



## Secondary gaseous metabolites analyses of wild *Artemisia annua* L.

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### ABSTRACT

*Artemisia annua* L. (sweet absinthe), besides artemisinin, synthesizes and accumulates secondary solids and liquids metabolites and other gaseous substances at ambient temperature. Some of them are biological active that justify their great world interest, mainly in the field of the traditional medicine. This work compares bibliographical information of some volatile components of that herb with wild plants growing in certain areas of the Argentine Republic. The identification and quantitative analyses of the volatile components at 70 °C of wild *Artemisia annua* L., were carried out using a gas chromatographic system connected to a mass selective detector. A 0.32 mm internal diameter quartz capillary column with phenylmethylsiloxane as stationary phase was used with an appropriate temperature program. Samples of dried leaves of the plants here studied show large values of 1,8-cineole,  $\alpha$ -terpineol and camphor, compared with those compounds found in other parts of the world. The content of *Artemisia ketone* (tujona) is relatively smaller in this case regarding their analogous samples obtained from cultivations. The composition of the essential oil suggests its utility as a camphor source and also brings the possibility to be used as flavoring agent and/or with medicinal applications.

**Keywords:** Wild *Artemisia annua* L. –gaseous metabolites - GC-MS analyses

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## Introduction

*Artemisia annua* L. (sweet absinthe, named Qinghao in China), can be included among the medicinal aromatic herbs. Now it is growing as a wild vegetable in Europe and America (Abba, 1975; Leonova, 1978). The herb is also cultivated in great scale with commercial interest in Vietnam, Turkey, India, Iran, Afghanistan and Australia. *Artemisia annua* L. is recognized by its pharmacological applications in the popular medicine for the treatment of paludism or malaria and in our days, in investigations carried out in the fight against cancer (Lai and Singh, 2001). The solid secondary metabolites has been widely studied (Gatti *et al.*, 2008). Besides artemisinin there are others substances, some of them could be their biosynthetic precursors, as well as volatile terpenes and compounds of different chemical characteristics, which contribute to make more interesting the study of that herb. Their main active principle, artemisinin is specifically toxic for the *Plasmodium falciparum*. The generation of free radicals structurally centered in carbon, for a mechanism of electronic transfer (Lai and Singh, 2001), in definitive drives to the the parasite destruction. Besides artemisinin, the plant biosynthesize and accumulates a great variety of gaseous secondary metabolites at ambient temperature, forming their essential oil. The investigation of these substances has allowed the identification of several sesquiterpenoids, flavonoids, cumarins, triterpenoids, steroids, phenolic compounds, purines, lipids and aliphatic compounds in samples coming from different parts of the world (Abba, 1975; Woerdenbag *et al.*, 1993). This work deals with the determination of the essential oil yield and the percentage of volatile components in wild *Artemisia annua* L. that grows very well in certain areas of the Argentine Republic, comparing these results with those coming from other regions of the world (Abba, 1975; Woerdenbag *et al.* 1993).

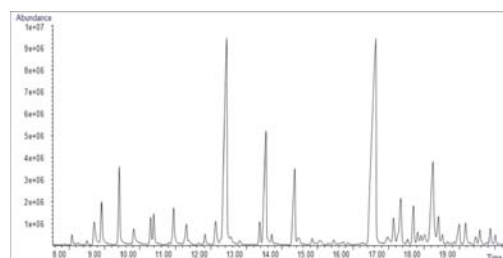
## Experimental

Leaves of wild *Artemisia annua* L. of ca 1.5 m of height were used, harvested before its flowering in the South area of the Province of Entre Ríos (Argentine Republic), dried off outdoors, at lower temperatures of 30 °C. Afterwards they were conveniently milled finally to ca 2 mm diameter particles. The quantitative determination of the total essential oil was made by molecular distillation at ca 1 Torr of its volatile components, collecting them at ca -78 °C. The

identification and quantitative analyses of the volatile components obtained at 70 °C, recovered directly from the dry leaves using the Head Space sampling technique, was done using a model 6890 Hewlett Packard chromatographic system connected to a 7290 model mass selective detector and to a NIST searching library. A quartz capillary column was used (30 m length and 0.32 mm internal diameter). A 0.5 mm film thickness of phenylmethylsiloxane was used as the stationary phase with a convenient temperature program in the 40 ° to 300 °C temperature range. Helium was used as the carrier gas. The mass spectra of the corresponding chromatographic peaks were obtained at 70 eV by the electronic impact technique.

## Results and Discussion

A typical gas chromatogram is shown (Figure 1) for the volatile components analysis at 70° C extracted directly from milled leaves of the wild *Artemisia annua* L.



**Figure 1.** Typical gas phase chromatogram (see in Experimental conditions of GC-MS analyses) of ca 20 cm<sup>3</sup> sample of the volatile compounds at 70 °C of wild *Artemisia annua* L.

(The total essential oil content of the herb studied (ca 16%, p/p) and the chemical nature of their volatile components (Table 1), compared with those of other parts of the world (Ram *et al.*, 1997; Woerdenbag *et al.*, 1992) are significantly different. This could be due mainly to the different environmental conditions of the regions where the cultivations were made.

