Journal of Spices and Aromatic Crops
Vol. 22 (2) : 181–187 (2013)
www.indianspicesociety.in/josac/index.php/josac

Indian Society for Spices


# Essential oil composition of *Artemisia annua* L. at different growth stages

V S Rana, K Abirami*, M A Blázquez[1] & S Maiti

*Directorate of Medicinal and Aromatic Plants Research,*
*Boriavi-387 310, Anand, Gujarat, India.*
**E-mail: abirami78@gmail.com*

Received 20 July 2012; Revised 19 October 2012; Accepted 2 February 2013

## Abstract

Chemical composition of the essential oils obtained from the aerial parts of *Artemisia annua* at vegetative, pre-bloom, bloom and post-bloom stages was determined using GC and GC/MS analysis. The yields of essential oil were 0.14%, 0.34%, 0.64% and 0.54% (w/w), respectively at different growth stages. A total of 67 compounds were identified. Oxygenated monoterpenes (39.0%–57.0%) constituted the main fraction of the oils followed by sesquiterpene hydrocarbons (11.8%–26.2%) and monoterpene hydrocarbons (4.2%–15.1%). The main compounds identified in all the analyzed samples were camphor (28.6%–31.7%), 1,8-cineole (2.1%–20.8%), germacrene D (3.8%–12.0%), β-caryophyllene (2.8%–6.9%), *trans*-β-farnesene (0.7%–4.5%), α-pinene (0.5%–2.4%), *p*-cymene (0.8%–2.3%) and terpinen-4-ol, (0.9%–2.1%). The results indicated considerable quantitative variations in both oil yield and chemical composition at different growth stages and artemisia ketone and artemisia alcohol were found to be absent in the oils.

**Keywords:** *Artemisia annua*, camphor, essential oil

## Introduction

*Artemisia* (Family: *Asteraceae*), is a genus of aromatic and bitter herbs or shrubs found in north temperate region, common in arid zones, notable in western United States, in the Asiatic Steppes, South Africa, South America and China. Around 300 species are found in different parts of the world (Bertea *et al.* 2005) and 27-37 species have been recorded in India (Hooker 1996). The essential oils obtained from the different species of *Artemisia* are used in perfumery, cosmetics and aromatherapy.

*A. annua* L. is annual erect, highly branched, glabrous and aromatic herb, found in Punjab, Peshawar to Waziristan upto an altitude of 5500 feet. In Asia, the herb has been used for many centuries in the treatment of fever and malaria (Brown *et al*. 2003). Its above-ground dried part is used for the treatment of malaria and jaundice in Chinese medicine and is known source of artemisinin used for the treatment of malaria (Tang & Eisenbrand 2011). This plant is native to China was introduced in India for cultivation (Gupta & Tandon 2004) and is

*Present address: Central Agricultural Research Institute, Port Blair-744 101, Andaman & Nicobar Islands, India.
[1]Department de Farmacologia, Facultat de Farmàcia, Universitat de València, Valencia, Spain.

mainly used for the production of antimalarial drug artemisinin and also for its highly fragrant essential oil. It's essential oil has been extensively studied by different workers from India and abroad and has been found to contain varied amounts of oil and major compounds (Ahmad & Misra 1994; Woerdenbag *et al*. 1994; Juteau *et al.* 2002; Goel *et al*. 2008; Brown ***et al.*** 2003; Emadi & Yassa 2009; Malik *et al.* 2009; Tzenkova *et al*. 2010; Padalia *et al*. 2011; Verma *et al.* 2011). Since there is a growing demand for by-products of *A. annua*, it is a remunerative crop for the farmers. Hence, this crop was introduced and cultivated in the experimental field of Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat, situated in the western part of India to study the adaptability of this crop to the semi arid region of Gujarat and also for the raw material production like artemisinin and essential oil. As part of the study, the chemical composition of the essential oils obtained from the aerial parts of *A. annua* at different growth stages were determined by GC and GC-MS analysis. The compositions of the oils were compared with published data.

