Supercritical Carbon Dioxide Extraction of Volatile Flavor Compounds from *Artemisia annua*

Hyang-Min Ahn, Md. Salim Uddin and Byung-Soo Chun*
Institute of Food Science, Faculty of Food Science and Biotechnology,
Pukyong National University, Busan 608-737, Korea
Fax: +82-51-629-5824, E-mail: bschun@pknu.ac.kr

ABSTRACT

The aromatic compounds of *Artemisia annua* were extracted by supercritical carbon dioxide. Supercritical carbon dioxide extraction of volatile flavor compounds from *Artemisia annua* was performed at pressure 150bar and temperature 40ºC. The flow rate of CO_2 was 20 g/min and the flow rate of ethanol as an entrainer was 5% of CO_2 used. The extraction time was 1 h. The trapped sample by cold trap 1 and 2 was analyzed by GC-MS. Identified major volatile compounds were artemisia ketone, artemisia alcohol, arteannuic acid, 1,8-cineole and so on. Artemisia ketone content was found in highest amount in both cold traps 1 and 2. Artemisia ketone was present in moderate amount. Arteannuic acid was present in small amount.

INTRODUCTION

Artemisia annua, a plant belonging to Asteraceae family, is an annual herb native to China and occurs naturally as a part of steppe vegetation in northern parts of Chahar and Suiyan province in China at 1000-1500 m above in the sea lavel [1]. The plant now grows in many countries such as Australia, Argentina, Bulgaria, France, Hungary, Italy, Spain and United States. It is single single stemmed with alternate branches reaching more than 2 meters in height. It leaves are deeply dissected and range from 2-5 cm in length. *Artemisia annua* is used for the crafting of aromatic wreaths [2], as a source of essential oils used in flavouring of vermouth [3] and also a source of artemisin, a potent anti-malarial drug [4]. The essential oil of *Artemisia annua* remarkably inhibited the growth of some Gram-positive bacteria and fungi. This oil has also antioxidant activity [5]. *Artemisia annua* synthesizes and accumulates a variety of secondary metabolites. Some of the biologically active

secondary metabolites substantiate the claim made in traditional system of medicine. Artemisinic acid, a well-known precursor for semisynthesis of artemisinin has shown antibacterial activity. The aim of this study was to extract oil by SCO_2 and to identify the biological active compounds from *Artemisia annua*.

MATERIALS AND METHODS

Material

Artemisia was collected from Africa. The sample was dried in a freeze-drier. The dried sample was crushed and sieved (800 ㎛) by mesh and stored at low temperature. This sample was used for SCO_2 extraction.

Methods

- SCO_2 extraction

A laboratory scale supercritical fluid extraction unit was used for extraction of oil with volatile flavour compound from *Artemisia* (Fig. 1). 30 g of *Artemisia* powder were packed into 180 mL stainless steel extraction vessel. CO_2 was supplied into the vessel by high pressure pump up to the desired pressure (150bar), which was regulated by a back pressure regulator. The vessel temperature was 40ºC maintained by heater. Flow rates and accumulated gas volume passing through the apparatus were measured using a gas flow meter. The flow rates of CO_2 were 20 g/min for 1 h. The extracted oil and volatile compound were collected by a cyclone separating vessel and cold trap 1 and 2, respectively.

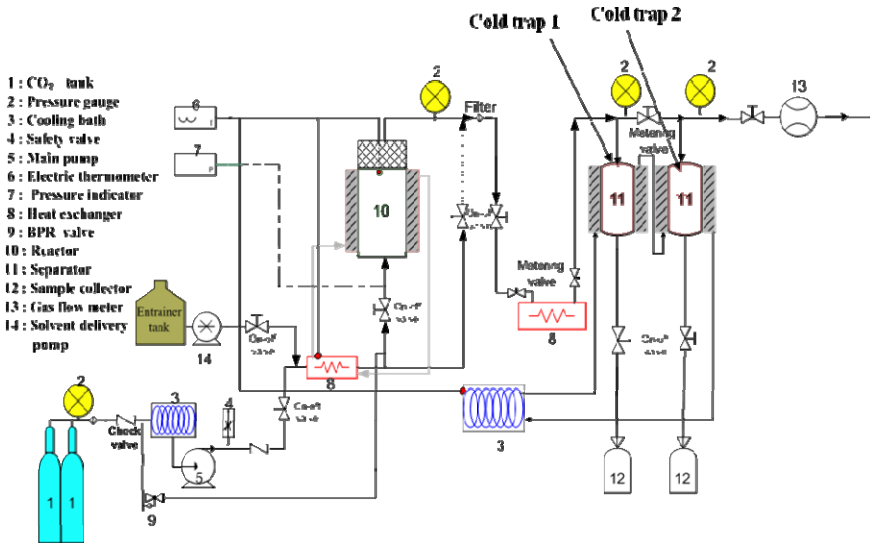

Fig. 1. Schematic diagram of supercritical Fluids Extraction process.

