

Basil Oil and Tarragon Oil: Composition and Genotoxicity Evaluation¹

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Abstract

The chemical composition of an Italian oil of tarragon and two commercial oils of basil were examined by GC/MS. The oils, which were found to contain 19 known compounds, possessed estragole contents of 60%, 8.1% and 16.5%, respectively. The genotoxicity of each oil was evaluated by the Zimmermann test. The oil of tarragon was found to be genotoxic, but the two basil oils exhibited no sign of genotoxicity. The genotoxicity, or lack of it, could not be demonstrated clearly to have a direct relationship to the estragole content only.

Key Word Index

Artemisia dracunculus, *Ocimum basilicum*, Labiatae, essential oil composition, genotoxicity, Zimmermann test.

Introduction

Tarragon and basil oil, which are obtained by steam distillation of *Artemisia dracunculus* L. and *Ocimum basilicum* L., respectively, are produced in commercial quantities in the temperate regions of the world. On one hand, tarragon oil is produced in Italy, Morocco, Yugoslavia, South Africa and France, whereas basil oil is produced in Madagascar, Comore Islands, Egypt, USA, Albania, Pakistan, Yugoslavia, Bulgaria, Morocco, Reunion and South Africa (1). Both oils are valued as raw materials in the flavor and fragrance industries. The similarity between these oils can be found in their estragole (methyl chavicol) content. In most studies, tarragon oil is found to contain estragole in amounts greater than 60% (2). The content of estragole is much less stable in basil oil, where it varies from 0.3-88.6% (3); however, many oils encountered are rich in estragole (4,5).

Over the past 15 years, a number of studies have been reported on the incidence of carcinoma induction in selected animals upon exposure to estragole (6-13). Other genotoxic studies have documented the mutagenicity of estragole (7,14-16). Recently, Ames, et al. (17) ranked estragole-containing plants (basil in particular) as possessing a greater risk as a carcinogenic hazard to man than pesticide residues or water pollution. As a result, we shall examine the genotoxicity of basil and tarragon oil in order to improve our knowledge base

of the biological activity of materials commonly used as food flavors. Prior to this, however, we will examine the chemical composition of both tarragon and basil oil. The genotoxicity of both oils, as measured by the Zimmermann test (18), will be evaluated in reference to the estragole content of the oils under study.

Experimental

The tarragon oil used in this study was produced commercially by steam distillation in the hilly region of Italy between Emily and Tuscany. The basil oils in this study were two commercial oils of European origin. GC analyses were performed on a Carlo Erba HRGC (FID) 5160 Mega Series using a 25 m x 0.32 mm, μ m OV-1 fused silica column which was temperature programmed as follows: 40°C for 10 min, 40°-130°C at 3°C/min, 130°-180°C at 10°C/min, and 180°C for 30 min. Quantitative results were obtained with the aid of a Shimadzu CR3A Chromatopac.

GC/MS analyses were performed on a Hewlett Packard MSD 5970B. The column used was 30 m x 0.32 mm, 0.4-0.45 μ m OV-1 fused silica column. It was programmed from 80°-120°C at 30°C/min, then from 120°-125°C at 3°C/min.

The genotoxicity evaluation was carried out using the *Saccharomyces cerevisiae* yeast strain D7 according to Zimmermann (18). The standard mutagenic substance used was EMS (ethylmethansulphonate) and the incubation time was 5 h.

¹Part of this study was given previously as an oral presentation at the 3rd C.N.A. Flavorings International Meeting, Lugano, 1988.