## Materials and methods

The seeds of *A. annua* were sown in raised nursery beds in the last week of December 2009. The seedlings were transplanted in field in the second week of February 2010. The fresh leaves of *A. annua* (1 kg) were collected at different phenological stages (Table 1) from the field at 90 (vegetative stage), 210 (pre-bloom stage), 240 (bloom stage) and 270 (post-bloom stage) days after transplanting (DAT) of the seedlings during the year 2010 and were subjected to hydrodistillation using a Clevenger type apparatus for 4 h. The distillate was extracted with diethyl ether, the ethereal layer was dried over anhydrous sodium sulphate and ether was removed on gently heated water bath. The oil content at vegetative, pre-bloom, bloom and post-bloom stages was found to be 0.14%, 0.34%, 0.64% and 0.54%, respectively on fresh weight basis (w/w) and were stored at 4ºC - 8ºC until analysis.

GC analysis was carried out on a Clarus 500 (Perkin Elmer) equipped with a flame ionization detector (FID) and a non-polar HP-1 (Crosslinked methylsilicone) capillary column (30 m × 0.2 mm, 0.33 mm film thickness). The oven temperature was held at 60°C for 5 min then programmed at 3°C/min to 180°C and then 20°C/min to 280°C, held for 20 min. Helium was used as carrier gas at a flow rate of 1 mL $min^{-1}$. The injection volume was 1 mL in split mode (1:25). The injector and detector temperatures were maintained at 220°C and 250°C, respectively. The volatile oil was analyzed using GC-MS (Varian 2000) equipped with a Varian CS. VA-5MS capillary columns (30 m × 0.25 mm, 0.25 mm film thickness). Chromatographic conditions were: helium as carrier gas at a flow-rate of 1 mL $min^{-1}$ (split mode); injection volume was 0.5 mL; injector temperature was 250°C. The column temperature was held at 60°C for 5 min., and programmed at 3°C $min^{-1}$ to 180°C and then 20°C $min^{-1}$ to 300°C and held for 20 minutes with split mode (1:25). The column was coupled directly to the quadrupole mass spectrometer and mass spectra was recorded in EI mode at 70eV with the mass range of 28-400 a.m.u range at 1 scan/s. Kovat's retention indices were calculated using co-chromatographed standard hydrocarbons. The individual compounds were identified by mass spectra and their identity was confirmed by comparing their retention indices, relatives to $C_7$-$C_{32}$ *n*-alkanes. Identification of individual compound was carried out by matching mass fragmentation pattern with those from the available authentic samples or with NIST 2005 Library and literature (Adams 2007).

## Results and discussion

The yield of the essential oils obtained at different growth stages *viz*., vegetative, pre-bloom, bloom and post-bloom stages was found to range from 0.14%–0.64% on fresh weight basis. The highest yield of the oil was found at bloom (0.64%) and minimum at vegetative (0.14%) stages. A total of 67 compounds were identified in analyzed oils including the compounds present in minor (0.9%–0.1%) and trace (<0.03%) amounts (Table 1). Oxygenated monoterpene (39.0%–57.0%) constituted the main fraction of the oils followed by sesquiterpene hydrocarbons (11.8%–26.2%)

