

Constituents of the Essential Oil from *Eclipta prostrata* L.

– Research Note –

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Abstract

The volatile aroma constituents of *Eclipta prostrata* L. (leaves, stems, and flowers) were isolated by hydro-distillation extraction method and analyzed by GC/MS. The yield of *Eclipta prostrata* L. essential oil was 0.1% (v/w), and its color was yellow. Sixty-eight volatile flavor compounds, which make up 71.15% of the total volatile composition of the essential oil were tentatively characterized. It contained 35 hydrocarbons (56.25%) with sesquiterpene predominating, 12 alcohols (3.05%), 8 ketones (3.83%), 9 aldehydes (1.86%), 2 oxides (6.03%), and 2 esters (0.13%). α -Humulene, 6,9-heptadecadiene, (*E*)- β -farnesene, and α -phellandrene were the major abundant aroma components in *Eclipta prostrata* L., aromatic and medicinal plant.

Key words: *Eclipta prostrata* L., hydro-distillation, essential oil, GC/MS

INTRODUCTION

Eclipta prostrata L. is one of the well-known species within this genus, belonging to the family *Asteraceae*, an annual, aromatic, and medicinal plant (1). There are numerous edible, aromatic, and medicinal herbaceous plants wild growing in Europe and Asia. The importance of aromatic medicinal plant is considerable owing to their application in folk medicine, and their potential for commercial value in various fields as food additives and enhancer, cosmetics, and pharmaceuticals etc. (2). It has been used whole body of the plants as a folk medicine for the treatment of various bleeding and inflammation, and dying for hair color (1,2). *Eclipta* genus are widely distributed in the field and mountain in Korea (1), and its importance to the pharmaceutical and food industries has stimulated its continual study (3,4). The essential oil from aromatic plants has been known since antiquity to possess biological properties. Because they included tremendous enriched with terpenoids which exert inhibitory action against micro organisms by disrupting their membranes (5,6). With the growing interest in the use of the plants essential oil from medicinal plants in both of the food and the pharmaceutical industry, a systemic examination on the volatile compounds of this plant has become increasingly important. In this paper, we report the aroma constituents of the *Eclipta prostrata* L. produced in Korea, an aromatic medicinal plant.

MATERIALS AND METHODS

Plant materials

Eclipta prostrata L. was purchased at Gyungdong

Herbal Market (Seoul, Korea) in April of 2007. This plant had been harvested in October of 2006 from Namwon (Jeonrabukdo) province in western Korea. The sample was kept at -70°C in air-tight bags until analysis was performed.

Separation of the essential oil

The dried *Eclipta prostrata* L. were crushed for 20 sec using a blender (NJ-8060SM, NUC Electronics, Seoul, Korea) and 1 kg samples were extracted by hydro distillation extraction method for 3 hr from setting by a Clevenger-type apparatus (Hanil Lab Tech Ltd., Incheon, Korea) (7). The essential oil obtained was dried over anhydrous sodium sulfate overnight, measured, and stored in hermetically sealed dark-glass containers in a freezer at -4°C until it was tested and analyzed by GC/MS.

GC-MS analysis

An Agilent 6890 gas chromatography/5973 mass selective detector (Agilent Co., Palo Alto, CA, USA) was employed. Analysis was carried out on a HP-5MS (5%-phenyl-methylpolysiloxane) capillary column (30 m length \times 0.25 mm I.d. \times 0.25 μm film thickness; Agilent Co., Palo Alto, CA, USA). Helium gas was used as carrier gas at a flow rate of 1.0 mL/min. The oven temperature was maintained at 40°C for 5 min and then programmed to increase as follows: from 40 to 150°C at a rate of $3^{\circ}\text{C}/\text{min}$ and holding at 150°C for 5 min, and then 150 to 220°C at a rate of $7^{\circ}\text{C}/\text{min}$ and holding at 220°C for 5 min. The temperatures of the injector and detector were 250 and 280°C , respectively. The sample 10^{-1} μL , previously dissolved in methylene chloride, was

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injected in split mode with a split ratio of 10:1. GC/MS analysis was carried out in triplicates. The MS conditions were: ionization energy of the mass selective detector was 70 eV, scanning interval 0.5 s and detector voltage 1.2 kV, and the mass scanning range was recorded at m/z 33 ~ 330.

