fractionated by preparative TLC with petroleum ether: AcOEt (6:4) to obtain two fractions (A_1-A_2). Compound **1** (7 mg) was obtained from A_1 using crystallization method. The fraction B (50 mg) was subjected to silica gel CC with petroleum ether: AcOEt (6:4) result in two fractions (B_1-B_2). Compound **2** (5 mg) was obtained from B_1 using preparative TLC followed by crystallization and Compound **3** (6 mg) was obtained from B_2. The pure compounds were dried under nitrogen gas. The vial placed in a small round-bottomed flask and the flask placed in a freeze dryer for 24h.

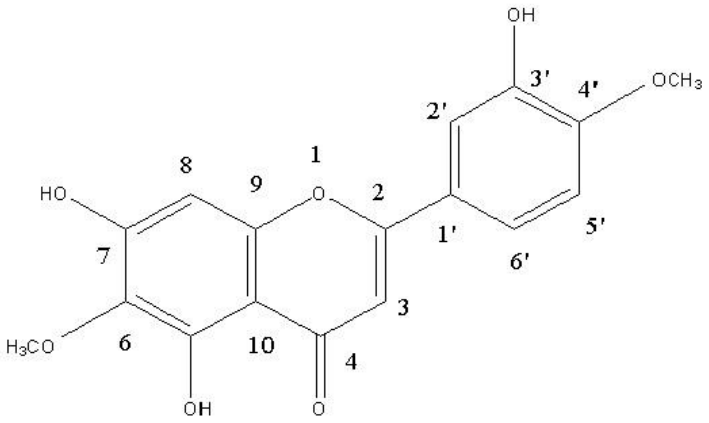
Brine Shrimp Cytotoxicity Bioassay

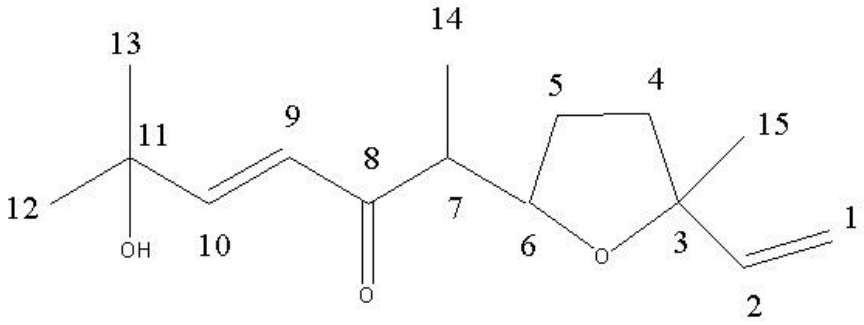
Cytotoxic activities of the *sesquiterpene lactone* were assessed by *Artemia salina* according to modified Mongelli method described by Saeidnia *et al.* (2009). Brine shrimp (*Artemia salina*) eggs were hatched in flask containing 300 ml artificial seawater made by dissolving distilled water in 29-30° C temperature and aerate condition. Different concentrations of each extract dissolved in normal saline were obtained by serial dilution. Four concentrations of each extract were prepared with (10, 100, 500 and 1000 µg/ml). Ten to 20 nauplii were added to each concentration of the extracts in 24 well chamber slides. Number of nauplii alive noted after 24 h. The mortality end point of the bioassay was determined as the absence of controlled forward motion during 30 seconds of observation. Seawater and berberine hydrochloride (LC_{50} = 26 µg/ml) were used as controls. Lethality percentage was determined and LC_{50} calculated based on Probit Analysis with 95% of confidence interval (Saeidnia *et al.*, 2009).