GC-MS analysis

GC-MS analysis were carried out on a Hewlett-Packard HP 6890 GC coupled to with an HP 5973 mass spectral detector (MSD). BP-20 column (50m x 0.25mm i.d. x 0.5µm df SGE, Melbourne, Vic) was used. Injector temperature was 250^{o}C. The oven temperature program was as follows; 40^{o}C for 10 min, followed by 7.5^{o}C/min ramp to 220^{o}C and held for 10 min. Helium carrier gas was set at a constant pressure of 151.5 kPa. MS conditions were as follows; ion source temperature 220^{o}C; MS quadrapoles temperature, 150^{o}C; electron multiplier, 1824 V; and the data rate is 20Hz. The transfer line was maintained at 220^{o}C.

RESULTS AND DISCUSSION

Almost 50 constituents were identified from *Artemisia annua*. Some phytochemicals of *Artemisia annua* in cold trap 1 and 2 are shown in Table 1 and 2. Artemisia ketone, which was reported to be the major constituent of *Artemisia annua*. Artemisia ketone content was very high in both cold trap 1 and 2. Artemisia ketone content in cold trap 1 and 2 were about 33 and 21%, respectively. Artemisia alcohol, 1,8-cineole, germacrene-D, caryophyllene oxide and phytol were also present in moderate amount in both cold trap. 1,8-Cineole is a terpenoid oxide and is non-toxic, non-irritant and nonsensitizing. Arteannuic acid was present in very small amount in cold trap 1, but higher amount in cold trap 2. Alpha-campholene aldehyde, terpinen-4-ol, myrtenal, eugenol, dihydroactinidiolide, torreyol and italicene were trapped in cold trap 1.

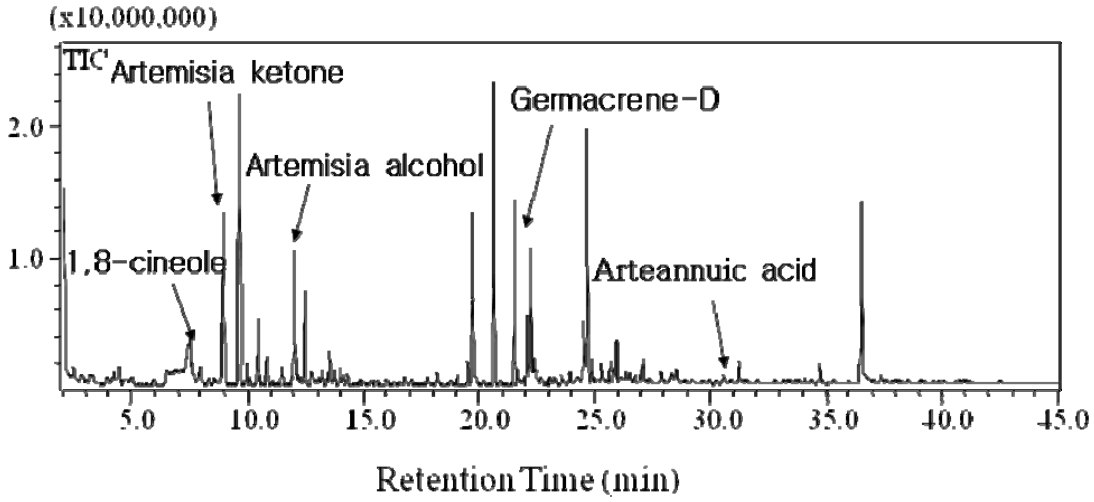

Fig. 2. Ionic chromatogram of essential oil from cold trap 1

Table 1: Phytochemicals of *Artemisia annua* in cold trap 1

Compounds	RT	Area %
1,8-cineole	8.98	3.09
Artemisia ketone	9.70	31.44
Trans-sabinene hydrate	9.97	0.39
Artemisia alcohol	10.47	5.33
Cis-sabinene hydrate	10.85	0.59
Alpha-campholene aldehyde	11.48	0.48
Terpinen-4-ol	13.24	0.35
Myrtenal	13.75	0.36
Eugenol	18.19	0.16
Copaene	19.53	0.29
Germacrene-D	22.25	1.97
Dihydroactinidiolide	22.48	0.25
Torreyol	23.05	0.10
Caryophyllene oxide	24.69	2.36
Italicene	25.97	0.45
Arteannuic acid	30.567	0.06
Phytol	36.53	4.60