Characterization of chemical compounds

The components of the hydro distilled oil from *Eclipta prostrata* L. were tentatively identified by means of comparison of their RIs on a HP-5MS capillary column, which were determined relative to the retention time of a homologous series of *n*-alkane with linear interpolation with those of authentic compounds. The constituents were also identified comparison of their RIs with those of other essential oils which had been identified earlier (8). And by comparison with the fragmentation pattern of the authentic compounds from the mass spectra with those in an on-line computer library (Wiley 275) (Agilent Co., Palo Alto, CA, USA). The RIs of the compounds, determined using *n*-alkanes [Alkane Standard Solution (04070, 04071), (C₈-C₂₀, C₂₁₋₄₀), Standard for GC, Fluka, Buchs, Switzerland] as external references, were compared with the published data (9,10). Especially several compounds were identified with those of the literature (11-13), and identification based on co-injection with authentic compounds (Acros, Sigma-Aldrich, St. Louis, MO, USA). The relative amount of individual components from the oil are expressed as peak area % relative to total peak area from the based on the ratio of the peaks obtained from the mass total ion chromatogram, and also marked quality percentage of the volatile flavor compounds from the GC/MS data.

RESULTS AND DISCUSSION

Profiles of the essential oil

The yield of the essential oil from 1 kg plants material of *Eclipta prostrata* L. was 0.1 % (v/w), and the color was yellow. The list of detected compounds in the hydro distilled oil of *Eclipta prostrata* L. with their retention time, relative percentage, retention indices, and percentage amounts of compound classes are given in Table 1. The data are mean values of triplicates. As shown in Table 1, sixty-eight volatile aroma components, which make up 71.15% of the total composition, were tentatively characterized in the essential oil of *Eclipta prostrata* L. They consist of 37 hydrocarbons with sesquiterpene predominating, 12 alcohols, 6 ketones, 9 aldehydes, 2 oxides, and 2 ester. α -Humulene (11.23%) was the predominantly abundant compounds, and 6,9-heptadecadiene (6.91%), (*E*)- β -farnesene (6.50%), and α -

phellandrene (5.13%) were the next abundant compounds. β -Gurjunene, β -caryophyllene, (-)-humulene epoxide II, sativene, caryophyllene oxide, *p*-cymene, germacrene D, and α -pinene were the compounds with concentrations higher than 2% as % peak area. And followed by β -ionone, methyl tridecyl ketone, 1,3-cyclooctadiene, ional, α -gurjunene, and δ -cardinene (>1% each).

Hydrocarbons

Thirty-five hydrocarbons, which make up 56.25%, were tentatively identified in the essential oil of *Eclipta prostrata* L. Nine monoterpene hydrocarbons were accounted for 10.96% of the total peak area. The major monoterpene hydrocarbons were detected in this essential oil, which were α -phellandrene (5.13%), *p*-cymene (2.20%), and α -pinene (2.02%). Monoterpenes are most often isolated as the major components of the aromatic plants (14). Monoterpenic compounds whose structures can be derived from the condensation of two isoprene units. The *p*-menthane skeleton appears to represent the most stable monoterpene structure. Six diones having the *p*-methane skeleton occur in nature, these being limonene, α -terpinene, α -phellandrene, β -phellandrene, γ -terpinene and terpinolene (15). Among them, 3 compounds (α -terpinene, α -phellandrene, and γ -terpinene) were characterized in the essential oil of *Eclipta prostrata* L. It is well known that γ -terpinene has slightly bitter-herbaceous flavor, but pleasant citrus flavor at low concentration, and has been used in the reconstruction of certain essential oils mainly for flavor purposes (14). α -Pinene, the third abundant compound in monoterpene hydrocarbon, was reported the most abundant volatile flavor of *Chrysanthemum indicum* L. produced in Korea (16), and described as the pine tree aroma and flavor, and it is very important as a starting material for perfume industry (17). This volatile flavor compound was also characterized in high contents (27.40%) of the *Juniperus oxycedrus ssp. oxycedrus* berry and wood oils from Lebanon, and it has reported anti-oxidant and hypoglycemic activities (18). We also detected twenty-two sesquiterpene hydrocarbons were accounted for 36.36% of the total peak area. The major sesquiterpenes with concentrations higher than 3% as % peak area were α -humulene, (*E*)- β -farnesene, β -caryophyllene, and β -gurjunene. Among them, α -humulene is the most abundant aroma compound with concentration 11.23%. Recently, it was reported that α -humulene of *Cordia verbenacea* oils was able to diminish the edema formation and has anti-cancer effect (19,20). And it had also reported that this compound was the major volatile components of the essential oil from *Myristica malabarica* L. and *Gymnacranthera canarica* (King) Warb. (21).

