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Composition and Antifungal Activity of the Oil of *Artemisia nilagirica* (Clarke) Pamp

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Abstract

The essential oil of the leaves of *Artemisia nilagirica* from South India was investigated by GC and GC/MS, which lead to the identification of 59 compounds including α-thujone (41.9%), borneol (10.8%), and β-thujone (9.1%). The leaf oil inhibited the growth of the plant pathogen *Phytophthora capsici*. This property has been attributed to the presence of thujones in the oil.

Key Word Index

Artemisia nilagirica, Asteraceae, essential oil composition, α-thujone, β-thujone, antifungal activity, *Phytophthora capsici*.

Introduction

The genus *Artemisia* is one of the largest and widely distributed genera of the family Asteraceae. The oil composition of several Indian species of *Artemisia nilagirica* (Clarke) Pamp found predominantly in the northern temperate regions of the world has been investigated by Thakur and Misra (1,2). *Artemisia nilagirica* C.B. Clarke (syn *A. vulgaris* L. var. *nilagirica* C.B. Clarke; *A. vulgaris* Hook. f.) is a tall, aromatic, shrubby herb commonly called Indian wormwood and found in the hilly regions of India. The herb is considered to be emmenagogue, anthelmintic and stomachic (3). The oil of *A. nilagirica* has been reported to exhibit biological properties (4,5). There are several chemotypes of this plant. The major components of the North Indian chemotype reported were camphor and 1,8-cineole, whereas the South Indian chemotype contained a high concentration of α-thujone (2,6).

The fungus *Phytophthora capsici* causes "foot rot" in pepper, which is the most destructive disease to pepper in all pepper growing countries. The aim of this study was therefore to identify the components present in the oil of *A. nilagirica*, to investigate the effect of it on the growth of *Phytophthora capsici* and to relate the antifungal property to the constituent components.

Experimental

Plant material: *Artemisia nilagirica* used for this study was identified by A.K. Pradeep. A voucher specimen has been deposited in the Herbarium of the Chemistry Department, Calicut University (No. 22). Fresh leaves were collected from Kerala, South India and air-dried. The dry leaves (350 g) were powdered in an electrical grinder and subjected to steam distillation for 5 h. The oil was extracted with diethyl ether (2 x 100 mL), dried over anhydrous sodium sulphate and the solvent removed by evaporation. Yield: 1 g (0.29%).

GC and GC/MS: The GC/MS analysis was carried out on a Varian 3400 GC fitted with an OPTIC injector and attached to a Finnigan ITS 40 ion trap mass spectrometer. The column employed for the analysis was an HP ultra 2 (50 m x 0.25 mm, film thickness 0.33 μm). Helium was used as the carrier gas with a flow rate of 1.6 mL/min. The temperature program was from 50°-270°C at the rate of 2°C/min.

Quantification and determination of relative retention indices were carried out using an HP 5890 GC fitted with an OPTIC injector and flame ionization detector. An HP-5 column (25 m x 0.2 mm, film thickness 0.5 μm) was used for the analysis. Nitrogen was used as the carrier gas at the rate of 0.3 mL/min. The oven temperature was programmed from 30°-280°C at the rate of 3°C/min. The compounds were identified by matching the mass spectra against published data (7) and in-house database, and by retention indices with those of reference compounds on the same HP-5 capillary column.

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Table I. Volatile constituents (%) of the leaf oil of *Artemisia nilagirica*