**Table 1.** Percentage composition of the essential oil of *Artemisia annua* L. at different growth stages

| Compound | RI | Area (%) | | | |
|---|---|---|---|---|---|
| | | VS | PBS | BS | PTBS |
| *Monoterpene hydrocarbons* | | 4.2 | 5.4 | 15.1 | 7.7 |
| Tricyclene | 929 | ND | t | t | t |
| α-Thujene | 930 | ND | t | t | t |
| α-Pinene | 939 | 0.6 | 0.5 | 2.4 | 1.5 |
| Camphene | 954 | 0.7 | 0.9 | 1.7 | 2.0 |
| Sabinene | 977 | t | 0.5 | 1.3 | 0.9 |
| β-Pinene | 980 | 0.4 | 0.2 | 0.3 | 0.2 |
| Myrcene | 992 | t | 0.1 | 2.2 | 1.0 |
| α-Terpinene | 1017 | t | 0.2 | 0.5 | 0.4 |
| *p*-Cymene | 1026 | 2.1 | 2.3 | 1.1 | 0.8 |
| Limonene | 1029 | 0.1 | ND | 4.5 | ND |
| γ-Terpinene | 1063 | 0.2 | 0.6 | 0.9 | 0.7 |
| Terpinolene | 1082 | 0.1 | 0.1 | 0.2 | 0.2 |
| *Oxygenated monoterpenes* | | 39.0 | 39.6 | 46.5 | 57.0 |
| 1,8-Cineole | 1032 | 2.1 | 5.0 | 12.9 | 20.8 |
| *cis*-Sabinene hydrate | 1071 | 0.1 | ND | ND | ND |
| *trans*-Sabinene hydrate | 1094 | 0.1 | 0.1 | ND | ND |
| α-Campholenal | 1128 | 0.4 | ND | ND | ND |
| *trans-p*-Mentha-2,8-dienol | 1145 | ND | ND | ND | ND |
| Camphor | 1149 | 31.2 | 28.8 | 28.6 | 31.7 |
| Pinocarvone | 1165 | 0.3 | 0.2 | ND | ND |
| Borneol | 1170 | 1.5 | 1.7 | ND | ND |
| *cis*-Pinocamphone | 1172 | t | t | ND | ND |
| Terpinen-4-ol | 1177 | 0.9 | 1.2 | 2.0 | 2.1 |
| *p*-Methylacetophenone | 1183 | t | ND | ND | ND |
| *p*-Cymen-8-ol | 1187 | 0.1 | ND | 0.1 | 0.1 |
| α-Terpineol | 1188 | 0.4 | 1.0 | 1.6 | 1.2 |
| Myrtenol | 1197 | 0.4 | 0.2 | 0.2 | 0.2 |
| *trans*-Carveol | 1218 | 0.8 | 0.7 | 0.3 | 0.3 |
| *cis*-Carveol | 1229 | 0.2 | 0.2 | ND | 0.1 |
| Cumin aldehyde | 1238 | ND | ND | 0.2 | 0.1 |
| Carvone | 1243 | 0.3 | 0.2 | 0.1 | 0.1 |
| Bornyl acetate | 1288 | t | 0.1 | ND | ND |
| Thymol | 1291 | 0.1 | 0.1 | 0.1 | ND |
| *p*-Cymen-7-ol | 1292 | t | ND | 0.1 | 0.2 |
| *p*-Mentha-1,4-dien-7-ol | - | ND | ND | 0.1 | 0.1 |
| Carvacrol | 1300 | 0.1 | 0.1 | ND | ND |
| *trans*-Carvyl acetate | 1342 | t | ND | ND | ND |

**Table 1** cont....

| Compound | RI | Area (%) | | | |
|---|---|---|---|---|---|
| | | VS | PBS | BS | PTBS |
| *Sesquiterpene hydrocarbons* | | 18.1 | 26.2 | 11.8 | 14.7 |
| α-Copaene | 1377 | 0.3 | 0.6 | 0.3 | 0.4 |
| β-Bourbonene | 1388 | t | ND | ND | ND |
| β-Elemene | 1394 | 0.4 | 0.5 | 0.2 | 0.2 |
| *cis*-Jasmone | 1396 | 0.2 | 0.2 | ND | ND |
| β-Caryophyllene | 1419 | 5.1 | 6.9 | 2.8 | 3.6 |
| α-Humulene | 1455 | 0.4 | 0.4 | 0.1 | 0.2 |
| *trans*-β-Farnesene | 1460 | 0.7 | 2.3 | 3.5 | 4.5 |
| Germacrene-D | 1484 | 9.0 | 12.0 | 3.8 | 4.5 |
| β-Selinene | 1490 | 0.9 | 0.6 | 0.4 | ND |
| Bicyclogermacrene | 1500 | 0.9 | 2.3 | 0.5 | 0.8 |
| α-Muurolene | 1502 | ND | ND | ND | 0.1 |
| γ-Cadinene | 1515 | ND | ND | t | 0.1 |
| δ-Cadinene | 1523 | 0.2 | 0.4 | 0.2 | 0.3 |
| *Oxygenated sesquiterpenes* | | 1.1 | 1.0 | 0.2 | 0.6 |
| Spathulenol | 1580 | 0.6 | 0.9 | 0.2 | 0.3 |
| *epi*-Cedrol | 1620 | 0.4 | ND | ND | 0.2 |
| Qinghaosu C | - | 0.1 | 0.1 | ND | 0.1 |
| *Oxygenated diterpenes* | 0.4 | 0.3 | 0.1 | ND | ND |
| Phytol | 1945 | 0.4 | 0.3 | 0.1 | t |
| *Aromatic compounds ($C_6$-$C_3$)* | | 0.3 | 0.5 | 0.4 | 0.2 |
| Eugenol | 1361 | 0.3 | 0.5 | 0.4 | 0.2 |
| *Other compounds* | | 1.2 | 1.1 | 1.6 | 1.3 |
| 2-Methyl ethyl butanoate | - | ND | ND | ND | t |
| 3-Hexen-1-ol | 853 | 0.1 | 0.2 | 0.1 | 0.1 |
| 1-Hexanol | 870 | t | t | t | t |
| Nonene | 900 | ND | ND | t | t |
| 4-Methylene-5-hexenal | - | ND | ND | t | t |
| 1-Octen-3-ol | 981 | 0.3 | 0.3 | ND | ND |
| 3-Octanone | - | t | t | ND | ND |
| Hexenyl acetate | 1003 | 0.2 | 0.2 | ND | ND |
| Hexyl acetate | 1007 | t | ND | ND | ND |
| 2-Methyl-butyl-2-methylbutanoate | 1103 | 0.1 | 0.2 | 1.1 | 0.6 |
| Hexenyl-2-methylbutanoate | 1233 | 0.1 | t | 0.2 | 0.3 |
| *cis*-3-Hexenyl tiglate | - | ND | ND | ND | 0.1 |
| Phenylmethyl-3-methylbutanoate | 1390 | 0.4 | 0.2 | 0.2 | 0.2 |
| Oil Yield (%) | | 0.14 | 0.34 | 0.64 | 0.54 |

