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# Antimutagenic Effect of the Major Volatile Compounds Identified from Mugwort (*Artemisia asictica nakai*) Leaves

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## Abstract

**Volatile aromatic compounds collected from raw and roasted mugwort (*Artemisia asictica nakai*) leaves by the Tenax trap and some major volatile compounds were separated and identified by GC-MS. The identified compounds were tested for the antimutagenic and mutagenic activities against aflatoxin $B_1$ ($AFB_1$) using their authentic compounds. Six compounds (myrcene, cineole, camphor, caryophyllen, coumarin, and farnesol) showed antimutagenic activities, but 2-pyrrolidine and thujone showed mutagenic activities. 1-Acetylpiperidine formed during roasting mugwort leaves exhibited mutagenic activities. When the mutagens and antimutagens were mixed, the mixture reduced the mutagenicity of $AFB_1$. These results suggested that the extract of mugwort leaves is not mutagenic and so the mugwort leaves might be used as a food and as medicinal sources without mutagenicity.**

**Key words** : mugwort leaves, volatile compounds, antimutagenic

## INTRODUCTION

Mugwort (*Artemisia asictica nakai*) has long been used as a food additive and a herb medicine in several oriental countries including Korea. European countries have also used its essential oil in perfumery. A recent study showed that the mugwort can also be utilized as a tea stuff after its leaves are dried and roasted. Excellence in the flavor of the wild mugwort grown in the cultural condition of Korea may increase enabling the Korean mugwort to be used for various purposes in foods as well as in perfumery. Its medicinal effect for curing stomachalgia, diarrhea, uterine hemorrhage, chronic hepatitis, phthisis, asthisis, chronic bronchitis, and chronic digestive ailments make the mugwort leaves efficient as a folk medicine in various forms. It was reported that essential oil containing most aromatic compounds of mugwort showed the antibacterial effects for the intestinal bacteria of rumenants[1].

The compounds which have such medicinal effects have not been identified, and the exact mechanisms for curing such varieties of diseases are not known either. Such remarkable range of medicinal effects of mugwort leaves prompted us to examine mutagenicity and antimutagenicity of some major aromatic compounds in raw mugwort leaves and mugwort tea. The volatile aromatic compounds collected from raw and roasted mugwort leaves by the Tenax trap[2] and some major volatile compounds were identified by GC-MS. The identified compounds were tested for mutagenic and antimutagenic activity against $AFB_1$, using authentic compounds.

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## MATERIALS AND METHODS

### Materials

Raw mugwort leaves were harvested in April 1991 and 1992 from the Kyeongnam area in Korea. Mugwort tea was made by the traditional green tea preparation method[3]. This includes washing mugwort leaves, roasting (100 ~ 150° C), and rubbing by hand. Roasting and rubbing steps were repeated 5 times. Raw mugwort leaves and mugwort tea were used for collecting the volatile compounds.

### Isolation of volatile compounds

Volatile compounds of raw mugwort leaves and mugwort tea were analyzed according to the method used by Dunn and Lindsay[4]. Mugwort samples (20g), each containing internal standard (1ug 4-decanol in ethanol ; Aldrich Chemical Co., Milwaukee, WI), were blended in a 250ml saturated sodium chloride solution with a Waring blender (Hartford, CT) at high speed for 30 seconds. After placing each of the slurries in a round bottom flask (500 ml), it was purged for 3h with nitrogen (300ml/min) onto Tenax (1g ; 60 ~ 80 mesh ; ENKA N.V. Holland) as shown in Fig. 1. Volatile compounds were eluted from the Tenax-GC traps with redistilled diethyl ether into a contrate tube (Laboratory Research Company, Los Angeles, CA), and then extracts were concentrated under a stream of nitrogen to about 10ul for GC-MS analysis. GC-MS analysis of concentrated volatile compounds in diethyl ether (1$\mu$l) was carried out with a HP5970 Mass spectrometer connected to a HP 5890 Gas chromatograph using a DB-5 capillary column (60m × 0.32mm, i.d., 0.25μm coating thickness ; Supelco Inc., Bellefonte, PA). The mass spectra were recorded at an electron energy of 70eV and the ion source temperature was 280° C. The column was operated with temperatures ranging from 50° C to 250° C at 2° C/min, and then held for 15min at 250° C. Helium was used as carrier gas (1ml/min, split ratio 1/25). Each peak was identified based on Chamstation (HP 91153C, NBS-REVEL) mass spectral data base and/or mass spectrum of authentic compound.