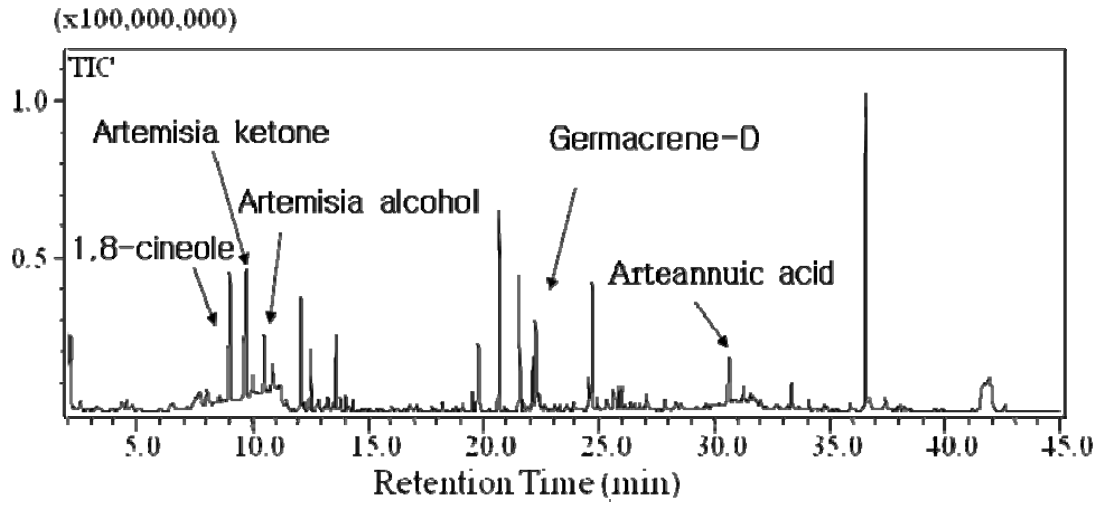

Fig. 3. Ionic chromatogram of essential oil from cold trap 2

Table 2: Phytochemicals of *Artemisia annua* in cold trap 2

Compounds	RT	Area %
1,8-cineole	9.01	4.26
Artemisia ketone	9.74	21.09
Trans-sabinene hydrate	9.99	0.76
Artemisia alcohol	10.51	8.30
Cis-sabinene hydrate	10.88	0.89
Alpha-campholene aldehyde	11.48	-
Terpinen-4-ol	13.24	-
Myrtenal	13.75	-
Eugenol	18.19	-
Copaene	19.54	0.45
Germacrene-d	22.28	2.28
Dihydroactinidiolide	22.48	-
Torreyol	23.05	-
Caryophyllene oxide	24.72	2.38
Italicene	25.97	-
Arteannuic acid	30.68	1.66
Phytol	36.57	11.70

CONCLUSIONS

Artemisia annua secondary metabolism appears to be a resource of many biologically active compounds. Studies on some of the other active compounds identified in *Artemisia annua* will hopefully give new therapeutic and agricultural products of commercial importance.

REFERENCES

[1] Wang., C. W., Maria moors cabot foundation, Harvard Univ., Cambridge, MA, No. 5, **1961**, p. 171-187

[2] Charles, D. J., Cebert, E., Simon, J. E., J Ess. Oil Res., Vol. 3, **1991**, p. 33-39

[3] ANON, Lancet, Vol. 339, **1992**, p. 649-651
[4] Dhingra, V., Rao, K. V., Narasu, M. L., Life Sciences, Vol. 66, **2000**, p. 279-300
[5] Juteau, F., Masotti, V., Bessiere, J. M., Dherbomez, M., Viano, J., Fitoterapia, Vol. 73, **2002**, p. 532-535