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Production of Volatile Oil Components by Cell Culture of *Agastache rugosa* O. Kuntze

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Abstract – To develop systems for economic production of useful essential oil compounds, callus was induced from the seedlings of *Agastache rugosa* and cultured on MS medium. The volatile oil fraction was extracted from the callus and investigated by mean of GC-MS. The composition of the oil was compared with that of the mother plant. As a result, sixty five compounds including ferruginol were identified in the essential oil fraction. The main component of the oil from the leaves of *Agastache rugosa* was methyl chavichol (53.6%). Methyl jasmonate and jasmonic acid were added to the culturing cell suspension, separately and the composition of induced oil were compared. The oils from cultured cells treated with jasmonates showed considerably different patterns. Especially, the peak of estragole was found in callus oil after treatment with methyl jasmonate as though the amount was limited to 0.58%. In general, the TIC pattern of GC-MS of the callus oil became more similar to the oil from the leaves after elicitation.

Key words – *Agastache rugosa,* essential oil, cell culture, methyl jasmonate, elicitor

Introduction

Agastache rugosa (Labiatae) is a perennial herb which is widely spread in the field of Korea. It has been used as one of the wild vegetables in the spring time and sometimes in folks medicine as bitter stomachics or for the treatment of anorexia. Futhermore, this plant is one of the most important Korean aromatic plant sources because of its unique aroma (Svoboda *et al.*, 1995; Ahn *et al.*, 1991; Wilson *et al.*, 1992). Recently, the cultivation or economic purpose became fairly popular in Korea followed by increasing tendency of aroma therapy and to supply the demand as additives in foods and medicines.

To develop systems for economic production of useful essential oil compounds, callus was induced from the seedlings of this plant and cultured on MS medium in various conditions.

In many cases, terpenoids are accumulated in specially differentiated tissues but generally not produced in undifferentiated callus tissues. Jasmonic acid and methyl jasmonate are known as representative compounds of jasmonates which have been reported to play an important role as signal transducer in defence mechanism of many plants. It is well known that externally provided jasmonates to the culture system can regulate the secondary metabolism of plant cells and induce the enhancement of biosynthesis of some special compounds (Shin *et al.*, 2000; Blechert *et al.*, 1995; Dicosmo *et al.*, 1990; Yamamda *et al.*, 1990). In many plants, it has been shown that the biotransformation of substrates exogenous to the cell culture system can also be used for this purpose (Suga *et al.*, 1990; Sakui *et al.*, 1992; Sakamoto *et al.*, 1994; Shin, 1995; Shin, 1996).

To challenge the control of biosynthesis of essential oil compounds in the cells by elicitors, the cultured cells in the medium were treated with methyl jasmonate and jasmonic acid in various illuminated condition. The changes of oil compositions were studied by GC-MS by eliciting process and by addition of several intermediates in shikimate pathway.

Materials and Methods

Culture – The callus tissues were derived from the young leaves and seeds of *Agastache rugosa* grown in the herbal garden at Duksung Women's University. The induced callus tissues were cultured on Murashige and Skoog's medium supplemented with 3% sucrose and yeast extract. We have subcultured the callus for two years on the medium containing

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various combinations of auxins and kinetin. The high productive callus lines for essential oils were selected for this study. The calluses (10 g/flask) were transferred into Murashige and Skoog's medium (80 ml) with casein (0.5 g) and sucrose (10 g), and then cultured on the shaker (100 rpm) for 72 hours.

As growth regulators, 2,4-D (1 ppm) and kinetin (0.1 ppm) were added to the media. The cells were treated with elicitors, 100 μM jasmonic acid and 100 μM methyl jasmonate at the beginning of the culture. The cultured cells were harvested from the medium by filtration three times at intervals of 24 hours. Dichloromethane was used for the extraction of essential oil from the cells after freeze drying.

GC-MS – Hewlett-Packard 6890 GC, Hewlett-Packard 5973 MSD, Ultra-2 capillary column (50 m ×0.2 mm×0.11 um), Temp. prog.: 70°C (5 min, 10°C/min)-230°C-270°C (5°C/min, 15 min), Carrier Gas: He (1.0ml/min), EI: 70 eV, CI: 200.