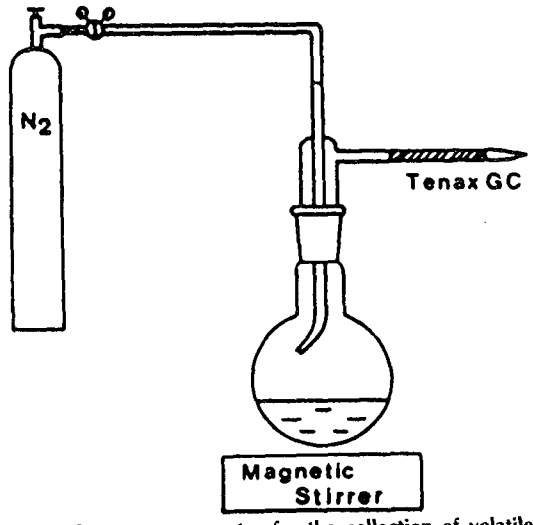



**Fig. 1. Tenax trap apparatus for the collection of volatile compounds from the mugwort leaves.**

### Antimutagenicity test

#### Bacterial strains

*Salmonella typhimurium* strains TA100 and TA98, histidine requiring mutants, were provided by Dr. B. N. Ames, University of California (Berkley, CA, USA) and were maintained as decribed by Maron and Ames[5]. The genotypes of tester strains were checked routinely for their histidine requirements, deep rough (*rfa*) character, UV sensitivity (*uvr* B mutation) and for the presence of R factor.

#### Chemicals

$AFB_1$ (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in spectrophotometric grade dimethyl sulfoxide (DMSO) (Aldrich Chemical Co. Milwaukee, WI, USA). N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was also obtained from Aldrich Chemical Co. These chemicals were sterilized through a millipore membrane filter or were autoclaved. Authentic compounds of farnesol, thujone, camphor, coumarin, cineole, myrcene, copaene, 2-pyrrolidinone, and 1-acetylpiperidine were obtained from Aldrich Chemical Co., and caryophyllen was purchased from Sigma Chemical Co.

**Mutagenicity test**

A modified plate incorporation test (liquid preincubation of the organisms with the test compound) was employed[6]. 0.5ml of S9 mix prepared by the method of Maron and Ames[5] was distributed to sterile capped tubes kept in an ice bath and then 0.1ml of testers from the overnight culture ($1 \sim 2 \times 10^9$ cells /ml) and 0.1ml of test compounds containing 1.5% and 3.0% respectively were added. The tubes were gently vortexed and preincubated at 37°C for 30min. Two ml of the top agar in each tube kept at 45°C were added and vortexed for 3 seconds. The resulting entire mixture was over-laid on the minimal agar plate. The plates were incubated at 37°C for 48hrs and then the revertant bacterial colonies on each plate were counted.

## RESULTS AND DISCUSSION

Among many compounds separated from raw mugwort leaves on DB-5 GC capillary column (Fig. 2), eight major compounds (myrcene, cineol, camphor, caryophyllen, coumarin, farnesol, 2-pyrrolidinone and thujone) shown in Table 1 were tested for the mutagenicity against aflatoxin $B_1$ ($AFB_1$) using their authentic compounds. The results are shown in Table 2. All six compounds showed inhibitory effects for the mutagenic activity of $AFB_1$ in *Salmonella typhimurium* TA98 and 100. Cineol (3.0% in DMSO) and farnesol (1.5% and 3.0% in DMSO) showed stronger inhibitory activity than other compounds tested with an inhibition ratio of 115% and 96%/99%, respectively (Table 2).

More extensive work has been done on the chemotherapeutic effect of cineole in mammary cancer of rats by Gould et al.[7] They reported that cineol did not extend tumor latency or decrease tumor number, while menthol did in the DMBA-induced mammary tumor model. They also suggested that monocyclic monoterpenoids are more effective chemopreventive agents than acyclic or bicyclic compounds. The addition of hydroxyl groups to the monocyclic compounds tends to increase chemoprevention activities[7].