RI=Retention index relative to $C_7$-$C_{32}$ *n*-alkanes on HP-1 capillary column; VS=Vegetative stage (90 DAT); PBS=Prebloom stage (210 DAT); BS=Bloom stage (240 DAT); PTBS=Post-bloom stage (270 DAT); DAT=Days After Transplanting; t=trace amounts (<0.03%); ND=Not Detected

and monoterpene hydrocarbons (4.2%–15.1%). Among the aromatic compounds (phenyl propanoids series) only eugenol (0.2%–0.5%) was found at different stages. Oxygenated diterpene, phytol (0.1%–0.4%) was absent in post-bloom stage. Aliphatic hydrocarbons were present in trace amounts while alcohols, ketone and mainly esters included in other compounds ranged from 1.1% to 1.6%. The main compounds identified in analyzed oils were camphor (28.6%–31.7%), 1,8-cineole (2.1%–20.8%), germacrene D (3.8%–12.0%), β-caryophyllene (2.8%–6.9%), *trans*-β-farnesene (0.7%–4.5%), α-pinene (0.5%–2.4%), *p*-cymene (2.1%–2.3%) and terpinen-4-ol (0.9%–2.1%).

In this analysis, 56 compounds (10 monoterpene hydrocarbons, 21 oxygenated monoterpenes, 11 sesquiterpene hydrocarbons, 3 oxygenated sesquiterpenes, the oxygenated diterpene phytol, the phenylpropanoid eugenol and 9 other compounds) were identified in the essential oil obtained at the vegetative stage. Camphor (31.2%), germacrene D (9.0%), β-caryophyllene (5.1%), 1-8-cineole (2.1%), *p*-cymene (2.1%) and borneol (1.5%) were identified as main compounds together with 38 minor and 12 compounds in trace amounts.

Forty eight compounds (11 monoterpene hydrocarbons, 15 oxygenated monoterpenes, 10 sesquiterpene hydrocarbons, 2 oxygenated sesquiterpenes, phytol, eugenol and 8 other compounds) were identified in the oil obtained at pre-bloom stage. The major compounds were camphor (28.8%), germacrene D (12.0%), β-caryophyllene (6.9%) and 1,8-cineole (5.0%) followed by *p*-cymene (2.3%), bicyclogermacrene (2.3%), *trans*-β-farnesene (2.3%), borneol (1.7%), terpinen-4-ol (1.2%) and α-terpineol (1.0) besides 32 minor and six trace compounds.

At bloom stage, 45 compounds (12 monoterpene hydrocarbons, 13 oxygenated monoterpenes, 10 sesquiterpene hydrocarbons, 1 oxygenated sesquiterpenes, phytol, eugenol and 7 other compounds) were identified. Fourteen compounds *viz*., camphor (28.6%), 1,8-cineole (12.9%), limonene (4.5%), germacrene D (3.8%), *trans*-β-farnesene (3.5%), β-caryophyllene (2.8%), α-pinene (2.4%), myrcene (2.2%), terpinen-4-ol (2.0%), camphene (1.7%), α-terpineol (1.6%), sabinene (1.3%), *p*-cymene (1.1%) and 2-methylbutyl-2-methylbutanoate (1.1%) were identified as the main compounds with 25 compounds in minor and 6 in trace amounts.