Results and Discussion

As shown in Fig. 1, ca. 300 peaks were identified by Wiley 275 library search in the essential oil extracted from the whole plant parts of *Agastache rugosa* with spontaneous distillation and extraction

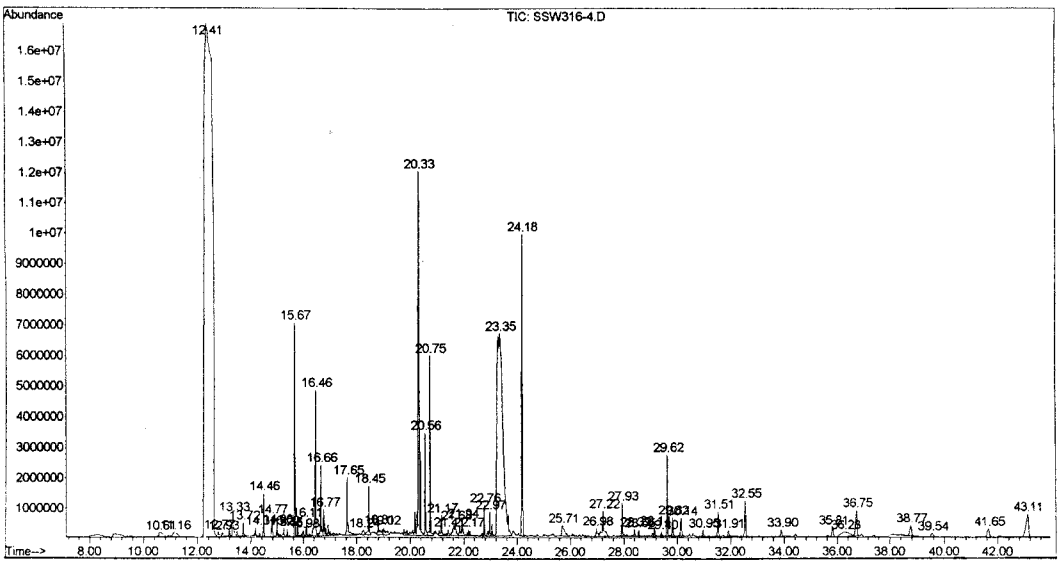

Fig. 1. TIC of essential oil from the mother plant of *Agastache rugosa* O. Kuntze.

Table 1. Identified compounds in essential oil from the mother plants of *Agastache rugosa* O. Kuntze

No.	Retention time	Compounds	No.	Retention time	Compounds
1.	12.41	methyl chavicol	16.	17.64	endo-1-bourbonanol
2.	13.33	chavicol	17.	20.33	3,7,11,15-tetramethyl-2-hexadecen-1-ol
3.	13.72	trans-anethole	18.	20.56	1-hexadecyne
4.	14.31	bicylcloelemene	19.	20.75	neophytadiene
5.	14.46	gamma-elemene	20.	21.16	hexadecanoic acid methyl ester
6.	14.77	eugenol	21.	22.76	dehydroabietane
7.	14.98	isoeugenol	22.	23.24	3,7,11,15-tetramethyl-2-hexadecen-1-ol
8.	15.22	beta-elemene	23.	24.18	neophytadiene
9.	15.35	methyl eugenol	24.	24.19	9-octadecyne
10.	15.67	beta-caryophyllene	25.	25.68	ferruginol
11.	15.98	germacrene-D	26.	27.93	3-nitro-1,2-benzene dicarboxylic acid
12.	16.10	beta-selinene	27.	28.55	1-heneicosyl formate
13.	16.44	beta-cubebene	28.	29.61	heptacosane
14.	16.65	bicyclogermacrene	29.	36.33	gamma-tocopherol
15.	16.77	2,6-bis(1,1-dimethylethyl)-4-methyl phenol	30.	38.09	vitamin E

