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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

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Effect of Drying Temperature on Essential Oil Content and Composition of Sweet Wormwood (*Artemisia annua*) Growing Wild in Iran

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Abstract: Studies were conducted to show the effect of different temperatures in the drying process on the amount and quality of essential oils of sweet wormwood (*Artemisia annua* L.). The sweet wormwood aerial parts were harvested in full blooming time from an area around the Siahkal city in north of Iran in September 2005. In order to complete drying, the aerial parts were placed at shade (room temperature) and in oven at 35, 45, 55 and 65°C temperatures. The aerial parts essential oil was extracted by hydrodistillation in a Clevenger apparatus and analyzed by GC/MS. Results showed that higher drying temperatures decreased the essential oil content, from 1.12% (room temperature) 0.88% (35°C), 0.55% (45°C) to 0.50% (55°C) and 0.37% (65°C). Thirty-five components were determined in essential oils, which were mostly monoterpenes. The drying temperatures had a significant effect on the essential oils composition and proportion of the various components, as when the temperature increased, the monoterpenes content gradually decreased and vice versa for sesquiterpenes. The major components were artemisia ketone and 1, 8 cineol for room and 45°C; artemisia ketone, 1, 8 cineol and camphor for 35 and 55°C and β -caryophyllene and germacrene D for 65°C temperatures.

Key words: *Artemisia annua*, sweet worm, essential oil, drying temperature, Iran

INTRODUCTION

The *A. annua* plant of the family Asteraceae is commonly known as sweet wormwood or Qinghao, indigenous to South East Asia, is an annual herb/shrub, which has become naturalized or is in cultivation as a horticultural or medicinal plant in many parts of Asia, Africa, Europe, America and Australia (Wright, 2002). The plant broadly grows in sea shores of Caspian sea in North of Iran (Zargari, 1989). The leaves and inflorescence of this plant are source of an antimalarial compound artemisinin (Qinghaosu) (Gupta *et al.*, 2002). The foliage and inflorescence of *A. annua* plants also yield an essential oil. The essential oil has potential to be used in perfumery, cosmetics and aromatherapy and also has been reported that it has antifungal and antimicrobial effects (Woerdenbag *et al.*, 1993; Wright, 2002). The yield of the oil generally varies between 0.3 and 0.4% (v/w) (Woerdenbag *et al.*, 1993; Holm *et al.*, 1997). Significant variations in the percentage occurrence of different constituents have also been reported. The oil has been subjected to extensive chemical study (Woerdenbag *et al.*, 1993; Ahmad and Misra, 1994; Holm *et al.*, 1997). Woerdenbag *et al.* (1993) reported that principal constituents of oil from Chinese plants were

artemisia ketone (63.9%), artemisia alcohol (7.5%), myrcene (5.1%), β -guainene (4.7%) and camphor (3.3%). Hethelyi *et al.* (1995) analyzed the oil content from fresh flowering shoots in Hungary. The oil content varied from 0.48-0.81% and was mainly composed of artemisia ketone and artemisia alcohol varying between 33-75 and 15-56%, respectively. The highest yield of artemisia ketone (80.9%) was found in *A. annua* grown in Bulgaria followed by a Netherlands variety (63.9%) and a USA variety (63.1%), (Ahmad and Misra, 1994). The other minor constituents detected were α and β -myrcene hydroperoxide (Rucker *et al.*, 1987; Herman *et al.*, 1993) camphene (Youan, 1955; Ma *et al.*, 2007), geraniol and allo-ocemene (Liu *et al.*, 1988), a-cadinene, trans- α -ocemene, α -selinene, α -thujone, α -farnesene, linalool, menthol and isomenthol (Lawrence, 1982; Herman *et al.*, 1993) α and β -pinene, sabinene and pinocarveol (Libby and Sturtz, 1989) and pinocarveol, borneol and limonene (Lawrence, 1982; Adams, 1995; Juteau *et al.*, 2002).

It has been observed that content and composition of essential oil of plants grown in various areas differs with respect to variety, soil, climate, drying conditions and harvesting time and method (Aflatuni, 2005). Drying process for storing of aromatic plants is important in order to maintenance quality, aroma and original taste.

With respect to lack of information about drying temperature effects on essential oil content and composition of sweet wormwood, this research was conducted and performed.

MATERIALS AND METHODS

Plant material: The sweet wormwood aerial parts were harvested in full blooming time from an area around the Siahkal city in north of Iran in September 2005. In order to complete drying, the aerial parts were placed at shade (20-25°C) and in ventilated oven at 35, 45, 55 and 65°C temperatures until establishment of weight. The process was repeated 3 times for each temperature treatment. Voucher specimens were deposited in the Herbarium of the faculty of agricultural sciences, Shahed University, Tehran, Iran.