Two compounds (2-pyrrolidinone and thujone) identified from the raw mugwort leaf and tested in this experiment showed mutagenic activity in *Salmonella typhimurium* TA100. The plate treated with thujone (1.5% and 3.0% in DMSO) had many small broken colonies, which is uncountable, indicating thujone has some mutagenicity. Thujone has

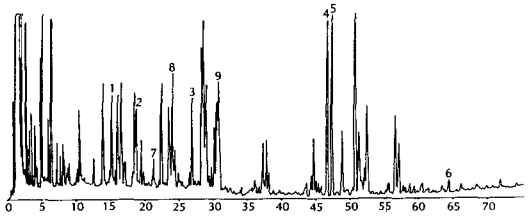
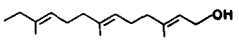
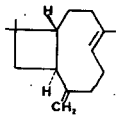
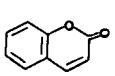



**Fig. 2. Gas chromatogram of Tenax trapped volatile compounds of raw mugwort leaves.**

**Table 1. Antimutagenic and mutagenic compounds identified from mugwort leaves by GC-MS and by matching their retention times with authentic compounds**

| Peak no.[a] | Compounds | Peak no.[a] | Compounds |
|---|---|---|---|
| 1 | Myrcene | 6 | Farnesol |
| |  | |  |
| 2 | Cineol | 7 | 2-Pyrrolodione |
| |  | |  |
| 3 | Camphor | 8 | Thujone |
| |  | |  |
| 4 | Caryophyllen | 9 | 1-Acetylpiperidine |
| |  | |  |
| 5 | Coumarin | | |
| |  | | |

[a]Peak numbers correspond to the peak numbers on Fig. 2.

**Table 2. Effects of compounds identified from mugwort leaves on the mutagenicity of aflatoxin $B_1$ ($AFB_1$, 1$\mu$g/plate) in *Salmonella typhimurium* TA100**

| | Revertants/plate | Inhibition ratio (%) |
|---|---|---|
| Spontaneous | 162 ±13 | |
| $AFB_1$ | 1177 ±231 | |
| $AFB_1$ + Myrcene | | |
| 1.5% | 517 ±51 | 65 |
| 3.0% | 439 ±44 | 73 |
| $AFB_1$ + Cineol | | |
| 1.5% | 742 ±40 | 43 |
| 3.0% | 7 ±0 | 115 |
| $AFB_1$ + Camphor | | |
| 1.5% | 669 ±45 | 50 |
| 3.0% | 443 ±41 | 72 |
| $AFB_1$ + Caryophyllen | | |
| 1.5% | 566 ±29 | 60 |
| 3.0% | 443 ±41 | 72 |
| $AFB_1$ + Coumarin | | |
| 1.5% | 964 ±79 | 21 |
| 3.0% | 560 ±24 | 61 |
| $AFB_1$ + Farnesol | | |
| 1.5% | 205 ±16 | 96 |
| 3.0% | 171 ±18 | 99 |
| $AFB_1$ + 2-Pyrrolidinone | | |
| 1.5% | 1185 ±64 | -1 |
| 3.0% | 1313 ±42 | -13 |
| $AFB_1$ + Thujone | | |
| 1.5% | –[a] | |
| 3.0% | –[a] | |
| $AFB_1$ + 1-Acetylpiperidine | | |
| 1.5% | 1430 ±39 | -25 |
| 3.0% | 1454 ±37 | -27 |

[a]Spore was destructed and appeared as very small particles

**Table 3. Effects of mixed authentic compounds identified from mugwort leaves on the mutagenicity of aflatoxin $B_1$ ($AFB_1$, 1ug/plate) in *Salmonella typimurium* TA100**

| | Revertants/plate | Inhibition ratio (%) |
|---|---|---|
| Spontaneous | 86 ±1 | |
| $AFB_1$ | 845 ±62 | |
| $AFB_1$ + Mixture 1 (3%)[a] | 72 ±4 | 102 |
| $AFB_1$ + Mixture 2 (3%)[b] | 83 ±6 | 100 |