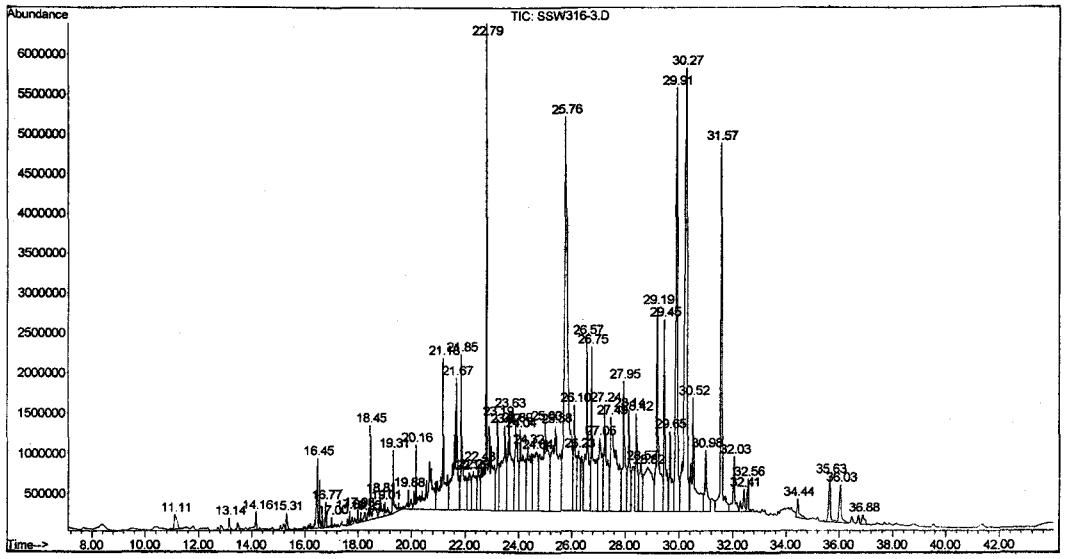

Fig. 2. TIC of essential oil from cells of *Agastache rugosa* cultured without elicitors in the dark condition.

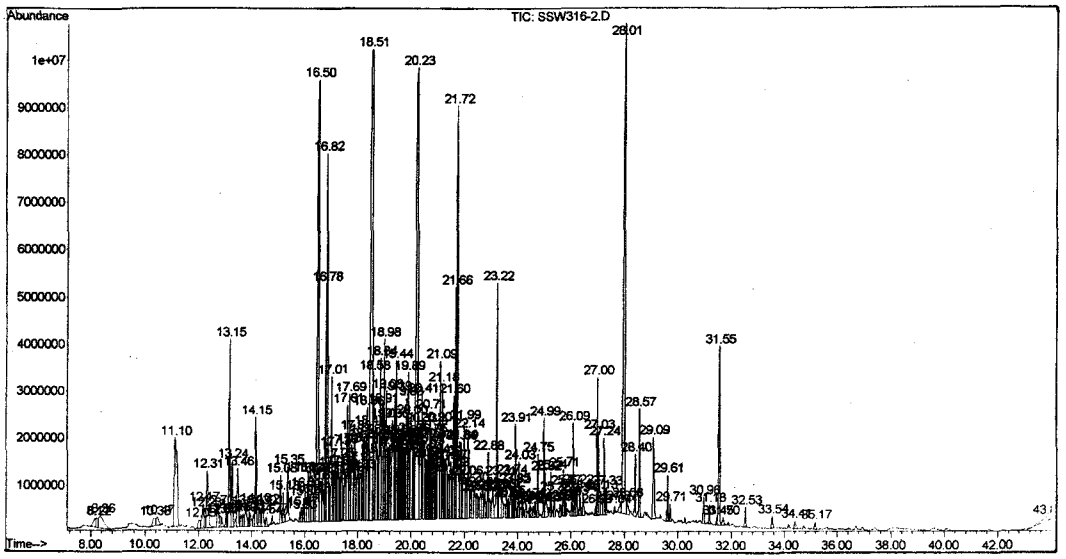

Fig. 3. TIC of essential oil from the cells of *Agastache rugosa* cultured treating with methyl jasmonate in the white light (800 Lux).

apparatus.

The main component of the oil from leaves was methyl chavichol (53.6%). Also phenol compounds, structurally related to this main component, eugenol, isoeugenol, methyl eugenol, anethol and chavichol were representing large proportion of this oil. The sesquiterpene compounds like bicycloelemene, gamma-elemene, beta-elemene, germacrene-D, alpha-humulene, beta- caryophyllene and gamma selinene were identified as other main components in the leaf oil of *Agastache rugosa* (Table 1).

When we compared the TIC and mass spectrum from GC-MS of the extracted oils, the composition of oil from the leaves of mother plants was very different from that of oil accumulated in the cultured callus (Fig. 2). As a result, sixty five compounds were identified including feruginol, one of the aromatic diterpenes which showed antimicrobial activity for

the phenol group in the structure. Valencene and endo-1-bourbonanol were also found in the callus oil. By the illumination of light, the pattern of chromatogram was drastically changed.

Most of the compounds identified in the callus oil cultured in the dark condition, were hydrocarbons and sesquiterpenes. After illumination of white light and elicitation with methyl jasmonate (100 μM) and jasmonic acid (100 μM), the composition of oil produced in the callus was significantly changed and more monoterpene peaks appeared than control (Fig. 3). Especially, the peak of estragole was found in callus oil after treatment with methyl jasmonate as though the amount was limited to 0.58%. In general, the TIC pattern of GC-MS of the callus oil became more similar to the oil from the leaves after elicitation.

However, feeding of the cells with cinnamic acid and eugenol resulted in no increase of estragole and other phenylpropane derivatives and the treatment with jasmonate and the illumination of light could not induce the production of these compounds. We assumed from this result the possibility that this plant has different phenylpropane biosynthetic pathway from those generally known in most plants or it needs special differentiation of cells for the processing of these reactions.

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