Isolation procedure: The essential oil was prepared by hydrodistillation for 2.5 h using a Clevenger-type apparatus. The oil was dried over anhydrous calcium chloride and stored in sealed vials at low temperature (2°C) before analysis.

Gas chromatography: GC analysis was performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30m×0.25 mm i.d., film thickness 0.25 µm). Oven temperature was held at 40°C for 5 min and then programmed to 250°C at a rate of 4°C min⁻¹. Injector and detector (FID) temperature were 260°C; helium was used as carrier gas with a linear velocity of 32 cm sec⁻¹.

Gas chromatography-mass spectrometry: GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (60 m×0.25 mm i.d.). Oven temperature was 40-250°C at a rate of 4°C min⁻¹, transfer line temperature 260°C, carrier gas helium with a linear velocity of 31.5 cm sec⁻¹, split ratio 1/60, ionization energy 70 eV, scan time 1 sec, mass range 40-300 amu.

Identification of components: The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds, or with data published in the literature (Adams, 1995; Shibamoto, 1987).

Statistical analysis: Analysis of variance was performed and Duncan's multiple test at 5% level was applied for means comparison using MSTATC software.

RESULTS

Effect of drying temperature on essential oil content: The results showed that increasing of drying temperature significantly decreased the essential oil content. The essential oil content was 1.12, 0.87, 0.55, 0.50 and 0.37% (V/W) at room temperature, 35, 45, 55 and 65°C, respectively. The maximum content was obtained at room temperature. Between effects of 45 and 55°C was not observed significant difference (Table 1).

Effect of drying temperature on essential oil composition: GC-MS analysis of *A. annua* essential oil resulted in the detection of 35 components consisting of 20 monoterpenes and 15 sesquiterpenes. The constituent's relative percent was varied with temperature treatment.

Table 1: Effect of drying temperature on chemical composition of *Artemisia annua* essential oil

Components	RI	Room temperature				
		35°C	45°C	55°C	65°C	
α-Pinene	938	7.2	4.1	4.0	4.6	1.2
Camphene	952	1.1	0.9	1.4	1.2	-
Sabinene	975	0.8	0.5	0.4	0.4	-
β-Pinene	980	0.8	0.5	0.5	0.5	-
Myrcene	987	1.1	0.3	0.8	0.6	0.8
Yomogi alcohol	997	0.7	0.7	0.7	0.9	0.8
α-Terpinene	1017	0.4	0.4	0.4	0.5	0.4
1,8-Cineole	1033	14.7	10.5	11.4	9.9	3.2
Artemisia ketone	1060	21.6	17.2	14.4	14.7	7.0
Cis-Sabinene hydrate	1068	0.7	1.0	0.6	0.8	0.6
Artemisia alcohol	1082	0.9	0.9	0.5	0.8	0.5
α-Campholenal	1124	0.7	0.3	-	0.3	-
Trans pinocarveol	1137	7.6	7.9	5.8	7.2	7.8
Camphor	1143	6.8	9.8	9.6	9.3	5.4
Pinocarpone	1160	8.8	9.0	6.3	8.2	6.8
Borneol	1165	0.9	0.7	1.6	1.1	1.0
Terpinen-4-ol	1177	-	1.5	1.3	1.5	1.4
α-Terpineol	1189	-	0.4	-	0.3	0.5
Myrtenal	1193	1.8	1.8	1.5	1.8	2.0
Trans pinocarvyl acetate	1296	0.5	0.5	0.8	0.8	0.9
α-Cubebene	1349	-	0.6	0.9	0.9	1.2
α-Copaene	1374	2.4	3.2	4.5	4.3	6.7
β-Caryophyllene	1418	3.6	5.6	8.2	7.8	12.5
(E)-β-Farnesene	1454	0.9	1.8	1.7	2.1	3.0
β-Chamigrene	1476	-	-	-	-	1.0
Germacrene D	1478	2.6	4.4	5.6	6.3	9.0
β-Selinene	1488	4.6	6.1	4.4	3.5	5.3
γ-cadinene	1512	-	-	-	-	0.8
Epi-Longipinanol	1561	-	-	-	-	0.7
Spathulenol	1572	-	-	-	-	0.7
Caryophyllene oxide	1576	2.1	2.4	4.7	2.0	3.0
β-Copaen-4-α-ol	1589	-	-	0.7	0.1	0.2
Epi-cedrol	1617	-	-	-	-	1.2
Epoxy allo-aromadendrone	1639	-	-	-	-	0.2
3-iso-Thujopsanone	1641	-	-	-	-	0.4
Total (%)		93.3	93.0	92.7	92.4	86.2
Monoterpene		77.1	68.9	62.0	65.4	40.3
Sesquiterpene		16.2	24.1	30.7	27.0	45.9