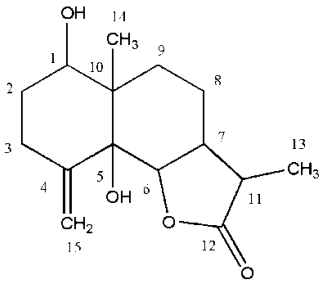
RESULTS AND DISCUSSION

In this study, active compounds of *A. santolina* were purified using combination of column chromatography (CC) and preparative thin layer chromatography (PTLC) from methanolic-extract of the aerial parts of the plant. The methanolic extract of the aerial parts of *A. Santolina* has been subjected to various chromatography separation methods to obtain two sesquiterpenes and one flavone as: artemin (**1**), 2-hidroxy-2,6,10-trimethyl-7,10-oxide-3,11-dodeca-dien-5-one (**2**) and jaceosidin (**3**), identified on the basis of the spectral data compared with those reported in the literature (Khafagy *et al.*, 1983; Agrawal, 1989; Rustaiyan *et al.*, 1989). Spectroscopic data of the compound **1** including ^{1}H-NMR, ^{13}C-NMR, HMBC, ^{1}H-^{1}H COSY and DEPT are shown in Table 1. The EI-MS spectrum of the compound **1** showed a molecular peak at m/z 266 indicating the molecular formula of the compound as $C_{15}H_{22}O_4$ and other prominent peaks were observed at m/z 248 $[M\text{-}H_2O]^+$, m/z 233 $[M\text{-}H_2O\text{-}CH_3]^+$, m/z 222 $[M\text{-}CO_2]^+$ and m/z 205 $[M\text{-}H_2O\text{-}CH_3\text{-}CO]^+$, corresponding to a backbone of eudesmanolid (one of the main class of sesquiterpene lactones) (Yang *et al.*, 2008). The ^{13}C-NMR spectrum of compound **1** shows 14 signals including of two methyl, five methylene, four methine and three quaternary carbon (Table 1). Since, carbons of compound **1** in position of 1 and 5 attached to hydroxyl groups they were appeared in 72.1 and 77.6 ppm, respectively. Explanation of the data from the HMQC, HMBC and ^{1}H-^{1}H COSY correlation (Table 1), that were initially stated, resulting in elucidation of planar structure of the compound **1** as shown in figure 1.

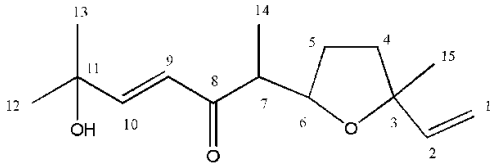




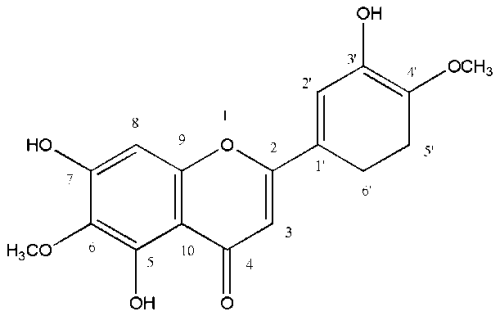




artemin (**1**)



2-hidroxy-2,6,10-trimethyl-7,10-oxide-3,11-dodecadien-5-one (**2**)



jaceosidin (**3**)

Figure 1
Chemical structures of the isolated compounds from *A. santolina*.

All spectroscopic data of compound **2** were presented in Table 2, in which data of ^{13}C-NMR, HMBC, ^{1}H-^{1}H COSY and DEPT spectra were reported for the first time. The structure of the isolated compound **2** was readily identified by comparing these spectral data with those reported in the literature (Figure 1). (Khafagy *et al.*, 1983) Compound **3** was isolated as yellow needles and its NMR data were compared to previous results, which are explained followed: ^{1}H-NMR data (500 MHz, aceton-d6), δ H (ppm): 3.89 (3H, s, OCH3), 4.01 (3H, s, OCH3), 6.65 (1H, s, H-8), 6.71 (1H, s, H-3), 7.02 (1H, d, J = 8Hz, H-5'), 7.65 (1H, d, J = 2, H-2'),7.61 (1H, dd, J = 2, H-6'); 13C NMR (500 MHz, Aceton), δ C (ppm): 164.6 (C-2), 103.4 (C-3), 182.1 (C-4), 153.4 (C-5), 131.6 (C-6), 157.1 (C-7), 94.3 (C-8), 105.3 (C-10), 123.1 (C-1´), 110.0 (C-2´), 150.9 (C-3´), 148.4 (C-4´), 115.9 (C-5´), 120.8 (C-6´), two methoxy groups in 56.1 and 60.2 (Agrawal, 1989).