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Table I. Chemical Composition of Italian Tarragon Oil

Peak No.	Compound	Percentage*	Peak No.	Compound	Percentage*
1	α -thujene	0.01	12	β -thujone	0.09
2	α -pinene	1.44	13	menthone	0.14
3	camphene	0.11	14	estragole (methyl chavicol)	60.46
4	sabinene	0.14	15	bornyl acetate	0.14
5	β -pinene	0.19	16	eugenol	0.30
6	myrcene	0.25	17	p-methoxycinnamaldehyde	0.11
7	limonene	4.65	18	methyl eugenol	0.51
8	ocimene	13.54	19	cinnamyl acetate	0.14
9	γ -terpinene	17.01	20	C ₁₅ H ₂₄	0.27
10	α -thujone	0.09	21	C ₁₄ H ₂₄	0.37
11	linalool	0.03			

* Area percent

Results and Discussion

Chemical composition: The results of quantitative analysis of Italian tarragon oil are shown in Table I. The chromatogram of this oil can be seen in Figure 1. The estragole (methyl chavicol) content of the oil compares favorably with the previously published data (2,19-24), albeit with some expected quantitative differences. The compounds identified in the two basis oils studied can be seen in Table II. It is interesting to note that the estragole content of the two oils studied was 8.1% and 16.5%, respectively. Like the tarragon oil analysis, the chemical composition of basil oil compares favorably with previously published information (3-5,25-29).

Genotoxicity evaluation: The results of the genotoxic evaluation of from 1-100 mL of tarragon oil against the yeast (strain D-7) *Saccharomyces cerevisiae* can be seen in Table III. As far as cytotoxicity is concerned, at a concentration of 1 μ L/mL, a survival of 98% was found; whereas, in a ten-fold increase in concentration (10 μ L/mL), a survival of 98% was found to yield only a 21% survival. There is no survival at treatment concentrations of 100 μ L/mL. At a concentration of 10 μ L/mL they are 8x and 10x higher than the control. At both concentrations, no increase in mitotic recombinations was observed.

The situation for basil oil is less clear and is shown in Table IV. The two basil oils (estragole content 8.1% and 16.5%) exhibited

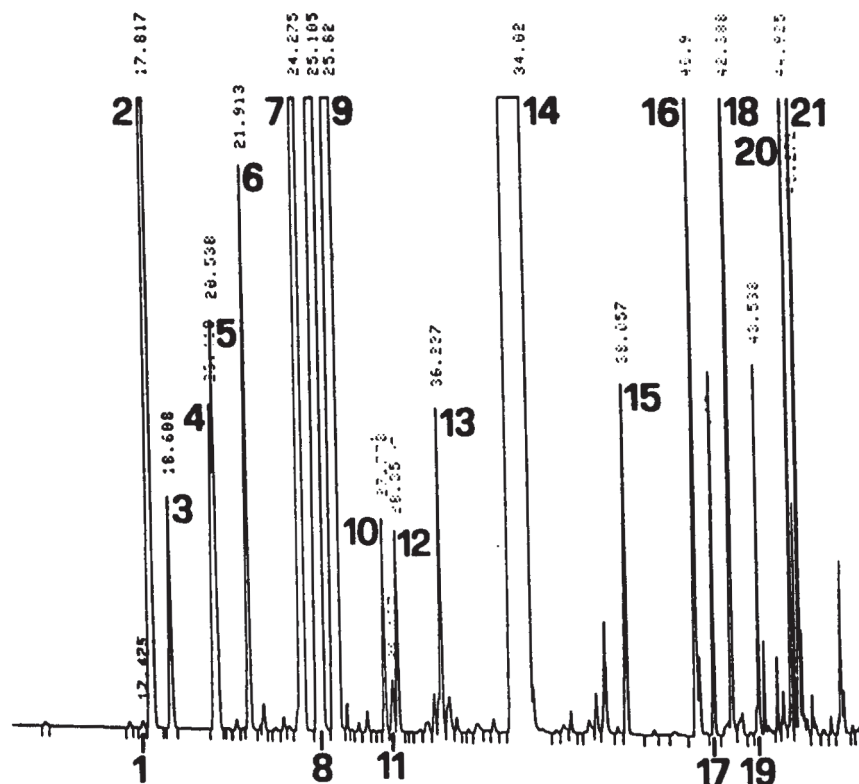


Figure 1. A chromatogram of the Italian tarragon oil; the peak numbers refer to the compounds identified in Table I