Similarly, 46 compounds (11 monoterpene hydrocabons, 12 oxygenated monoterpenes, 10 sesquiterpene hydrocarbons, 3 oxygenated sesquiterpenes, eugenol and 9 other compounds) were also identified in the oil obtained at post-bloom stage. Camphor (31.7%), 1-8-cineole (20.8%), *trans*-β-farnesene (4.5%), germacrene D (4.5%), β-caryophyllene (3.6%), terpinen-4-ol, (2.1%), camphene (2.0%), α-pinene (1.5%) and α-terpineol (1.2%) were the main compounds along with 30 minor (0.1%-0.9%) and 7 compounds in trace amounts. Number of compounds identified in the oil obtained at one stage and not detected in the other stages were found to vary from 11 to 21. In analyzed oils, the amounts of oxygenated sesquiterpenes were found to range from 0.2% to 1.1%. Phytol was absent at post-bloom stage, however, its amount in the oils obtained at vegetative, pre-bloom and bloom stages was found to vary from 0.1%–0.4%.

Further, common major compounds identified in all stages were monoterpene hydrocarbons, α-pinene (0.5%–2.4%), camphene (0.7%–2.0%), *p*-cymene (0.8%–2.3%); oxygenated monoterpenes, 1,8-cineole (2.1%–20.8%), camphor (28.6%–31.7%), terpinen-4-ol (0.9%–2.1%), α-terpineol (0.4%–1.6%); sesquiterpene hydrocarbons, β-caryophyllene (2.8%–6.9%), *trans*-β-farnesene (0.7%–4.5%), germacrene-D (3.8%–12.0%), bicyclogermacrene (0.5%–2.3%) and oxygenated sesquiterpene, spathulenol (0.2%–0.9%).

Comparing the major compounds identified in the essential oils analyzed at different stages, great quantitative variation in camphor (28.6%–31.7%), 1-8-cineole (2.1%–20.8%), germacrene D (3.8%–12.0%), β-caryophyllene (2.8%–6.9%), *trans*-β-farnesene (0.7%–4.5%), α-pinene (0.5%–2.4%), *p*-cymene (0.8%–2.3%) and terpinen-4-ol, (0.9%–2.1%) were observed. Results also

showed that the vegetative and post-bloom (31.2% & 31.7%) and pre-bloom and bloom (28.8% & 28.6%) stages contained similar amount of camphor. Camphor was found to be the major compound in the oils in all growth stages. The amount of 1,8-cineole was higher at post-bloom (20.8%) and minimum (2.1%) at vegetative stages. It was noticeable that the amount of 1,8-cineole increased from vegetative (2.1%) to post-bloom (20.8%) stages.

Comparing the main compounds of the essential oils analyzed with those reported earlier in *A. annua* (Ahmad & Misra 1994; Juteau *et al.* 2002; Goel *et al*. 2008; Emadi & Yassa 2009; Malik *et al.* 2009; Tzenkova *et al*. 2010; Padalia *et al*. 2011; Verma *et al*. 2011), considerable quantitative variations were found among the major compounds. Artemisia ketone and artemisia alcohol reported earlier (Woerdenbag *et al*. 1994; Juteau *et al*. 2002; Goel *et al*. 2008; Malik *et al*. 2009; Tzenkova *et al*. 2010) as major compounds in *A. annua* essential oil were absent in the oils at different growth stages. Nevertheless, camphor the main compound detected in all samples, has also been reported as one of the main compound in *A. annua* oil (Padalia *et al*. 2011; Verma *et al.* 2011; Woerdenbag *et al*. 1994; Juteau *et al*. 2002). Among the compounds identified, the amounts of 1,8-cineole (2.1% to 20.8%) and *trans*-β-farnesene (0.7% to 4.5%) were found to increase with growth stages. Artemisia ketone (0.3%–0.9%) and artemisia alcohol (0.1%–1.0%) identified recently in *A. annua* were absent in the analyzed oil (Padalia *et al*. 2011). The highest amount of 1, 8-cineole, an antibacterial compound and permeation enhancer was found at the post-bloom stage (Hendry *et al*. 2009) while camphor was found at the vegetative and post-bloom stages.