RI: Retention index on DB-5 column

The major constituents were artemisia ketone and 1,8-cineole at room temperature, 35 and 45°C, artemisia ketone, 1,8-cineole and camphor at 55°C and β -caryophyllene and germacrene D at 65°C. Essential oil composition was affected by increasing of drying temperature. Maximum and minimum number and diversity of constituents were found at 65°C and room temperature, respectively. Some components such as α -pinene, 1,8-cineole and artemisia ketone significantly decreased when temperature increased, while the components such as α -copaene, β -Caryophyllene and germacrene D increased and there was no obvious trend for some of them such as Caryophyllene oxide, β -Selinene and Cis-Sabinene hydrate. Some constituents such as β -chamigrene, γ -cadinene, Epi-longipinanol, Spathulenol, 3-iso-thujopsanone, Epi-cedrol and Epoxy alloaromadendrone were only observed at 65°C.

DISCUSSION

Effect of drying temperature on essential oil content: The results showed that increasing of drying temperature significantly decreased the essential oil content. It has been reported that higher drying temperature sharply decreased the essential oil content (%v/w) from 1.0% (40°C) to 0.14% (60°C) and 0.12% (80°C) for peppermint (*Mentha piperita* L.) and from 2.13% (40°C) to 1.62% (60°C) and 1.09% (80°C) for rosemary (*Rosmarinus officinalis* L.) (Blanco *et al.*, 2002a, b). Buggle *et al.* (1999) found that increasing temperature from 30 to 90°C resulted in decreasing in essential oil content of *Cymbopogon citrates* (DC) Stapf. In comparison of shade (22-25°C) and oven (40°C), drying methods, for *Chamaemelum nobile* var. *flora plena* plant, the essential oil content was 1.9 and 0.9%, respectively (Omidbaigi *et al.*, 2004). Essential oil content of *Piper hispidinervium* C. DC was affected by the removal of moisture from leaves. Under the operating conditions used, drying resulted in a higher essential oil content for temperatures up to 50°C but for drying temperatures above 50°C, this parameter was decreased (Braga *et al.*, 2005). In aromatic plants, at beginning of drying, moisture moves by diffusion to the leaf surfaces and drags essential oil with it. The proposed mechanism explains the loss of essential oil (Cremasco, 2003).

Of course there are some reports which are not confirmed the above findings, for example in *Lippia alba* plant when drying temperature was increased from 40 to 70°C, the content of essential oil increased (Castro *et al.*, 2001). Sefidkon *et al.* (2006) reported that there is no difference between shade (22-25°C) and oven (45°C)

drying methods on essential oil content *Satureja hortensis*. These opposite results may be due to differences in plant species, secretory structures and their position in plant body and chemical composition of essential oil.

Effect of drying temperature on essential oil composition:

Drying temperature affected the essential oil composition. When temperature increased, some components decreased, while others increased or showed no obvious trend. There are some reports that corroborate these findings. Blanco *et al.* (2002a) found that higher drying temperatures affected the essential oil composition of peppermint (*M. piperita* L.), decreasing the contents of 1, 8 cineol and citronelal until 80°C and increasing the contents of menthol and neomenthol until 60°C. These authors also found that decreasing in concentrations of α -pinene, β -myrcene and camphor in essential oil of rosemary (*R. officinalis* L.) occur when comparing essential oil composition from the 40 and 80°C treatments (Blanco *et al.*, 2002b). It has been observed that safrole content, which constitutes 90-94% essential oil of *P. hispidinervium* C. DC, was affected by the drying temperature, increased until 45 and 50°C and then decreased at higher temperatures (Braga *et al.*, 2005). Omidbaigi *et al.* (2004) in comparison of shade (22-25°C) and oven (40°C) drying methods, for *C. nobile* var. *flora plena* plant found significant effect on the proportion of the various components of essential oil. In general by increasing of drying temperature monoterpenes in essential oil composition gradually were decreased while sesquiterpenes increased and their proportion was noticeable (Table 1). This may be due to low molecular weight of monoterpenes in compare to sesquiterpenes, which at higher temperature more rapidly leave the plant organs.

ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support of the University of Shahed.

REFERENCES

- Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, IL., pp: 469.
- Aflatuni, A., 2005. The yield and essential oil content of mint (*Mentha* sp.) in Northern Ostrobothnia. M.Sc. Thesis, University of Oulu, Finland.