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Table II. Chemical composition of basil oil

Compound	Percentage		Compound	Percentage	
	1*	2*		1*	2*
α-pinene	0.5	0.3	linalyl acetate	0.2	0.1
camphene	0.1	0.1	estragole	8.1	16.5
β-pinene + sabinene	0.8	0.7	neral	trace	trace
myrcene	0.5	0.2	α-terpineol	0.6	2.8
1,8-cineole	4.2	4.0	geranial	0.1	0.2
limonene	0.5	0.4	geranyl acetate	1.6	0.9
ocimene	0.1	-	methyl eugenol	1.6	0.5
γ-terpinene	0.3	0.1	eugenol	2.5	2.5
linalool	46.0	50.0	bornyl acetate	1.0	0.4

* Commercial oils from two different sources

Table III. Cytotoxicity and genotoxicity evaluation*
Response of *Saccharomyces cerevisiae* to treatment with tarragon oil

Concentration μL/mL	Survivors %	Crossing overs %	Revertants / 10 ⁶ m.o.	Convertants / 10 ⁵ m.o.
Control	100.0	0.000	2.8	3.4
+ EMS 10	40.3	2.300	916.0	216.0
+ Tarragon				
1	97.9	0.000	6.6	16.5
10	21.0	0.970	16.7	34.0
100	0.7	0.000	0.0	0.0

EMS = ethylmethansulphonate (standard mutagenic substance); * Zimmermann test (18)

Table IV. Cytotoxicity and genotoxicity evaluation*
Response of *Saccharomyces cerevisiae* to treatment with basil oil

Concentration μL/mL	Survivors %	Crossing overs %	Revertants / 10 ⁶ m.o.	Convertants / 10 ⁵ m.o.
Control	100.0	0.000	2.8	3.4
+ EMS 10 5h	40.3	2.300	916.0	216.0
+ Basil				
Sample 1a 1 5h	85.5	0.000	3.2	2.5
1 10 5h	18.2	0.000	2.1	3.9
1 100 5h	<0.01	0.000	0.0	0.0
Sample 2a 1 5h	63.7	0.000	1.3	2.8
2 10 5h	6.9	0.000	0.0	0.0
2 100 5h	<0.01	0.000	0.0	0.0

EMS = ethylmethansulphonate (standard mutagenic substance); * Zimmermann test (18); ^a for chemical composition, see Table II

survival levels of 64% and 86%, respectively, on treatment of *Saccharomyces cerevisiae* with 1 μL/mL oil. The survival rates decreased to 7% and 18%, respectively, when the treatment was increased to 10 μL/mL. At all concentrations, no crossing over nor revertant or convertant vales (mitotic recombinations or genotoxicity) were detected at the survival values found.

General discussion: Earlier in this report, we noted that a carcinogenic risk assessment has been expressed by Ames, et al (17). In the discussion of this risk assessment study, the authors stated that although animal cancer tests cannot be used to predict absolute human risks, such tests, especially with rodents, may be used to indicate those chemicals that might be of concern to human health. The authors noted that humans are exposed to numerous

carcinogens at low levels on a daily basis. Such carcinogens differ enormously in potency. Using an animal-derived method of potency (TD₅₀), the daily dose rate (mg/kg) to halve the percent of tumor-free animals (30), a carcinogenic potency data base has been developed (31). To relate this information to humans or to calculate an index of possible hazard, each human exposure is expressed in daily lifetime dose (mg/kg) as a percentage of the rodent TD₅₀ for each carcinogen. This is known by the acronym HERP (Human Exposure/Rodent Potency Dose). The authors stated that it would be a mistake to use the HERP index as a direct estimate of human risk (hazard). This is particularly true because of the difference in dose susceptibility between rodents and humans and the dose response relationship. As a result, it is perhaps best to look at the HERP percent from