Table 1. The volatile flavor compounds of *Eclipta prostrata* L.

Compounds	RT ¹⁾	RI ²⁾	PA% ³⁾	Method of ID ⁴⁾	Compounds	RT ¹⁾	RI ²⁾	PA% ³⁾	Method of ID ⁴⁾
(<i>E</i>)-2-Hexanal	06.39	0826	tr	MS/RI	α -Copaene	31.17	1403	0.06	MS/RI
α -Pinene	10.06	0942	2.02	MS/RI	Sativene	31.96	1416	2.84	MS/RI ⁷⁾
Camphene	10.58	0949	0.01	MS/RI	<i>allo</i> -Aromadendrene	32.34	1424	0.08	MS/RI
Benzaldehyde	11.30	0960	0.03	MS/RI	α -Cederene	32.60	1426	0.20	MS/RI
β -Pinene	12.04	0970	0.64	MS/RI ⁵⁾	Methyl eugenol	32.66	1428	0.12	MS/RI
Myrcene	13.07	0985	0.34	MS/RI	β -Caryophyllene	33.22	1437	3.92	MS/RI
α -Phellandrene	13.79	0996	5.13	MS/RI	β -Cubebene	33.37	1442	0.45	MS/RI
2,4-Heptadienal	14.01	1001	0.01	MS/RI	(+)-Aromadendrene	34.12	1452	0.19	MS
α -Terpinene	14.18	1006	0.58	MS/RI ⁶⁾	α -Humulene	34.90	1465	11.23	MS/RI
<i>p</i> -Cymene	14.75	1019	2.20	MS/RI	(<i>E</i>)- β -Farnesene	35.24	1471	6.50	MS/RI
γ -Terpinene	16.34	1060	0.04	MS/RI*	Germacrene D	35.91	1481	2.13	MS/RI
3,5-Octadiene-2-one	17.08	1077	tr	MS/RI ⁷⁾	β -Ionone	36.03	1483	1.65	MS
α -Terpinolene	17.81	1095	tr	MS	β -Selinene	36.30	1488	0.28	MS/RI
Linalool	18.59	1109	0.02	MS	α -Muurolole	36.51	1491	0.34	MS/RI
Nonanal	19.29	1121	0.04	MS	Ional	37.26	1505	1.30	MS
1,7-octadiene-3-one	19.39	1123	0.01	MS	α -Selinene	37.57	1511	0.22	MS/RI
Camphor	20.41	1141	0.02	MS/RI	Valencene	37.69	1517	0.49	MS
Terpinen-4-ol	22.46	1174	0.09	MS/RI*	δ -Cardinene	38.22	1530	1.16	MS/RI
<i>p</i> -Cymen-8-ol	22.63	1178	0.05	MS	Germacrene B	38.64	1541	0.62	MS/RI
Estragol	23.25	1199	0.20	MS/RI*	Santalol	39.30	1558	0.75	MS
Decanal	23.71	1208	0.04	MS	Caryophyllene oxide	39.76	1576	2.85	MS/RI
<i>p</i> -Cumamol	24.92	1222	0.03	MS	Salvenone	40.04	1561	0.46	MS
Cuminal	25.09	1226	0.14	MS/RI	<i>cis</i> - α -Bisabolol	40.25	1565	0.79	MS
Carvone	25.69	1243	0.02	MS/RI	(-)-Humulene epoxide II	40.89	1596	3.18	MS
Phellandral	26.68	1266	0.20	MS	β -Gurjunene	41.64	1612	3.97	MS/RI
(<i>E</i>)-Anethol	27.30	1282	0.45	MS/RI	δ -Selinene	41.89	1618	0.32	MS/RI ⁶⁾
Tridecane	28.07	1300	0.04	MS	α -Gurjunene	42.49	1635	1.19	MS/RI ⁷⁾
Carvacrol	28.52	1315	0.15	MS/RI	6,9-Heptadecadiene	43.55	1660	6.91	MS
(<i>E</i>)-2,4-Decadienal	28.73	1323	0.10	MS/RI	1,3-Cyclooctadiene	43.74	1664	1.83	MS
α -Cubebene	30.06	1368	0.08	MS	Valerenol	48.27	1756	0.12	MS
α -Ionone	30.25	1375	0.02	MS	Methyl tridecyl ketone	50.72	1854	1.65	MS
Citronellyl acetate	30.45	1382	0.01	MS/RI	Methyl linoleate	55.68	2043	0.12	MS/RI
Cyclosetivene	30.59	1386	0.05	MS	Phytol	58.09	2118	0.28	MS/RI
α -Yalangene	30.97	1401	0.04	MS	14 β -Pregnane	59.95	2210	0.15	MS