The essential oils yields of *Artemisia annua* L. coming from other parts of the world are comprised between 0.3% and 1.4% (p/p) referred to the dried plants. However, also they have been informed (Lai and Singh, 2001; Libbey and Sturtz, 1989) large yields of oils coming from seeds of China and Vietnam. The volatile components yields and also the chemical nature of China samples show differences (*e.g.* artemisia ketone content ca 64% of the total of the volatiles), artemisia alcohol (ca 7%), myrcene (ca 5%) and camphor (ca 3%). The Vietnan essential oil



(Libbey and Sturtz, 1989) show mainly camphor (21,8%) and 1,8-cineole (3,1%) although in this case *artemisia ketone* is not observed. In the wild *Artemisia annua* L. here studied (TABLE), the relative abundances of camphene and of camphor are more important than those of plants coming from other parts of the world (Woerdenbag *et al.*, 1994; Woerdenbag *et al.*, 1993; Woerdenbag *et al.*, 1992). Here (TABLE) the content of *artemisia ketone* (tujona) it is relatively small (8,3%) regarding those cultivated in other regions (Woerdenbag *et al.*, 1993; Ahmad and Misra, 1994). Benzaldehyde, 2-carene, 3,3,6-trimethyl-1,5-heptadien-4-ol, 3-methylhexanone-2, Santolinatriene, tricyclene and the *Yomogi alcohol* don't appear in others *Artemisia annua* (Woerdenbag *et al.*, 1994). However, in those species it is important the content of 1,8-cineole like the values here reported (TABLE), excepting those of North America (Libbey and Sturtz, 1989; Charles *et al.*, 1991). With myrcene it happens the same that with the *artemisia ketone* component, where their concentration is still smaller that those of the American coast and of the chinese cultivations (Libbey and Sturtz, 1989; Brown, 1994). The content of  $\beta$ -pinene is similar to the the plants cultivated in India (Ahmad and Misra, 1994) and in the North American coast (Libbey and Sturtz, 1989). Here (TABLE) it is higher the content of  $\alpha$ -terpineol that in the species from Vietnam (Woerdenbag *et al.*, 1994) as well as of the India (Ahmad and Misra, 1994). These two regional varieties shows similar contents of  $\gamma$ -terpinene with that of our region, which overcomes those of the plants from Vietnam (Woerdenbag *et al.*, 1994).

### Conclusions

The wild vegetable here studied it could be a potential source of camphor. Also, for their significant myrtenal concentration, their essential oil could possess therapeutic effects in some medical treatments. On the other hand, the high natural concentration of the essential oil of wild *Artemisia annua* L. can be an economic source of valuable perfuming for its tenors of  $\gamma$ -terpinene, *Yomogi alcohol* and  $\beta$ -pinene.

Note: Part of this study was presented at the 'II Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (Second Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2009.

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**Table 1.** Main volatile Compounds extracted at 70° C from wild *Artemisia Annu* L. harvested in the Argentine Republic. <sup>a</sup> GC retention time values; <sup>b</sup> total % GC peak area; <sup>c</sup> % accuracy of the solute identification parameter values related to those showed in the 7290 model Hewlett Packard MS library; <sup>d</sup> similarity parameters values of the corresponding cracking patterns of the analysed compounds respect to the reference samples; <sup>e</sup> 3-ethenyl-2,5-dimethyl-1,4-hexadiene; <sup>f</sup> 2,5,5-trimethyl-hepta-3,6-dien-2-ol (E); <sup>g</sup> 3,3,6-trimethyl-1,5-heptadien-4-one; <sup>h</sup> 3,3,6-trimethyl-1,5-heptadien-4-ol; <sup>i</sup> 6,6-dimethyl-bicyclo [3.1.1]-hept-2-ene-2-carboxaldehyde.

Substance	RT/ min <sup>a</sup>	% total GC peak area <sup>b</sup>	% accuracy <sup>c</sup>	Concordance <sup>d</sup>
<i>Santolinatriene</i> <sup>e</sup>	8,1	0,5	38,8	917
Tricyclene	8,5	0,2	31,9	940
Canphene	9,4	4,2	46,6	945
Benzaldehyde	9,8	1,2	65,5	934
Sabinene	10,3	1,3	27,8	935
$\beta$ -pinene	10,4	1,5	26,8	930
Myrcene	11,0	2,3	34,1	882
<i>Yomogi alcohol</i> <sup>f</sup>	11,3	1,2	77	876
2-Carene	11,8	0,5	12,5	903
1,8-Cineole	12,4	18,4	19,9	962
$\gamma$ -terpinene	13,4	1,1	45,2	949
<i>Artemisia ketone</i> <sup>g</sup>	13,5	8,3	52,8	911
<i>Artemisia alcohol</i> <sup>h</sup>	14,4	5,6	73,3	925
Camphor	16,6	29,0	42,9	960
6-Canphenol	17,3	2,7	26,5	744
Terminen-4-ol	17,7	2,1	49,7	923
$\alpha$ -terpineol	18,3	7,6	34,4	901
<i>Myrtenal</i> <sup>i</sup>	18,4	1,5	74,7	915
3-methyl-hexanone-2	19,0	1,4	24,1	741