Table 1
The NMR data of artemin (1), a sesquiterpene lactone, isolated from *A. santolina* in chloroform-*d*.

No	$^{1}H_{NMR}$	$^{13}C_{NMR}$	HMBC	COSY	DEPT
1	4.2(dd, j=5)	72.1	H-2,H-3, H-14	H-2, H-3	CH
2	1.6, 2.2	30.7	H-3 , H-14	H-1, H-3	CH_2
3	1.9, 2.7	30.0	H-2 , H-15	H-1, H-2, H-15	CH_2
4	----------	145.3	H-3 , H-15		C
5	----------	77.6	H-2 , H-9 H-14 ,H-15		C
6	4.3(d, j=10)	82.1	H-7 , H-8 H-11	H-7, H-11	CH
7	2.4(m, j=6)	45.8	H-9 , H-13	H-6, H-8, H-11	CH
8	1.6, 1.9	23.2	H-9	H-9, H-7	CH_2
9	1.79	30.3	H-14	H-8	CH_2
10	----------	44.9	H-14		C
11	2.4(m, j=6)	41.6	H-6 , H-7 H-13	H-13, H-6	CH
12	----------	179.7	H-11, H-13		C
13	1.28(d, 3H)	12.8		H-11	CH_3
14	0.95(s, 3H)	13.6	H-9		CH_3
15	5.1(d, 2H)	112.9	H-6	H-3	CH_2

Table 2
The NMR data of 2-hidroxy-2,6,10-trimethyl-7,10-oxide-3,11-dodecadien-5-one (2), sesquiterpene, isolated from *A. santolina* in chloroform-*d*.

No	$^{1}H_{NMR}$	$^{13}C_{NMR}$	HMBC	COSY	DEPT
1	5.00(d),5.20(d)	111.9	H-15	H-2	CH_2
2	5.90m	145.0	H-1, H-4,H-15	H-1	CH
3	----------	83.3	H-1, H-2,H-4, H-5,H-6, H-15		C
4	1.78(m),1.91(m)	38.0	H-2, H-5,H-6, H-15	H-5	CH_2
5	1.65(m),2.05(m)	29.8	H-4, H-6,H-7, H-15	H-4 , H-6	CH_2
6	4.3(m)	80.9	H-4, H-5,H-7, H-14	H-5, H-7	CH
7	3.00	50.2	H-5, H-6,H-9, H-14	H-6,H-14	CH
8	----------	203.4	H-6,H-7,H-9, H-10,H-14		C
9	6.45d	125.6	H-7, H-10 H-12, H-13	H-10	CH
10	6.95d	153.1	H-7, H-9 H-12, H-13	H-9	CH
11	----------	71.3	H-9, H-10 H-12, H-13		C
12	1.40s	29.71	H-9, H-10 H-13		CH_3
13	1.40s	29.74	H-9, H-10 H-12		CH_3
14	1.05d	13.4	H-6, H-7	H-7	CH_3
15	1.27s	26.9	H-2, H-4		CH_3

The results of brine Shrimp cytotoxicity bioassay showed that the artemin (**1**) indicated a toxicity effect against *A. salina* nauplii (LC_{50} = 26 µg/ml). The equations of the regression lines from probit mortality versus log dosage plots and the lower and upper of the LC_{50}'s with 95% confidence limit are 4.97,8.34. In this test, sesquiterpene lactone (**1**) has shown high toxicity with LC_{50}'s of 6.44µg/mL against *Artemia salina* larvae.