[a]Myrcene + cineol + camphor + caryophyllen + coumarin + farnesol

[b]Myrcene + cineol + camphor + caryophyllen + coumarin + farnesol + 2-pyrrolidinone + thujone + 1 - acetylpiperidine

been known as a toxic compound[8] ; the fact is that it causes problems to use essential oil from mugwort for food application. The content of thujone in raw mugwort leaves vary depending on harvesting time[9] and the area harvested (unpublished data). The concentrations of thujone in mugwort leaves harvested on March, April, and May were 17.248%, 1.508%, and 1.131% of total volatiles, respectively[9]. This indicates that the concentration of thujone in mugwort leaves decrease by age. Since the mugwort leaves used for medicinal purpose are harvested in April or May rather than March, the concentration of thujone is relatively low. 1-Acetylpiperidine was formed during the roasting process of mugwort leaves and considered as an important volatile compound contributing roasted flavor to mugwort tea. 1-Acetylpiperidine turned out to be a mutagenic compound in *Salmonella typhimurium* strains (Table 2). The formation of N-containing heterocyclic compound is a well known process during cooking foods, and some of them such as heterocyclic amines are known as mutagens[10~13].

Since mugwort leaves contained both antimutagens and mutagens (like other natural products), we examined whether the mixtures of authentic compounds possessed mutagenic or antimutagenic activity. Two mixtures were prepared. Mixture 1 containing equal amounts each of myrcene, cineol, camphor, caryophyllen, coumarin, and farnesol showed antimutagenic effects. Mixture 2 consisted of equal amounts of six antimutagenic compounds (myrcene, cineole, camphor, caryophyllen, coumarin, farnesol) and three mutagenic compounds (2-pyrrolidinone, thujone, 1-acetylpiperidine). Three percent of mixture 1 and 2 in DMSO were tested for the mutagenicity of aflatoxin $B_1$ in *Salmonella typhimurium* TA100. As shown in Table 3, both mixtures 1 and 2 inhibited mutagenicity of aflatoxin $B_1$ in *Salmonella typhimurium* TA100. Although individually 2-pyrrolidinone, thujone, 1-acetylpiperidine exhibited strong mutagenic activity, they are no longer considered as a mutagen when mixed with antimutagens (myrcene, cineole, camphor, caryophyllen, coumarin, farnesol). These results tell us that we need not hesitate in using mugwort for

the food application as only a reason for the mugwort leave contained mutagenic compounds when tested by individual compounds. This consideration should also apply to all other natural products.

In conclusion, among major volatile flavor compounds, myrcene, cineole, camphor, caryophyllen, coumarin, and farnesol exhibited antimutagenic effects against aflatoxin $B_1$ in *Salmonella typhimurium* TA100. 2-Pyrrolidinone, thujone, and 1-acetylpiperidine showed mutagenic effects in *Salmonella typhimurium* TA100. 1-Acetylpiperidine was found in roasted mugwort tea, but not in raw mugwort leaves. Not only the mixture of six antimutagenic compounds, but three mutagenic compounds with six antimutagenic compounds strongly reduced mutagenic activity of aflatoxin $B_1$ in *Salmonella typhimurium* TA100.

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# 쑥의 휘발성분에서 동정된 물질의 항돌연변이 효과

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## 요 약

쑥의 주요 휘발성분이 *Salmonella typhimurium* TA100에서 aflatoxin $B_1$의 돌연변이 유발성에 미치는 영향을 조사하기위해 쑥 (*Artemisia asictica nakai*)의 생엽과 볶음 쑥차로 부터 휘발성향기성분을 Tenax trap으로 포집하여 분리하였다. GC-MS로 동정된 9가지 주요화합물중 myrcene, cineole camphor, caryophyllen, coumarin, farnesol은 돌연변이 유발을 억제시키는 효과가 있었으며, 2-pyrrolidine, thujone, 그리고 1-acetylpiperidine은 증가시키는 효과가 있었다. 그러나 이들 9가지 화합물을 혼합하여 시험하였을때는 돌연변이 억제효과만 현저하였다. 따라서 2-pyrrolidine, thujone, 그리고 1-acetylpiperidine의 돌연변이 증가효과는 쑥중의 항돌연변이 물질에의해 그 효과를 나타내지못함을 알 수 있었다.