The results suggested that the essential oils from *A. annua* of varied with origin and from other species of genus *Artemisia*, even for the major compounds. Our results indicated that the growth stages have significant effect on the quantity and quality of the essential oil and the components of *A. annua*. In the present study, artemisia ketone and artemisia alcohol were absent in the analysed oils. It also showed the distinction of different chemotypes of *A. annua* that may be the result of an adaptive process to ecological conditions from different origins.

## References

Adams R P 2007 Identification of Essential Oil Components by Gas-chromatography / Mass spectrometry, Allured Publishing Co., Carol Stream, Illinois, USA.

Ahmad A & Misra L N 1994 Terpenoids from *Artemisia* annua and constituents of its essential oil. Phytochem. 37: 183–186.

Bertea C M, Freije J R, Woude, V D H, Verstappen F W A, Perk L, Marquez V, Kraker D J W, Posthumus M A, Jansen B J M, Groot D A, Franssen M C R & Bouwmeester H J 2005 Identification of intermediates and enzymes involved in the early steps of artemisinin biosynthesis in *Artemisia annua*. Planta Med. 71: 40–47.

Brown G D, Liang G & Sy L 2003 Terpenoids from the seeds of *Artemisia annua.* Phytochem. 64: 303–323.

Emadi F &Yassa N 2009 Chemical composition of Iranian *Artemisia annua* L. essential oil and its antibacterial, antifungal and antioxidant effects. Planta Med. 75: 91.

Goel D, Mallavarupu G R, Kumar S, Singh V & Ali M 2008 Volatile metabolite compositions of the essential oil from aerial parts of ornamental and artemisinin rich cultivars of *Artemisia annua.* J. Essent. Oil Res. 20: 147–152.

Gupta A K & Tandon N 2004 Reviews on Indian Medicinal Plants. Indian Council of Medicinal Research, New Delhi, Vol. 3 (Are-Azi), p.131.

Hendry E R, Worthington T, Conway B R & Lambert P A 2009 Antimicrobial efficacy of eucalyptus oil and 1,8-cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures. J. Antimicrob. Chemother. 64: 1219–1225.

Hooker J D 1996 The British Flora of India, Bishen Singh Maherdra Pal Singh, Dehra Dun (Uttrakhand), India, Vol. III, pp.321–330.

Juteau F, Masotti V, Bessie're J M, Dherbomez M & Viano J 2002 Antibacterial and antioxidant activities of *Artemisia annua* essential oil. Fitoterapia 73: 532–535.

Lutz D L, Alviano D S, Alviano C S & Kolodziejczyk P P 2008 Screening of chemical composi-

tion, antimicrobial and antioxidant activities of *Artemisia* essential oils. Phytochem. 69: 1732–1738.

Malik A A, Ahmad J, Mir S R, Ali M & Abdin M Z 2009 Influence of chemical and biological treatments on volatile oil composition of *Artemisia annua* Linn. Ind. Crop Prod. 30: 380–383.

Padalia R C, Verma R S, Chauhan A, Chanotiya C S & Yadav A 2011 Variation in the volatile constituents of *Artemisia annua* var. CIM-Arogya during plant ontogeny. Nat. Prod. Commun. 6: 239–242.

Tang W & Eisenbrand G 2011 Handbook of Chinese Medicinal Plants: Chemistry Pharmacology Toxicology, Wiley-VCH Verlag GmbH & Co., KGaA, Germany, 1: 192–201.

Tzenkova R, Kamenarska Z, Draganov A & Atanassov A 2010 Composition of *Artemisia annua* essential oil obtained from species growing wild in Bulgaria. Biotechnol. Equipt. 24: 1833–1835.

Verma R K, Chauhan A, Verma R S & Gupta A K 2011 Influence of planting date on growth, artemisinin yield, seed and oil yield of *Artemisia annua* L. under temperate climatic conditions. Ind. Crop Prod. 34: 860–864.

Woerdenbag H J, Pras N, Chan N G, Bang B T, Bos R, Uden W V, Pham V Y, Nguyen V B, Boi N V, Batterman S & Lugt C B 1994 Artemisinin related sesquiterpenes, and essential oil in *Artemisia annua* during a vegetation period in Vietnam. Planta Med. 60: 272–275.