Three compound identified as artemin (**1**), 2-hidroxy-2,6,10-trimethyl-7,10-oxide-3,11-dodeca-dien-5-one (**2**) and jaceosidin (**3**) are investigated but among them, artemin (**1**) was the most active one.

CONCLUSION

The present work is the first report of the isolation and identification of the above mentioned compounds (**1-3**) from *A. santolina*. Artemin (**1**) has been isolated from other *Artemisia* species, such as *A. diffusa* (Yang *et al.*, 2008), *A. pontica* (Trendafilova *et al.*, 1996), *A. hugueti* (Marco *et al.*, 1994), *A. caeralescens* (Kawatani *et al.*, 1956) and *A. halophile* (Arkhipova *et al.*, 1970), and *A. taurica*, and has reported to show antimicrobial activity (Konovalov *et al.*, 2002), moreover artemin and its isomer, arsubin, have been reported to possess anti tuberculosis activity against *Mycobacterium aurum* (Ntutela *et al.*, 2009). In addition, the compound **2** has been already isolated and identified from *A. inculta* (Khafagy *et al.*, 1983). Jaceosidin is an active flavonoid with high biological activity, as one of the major constituent of the medicinal herbs of the genus *Artemisia* and has been exhibited anti-inflammatory (Clavin *et al.*, 2007; Kim *et al.*, 2008), antioxidant (Kim *et al.*, 2008), anti-microglial and anti-neuroinflammatory, (Nam *et al.*, 2013) anti-cancer (Lv *et al.*, 2008) and anti-allergic (Lee *et al.*, 2007) activities.

REFERENCES

Agrawal PK. 1989. **Carbon-13 NMR of flavonoids**. Elsevier, Amsterdam,

Aniya Y, Shimabukuro M, Shimoji M, Kohatsu M, Gyamfi MA, Miyagi C, Kunii D, Takayama F, Egashira T. 2000. Antioxidant and hepatoprotective actions of the medicinal herb *Artemisia campestris* from the Okinawa Islands. **Biol Pharm Bull** 23: 309 - 312.

Arkhipova LI, Kasymov SZ, Sidyakin GP. 1970. Sesquiterpenoid lactones from *Artemisia halophila*. **Khim Prir Soedin** 6: 480 - 481.

Clavin M, Gorzalczany S, Macho A, Munoz E, Ferraro G, Acevedo C, Martino V. 2007. Anti-inflammatory activity of flavonoids from *Eupatorium arnottianum*. **J Ethnopharmacol** 112: 585 - 589.

Emami SA, Zamanai Taghizadeh Rabe S, Ahi A, Mahmoudi M. 2012. Inhibitory activity of eleven *Artemisia* species from iran against leishmania major parasites. **Iran J Basic Med Sci** 15: 807 - 811.

Juteau F, Masotti V, Bessiere JM, Dherbomez M, Viano J. 2002. Antibacterial and antioxidant activities of *Artemisia annua* essential oil. **Fitoterapia** 73: 532 - 535.

Kawatani T, Vodopivec S, Mizukami S. 1956. Communications to the editor. **Yakqaku Zusshi** 76: 1214.

Khafagy SM, Al-Yahya MA, Ziesche J, Bohlmann F. 1983. An unusual nerolidol derivative from *Artemisia inculta*. **Phytochemistry** 22: 1821 - 1822.

Kim MJ, Han JM, Jin YY, Baek NI, Bang MH, Chung HG, Choi MS, Lee KT, Sok DE, Jeong TS. 2008. *In vitro* antioxidant and anti-inflammatory activities of Jaceosidin from *Artemisia princeps* Pampanini cv. Sajabal. **Arch Pharm Res** 31: 429 - 437.

Konovalov DA, Starykh VV, Shkhanukov YZ. 2002. Phytotoxic and antifungal activites of lactone sum of *Artemisia taurica* Willd. **Rastit Res** 38: 77 - 81.