- Ahmad, A. and L.N. Misra, 1994. Terpenoids from *Artemisia annua* and constituents of its essential oil. *Phytochemistry*, 37 (1): 183-186.
- Blanco, M.C.S.G., L.C. Ming, M.O.M. Marques and O.A. Bovi, 2002a. Drying temperature effects in peppermint essential oil content and composition. Latin-American Symposium on the Production of Medicinal, Aromatic and Condiments Plants. *Acta Hortic.*, 569: 1.
- Blanco, M.C.S.G., L.C. Ming, M.O.M. Marques and O.A. Bovi, 2002b. Drying temperature effects in rosemary essential oil content and composition. Latin-American Symposium on the Production of Medicinal, Aromatic and Condiments Plants. *Acta Hortic.*, 569: 1.
- Braga, N.P., M.A. Cremasco and R.C.C.R. Valle, 2005. The effects of fixed-bed drying on the yield and composition of essential oil from long pepper (*Piper hispidinervium* C. DC) leaves. *Braz. J. Chem. Eng.*, 22 (02): 257-262.
- Buggle, V., L.C. Ming, E.L. Furtado, S.F.R. Rocha and M.O.M. Marques, 1999. Influence of different drying temperatures on the amount of essential oils and citral content in *Cymbopogon citrates* (DC) Stapf. *Acta Hortic.*, 500.
- Castro, M.D., L.C. Ming, M.O.M. Marques and S.R. Machado, 2001. Effect of different drying temperature on *Lippia alba* essential oil production and yield. In: Proceeding of the 5th National Symposium New Crops and New uses, 8-11 November 2001 Atlanta, USA.
- Cremasco, M.A., 2003. Influence of fixed-bed drying on the saffrole content in essential oil from long pepper (*Piper hispidinervium* C. DC) Final report, FAPESP Project., No. 00/12422-7.
- Gupta, S.K., P. Singh, P. Bajpai, G. Ram, D. Singh, M.M. Gupta, D.C. Jain, S.P. Khanuja and S. Kumar, 2002. Morphogenetic variation for artemisinin and volatile oil in *Artemisia annua*. *Ind. Crops Prod.*, 16(3): 217-224.
- Herman, J.W., R. Bos, M.C. Salomons, H. Hendrik, N. Pras and T.M. Malingre, 1993. Volatile constituents of *Artemisia annua* L. (Asteraceae). *Flavour Fragr. J.*, 8 (3): 131-137.
- Hethelyi, E.B., I.B. Cseko, M. Grosy, G. Mark and J.J. Palinkas, 1995. Chemical composition of *Artemisia annua* essential oils from Hungary. *J. Ess. Oil. Res.*, 7: 45-48.
- Holm, I., I. Laakso, R. Hiltunen and B. Galambosi, 1997. Variation in the essential oil composition of *Artemisia annua* L. of different origin cultivated in Finland. *Flavour Fragr. J.*, 12: 241-246.
- Juteau, F., V. Masotti, J.M. Bessiere, M. Dherbomez and J. Viano, 2002. Antibacterial and antioxidant activities of *Artemisia annua* essential oil. *Fitoterapia*, 73 (6): 532-535.
- Lawrence, B.M., 1982. Progress in essential oils. *Perfum. Flavor.*, 7 (5): 43-44.
- Libby, L.M. and J. Sturtz, 1989. Unusual essential oils grown in Oregon, II *Artemisia annua*. *J. Ess. Oil. Res.*, 1: 201-202.
- Liu, Q., Z.Y. Yang, Z.B. Deng, G.H. Sa and X.J. Wang, 1988. Preliminary analysis on chemical constituents of essential oil from inflorescence of *Artemisia annua*. *Acta Botanica*, 30: 223-225.
- Ma, C., H. Wang, X. Lu, H. Li, B. Liu and G. Xu, 2007. Analysis of *Artemisia annua* L. volatile oil by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. *Jchroma*, 1150 (1): 50-53.
- Omidbaigi, R., F. Sefidkon and F. Kazemi, 2004. Influence of drying methods on the essential oil content and composition of Roman chamomile. *Flavour Fragr. J.*, 19: 196-198.
- Rucker, G., R. Mayer and D. Manns, 1987. α and β -myrcene hydroperoxide from *Artemisia annua*. *J. Nat. Prod.*, 50: 287-289.
- Sefidkon, F., K. Abbasi and K. G. Bakhshi, 2006. Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Satureja hortensis*. *Food Chem.*, 99 (1): 19-23.
- Shibamoto, T., 1987. Retention Indices in Essential Oil Analysis. In: *Capillary Gas Chromatography in Essential Oil Analysis*, Sandra, P. and C. Bicchi (Eds.). Huethig, Verlag, New York, USA.
- Woerdenbag, H.J., R. Bos, M.C. Salomons, H. Hendriks, N. Pras and T.M. Malingre, 1993. Volatile constituents of *Artemisia annua* L. (Asteraceae). *Flavour Fragr. J.*, 8: 131-37.
- Wright, C.W., 2002. *Artemisia*. Taylor and Francis Inc., New York.
- Youan, T., 1955. Essential oil of *Artemisia annua*. *Perfum. Essent. Oil. Res.*, 46: 75-78.
- Zargari, A., 1989. Medicinal Plants. University of Tehran Press, Tehran (In Persian).