¹⁾RT is retention time. ²⁾R.I.: Retention indices were determined by using n-alkanes (C₈-C₂₂) as external references. ³⁾PA% means peak area %, average (n=3) of the relative percentage of the peak area in the MS total ion chromatogram. tr, trace; mean value <0.01%. ⁴⁾Method of identification based on reference no. 9, 10. MS: mass spectrum was consistent with that of Wiley mass spectrum database (2001, Hewlett Packard Co., Palo Alto, USA). RI was consistent with that of the literature. ⁵⁾Identification based on reference no. 11. ⁶⁾Identification based on reference no. 12. ⁷⁾Identification based on reference no. 13. *Identification based on co-injection with authentic compounds (Acros, Sigma-Aldrich, St. Louis, MO, USA).

Alcohols and aldehydes

Various alcohol compounds were detected in this sample by our experimental results. Though most of the alcohol compounds were found in small amounts, there were twelve alcohol compounds (3.05%) in the volatile flavor compounds of *Eclipta prostrata* L. oil. Among them, terpinen-4-ol called as 4-terpineol, this volatile flavor compound has been used for aroma therapy with lavender oil, recently. And it was known to one of the major volatile flavor of the tea tree oil, and the quality of the tea tree oil depend upon the percentage of this compound (22). Aldehydes are intermediates between the alcohols and the acids. The aldehydes having lower molecular weight are characterized by their unpleasant

and pungent odors and irritant effect in the nose. As the molecular weight increases, the odor profile gradually leads a more pleasant fruity character. Especially, aldehyde C₈ to C₁₀ have very attractive floral odor (15). Nine aldehydes including (*E*)-2-hexanal, benzaldehyde, 2, 4-heptadienal, nonanal, decanal, cuminal, phellandral, (*E*)-2,4-decadienal and ional were detected in the essential oil of *Eclipta prostrata* L., accounting for 1.86%.

Ketones and esters

Ketone constituted 3.83% of *Eclipta prostrata* L. distilled oil consisting of camphor, 3,5-octadiene-2-one, α -ionone, 1,7-octadiene-3-one, β -ionone, methyl tridecyl ketone, salvenone, and carvone, and with methyl tridecyl ketone and β -ionone as the main components. The ke-

tones give a wide range of aromatic effects, and most of them are pleasing. Those of higher molecular weight have a marked floral character (14). Camphor is a saturated ketone having camphene skeleton, and is obtained industrially via the pinene, camphene, and isoborneol (15). It was characterized in *Eclipta prostrata* L. oil in small amount, and we also detected camphene in this study by GC/MS. The esters detected in this sample were small amounts, which make up 0.13% with citronellyl acetate and methyl linoleate. Acetate is of prime importance in the formulation of imitation flavors (15).

In this study, we determined the volatile constituents of the essential oil from *Eclipta prostrata* L. which was isolated by hydro-distillation extraction method. The common characteristics of the distilled oil from *Eclipta prostrata* L. tentatively identified in this work were its high contents of terpene hydrocarbon with sesquiterpene predominating, especially α -humulene, and its low contents of other functional groups. Sixty eight volatile flavor compounds, which make up 71.15% of the total volatile composition of the essential oil, were characterized. It contained 35 hydrocarbons, 12 alcohols, 8 ketones, 9 aldehydes, 2 oxides, and 2 esters. α -Humulene, 6,9-heptadecadiene, (*E*)- β -farnesene, and α -phellandrene were the major abundant aroma components in *Eclipta prostrata* L. We envision a possible use of the essential oil from *Eclipta prostrata* L. in the food and pharmaceutical industries because of their bio-functional properties.

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