Lee SH Bae EA, Park EK, Shin YW, Baek NI, Han EJ, Chung HG, Kim DH. 2007. Inhibitory effect of eupatilin and jaceosidin isolated from *Artemisia princeps* in IgE-induced hypersensitivity. **Int Immunopharmacol** 17: 678 - 684.

Lv W, Sheng X, Chen T, Xu Q, Xie X. 2008. Jaceosidin induces apoptosis in human ovary cancer cells through mitochondrial pathway. **J Biomed Biotechnol** 2008: 394802.

Marco JA, Sanz-Cervera JF, Pareja JM, Sancenon F, Valles-Xirau J. 1994. Sesquiterpene lactones from North African *Artemisia* species. **Phytochemistry** 37: 477 - 485.

Mirhydar H. 1995. **Maaref giahi.** Daftar Nashre Farhang Eslami publication, Tehran, Iran.

Mozafarian V. 1996. A dictionary of Iranian plant names. Farhang Moasser publication, Tehran, Iran,

Nam Y, Choi M, Hwang H, Lee MG, Kwon BM, Lee WH, Suk K. 2013. Natural flavone jaceosidin is a neuro inflammation inhibitor. **Phytother Res** 27: 404 - 411.

Nezhadali A, Lari J, Asili J, Mahmoudabadi M. 2010. Chemical composition of the essential oil of *Artemisia santolina*. **J Essent Oil Bearing Plants** 13: 738 - 741.

Ntutela S, Smith P, Matika L, Mukinda J, Arendse H, Allie N, Estes DM, Mabusela W, Folb P, Steyn L, Johnson Q, Folk WR, Syce J, Jacobs M. 2009. Efficacy of *Artemisia afra* phytotherapy in experimental tuberculosis. **Tuberculosis** 89 Suppl 1: S33 - S40.

Rustaiyan A, Balalaei S, Mohammadi F, Masoudl S, Yari M. 2000. Comparison of the volatile oils of *Artemisia santolina* Schrenk and *Artemisia gypsacea* Krasch., M. Pop. et Lincz. ex Poljak. from Iran. **J Essent Oil Res** 12: 330 - 332.

Rustaiyan A, Masoudi S. 2011. Chemical constituents and biological activities of Iranian Artemisia species. **Phytochem Lett** 4: 440 - 447.

Rustaiyan A, Sigari H, Jakupovic J, Grenz M. 1989. A sesquiterpene lactone from *Artemisia diffusa*. **Phytochemistry** 28: 2723 - 2725.

Saeidnia S, Gohari AR, Shahverdi AR, Permeh P, Nasiri M, Mollazadeh K, Farahani F. 2009. Biological activity of two red algae, *Gracilaria salicornia* and *Hypnea flagelliformis* from Persian Gulf. **Pharmacogn Res** 1: 428 - 430.

Tan RX, Zheng WF, Tang HQ. 1998. Biologically active substances from the genus Artemisia. **Planta Med** 64: 295 - 302.

Trendafilova AB, Todorova MN, Gussev CV. 1996. Eudesmanolides from *Artemisia pontica*. **Phytochemistry** 42: 469 - 471.

Yang X, Wu Q, Zou L, Fu D, Chang Y, Lu Y, Zhang

Q. 2008. Characterization of nmr signals of tanacetin and artesin isolated from the whole herbs of *Crossostephium chinense*. **Chin J Magn Reson** 25: 127 - 131.

Yu HH, Kim YH, Kil BS, Kim KJ, Jeong SI, You YO. 2003. Chemical composition and antibacterial activity of essential oil of *Artemisia iwayomogi*. **Planta Med** 69: 1159 - 1162.

Zamanai S, Mahmoudi M, Ahi A, Emami SA. 2011. Antiproliferative effects of extracts from Iranian Artemisia species on cancer cell lines. **Pharmaceut Biol** 49: 962 - 969.

Zargari A. 1997. **Medicinal Plants.** 6th edn. Tehran University publications, Tehran, Iran.