	Components identified	Relative retention index on HP-5*	% RPA		Components identified	Relative retention index on HP-5*	% RPA
1	isoamyl alcohol	729	0.1	32	linalool	1101	0.2
2	2-methylbutanol	733	t	33	nonanal	1104	t
3	hexanal	800	t	34	α-thujone	1113	41.9
4	(E)-2-hexenal	851	t	35	2-phenylethyl alcohol	1120	t
5	(Z)-3-hexenol	852	t	36	β-thujone	1123	9.1
6	hexanol	865	t	37	*cis*-2,8-p-menthadien-1-ol	1126	1.2
7	2-heptanone	891	t	38	*trans*-pinocarveol	1148	1.5
8	(Z)-4-heptenal	900	t	39	camphor	1155	0.6
9	heptanal	901	t	40	pinocarvone	1170	0.7
10	α-thujene	930	t	41	borneol	1176	10.8
11	α-pinene	938	t	42	terpinen-4-ol	1184	3.3
12	camphene	954	t	43	p-cymen-8-ol	1190	0.3
13	sabinene	977	t	44	α-terpineol	1197	0.8
14	1-octen-3-ol	978	0.1	45	myrtenol	1203	0.9
15	β-pinene	982	t	46	myrtenal	1206	0.3
16	6-methyl-5-hepten-2-one	986	t	47	cuminaldehyde	1249	0.2
17	myrcene	993	0.1	48	carvone	1250	0.2
18	6-methyl-5-hepten-2-ol	993	t	49	perillaldehyde	1270	0.7
19	dehydro-1,8-cineole	993	0.1	50	iso bornyl acetate	1296	0.8
20	octanal	1003	t	51	dihydrocuminyl alcohol	1304	0.7
21	α-phellandrene	1009	t	52	eugenol	1367	0.3
22	α-terpinene	1021	t	53	β-caryophyllene	1431	1.9
23	p-cymene	1029	0.1	54	α-humulene	1472	0.3
24	limonene	1033	t	55	β-selinene	1497	0.4
25	1,8-cineole	1037	6.2	56	ar-curcumene	1498	0.5
26	phenylacetaldehyde	1048	0.1	57	germacrene D	1499	0.2
27	γ-terpinene	1063	0.1	58	caryophyllene oxide	1604	2.6
28	*trans*-sabinene hydrate	1072	0.2	59	β-eudesmol	1670	1.6
29	m-cresol	1075	t				
30	*cis*-linalool oxide (furanoid)	1076	t		Total		88.19%
31	*trans*-linalool oxide (furanoid)	1092	t				

RPA = relative peak area; t = trace (< 0.1%); * = retention indices relative to n-alkanes

To investigate the antifungal property of the oil, *P. capsici* cultures for inoculation were prepared by growing them in carrot agar medium (8).

Discs of 0.5 cm diameter were taken from the growing edges of 48 h old culture plates using sterilized borer. For testing the antifungal activity, oil solutions of different concentrations were prepared in acetone, and acetone alone was used as control. Appropriate quantities of the oil solution in acetone was added to carrot agar medium in order to get a concentration of 50 ppm, 100 ppm, 200 ppm and 400 ppm of oil in the medium. The Petriplates were autoclaved at 15 psi for 30 min and cooled to 40°C. Mycelial discs of 0.5 cm were placed at the centre of these Petriplates and incubated in the dark at 20°-22°C for 72 h. The radial growth in each Petriplate was measured and converted to percent inhibition using the formula: Percent inhibition = (a-b/a) x 100, where "a" is the radial growth of the colony in the control medium and "b" is the radial growth in the test medium.

Similar studies were conducted using borneol (Sigma-Aldrich, Dorset, UK) and an isolate from cedarleaf oil (*Thuja occidentalis*) containing α-thujone (54%), β-thujone (8%) and fenchone (14%) in place of the *A. nilagirica* oil.

Results and Discussion

By GC and GC/MS analysis of *A. nilagirica* leaf oil, 59 components could be identified making a total of 88.89% of the oil (Table I). The major constituents were found to be α-thujone (41.9%), borneol (10.8%), β-thujone (9.1%) and 1,8-cineole (6.2%). The antifungal study showed that 100 ppm of the oil in the carrot agar medium completely inhibited the mycelial growth of *P. capsici*. On the assumption that the major constituents, α-thujone and β-thujone were responsible for the effect, a sample of α-thujone (54%), β-thujone (8%) and fenchone (14%) isolated from cedarleaf oil was tested for activity against *P. capsici* and found to be equally effective as the *A. nilagirica* oil. Other oils rich in α- and β-thujone have also been shown to have antifungal properties (9). Borneol was found not to inhibit the growth of *P. capsici* (Table II).

From this work, it was also possible to compare the composition of the oil with those reported earlier from other

Table II. Inhibition of *Phytophtora capsici* by *Artemisia nilagirica* oil and its major components

Concentration	50 ppm	100 ppm	200 ppm	400 ppm
A. nilagirica oil	70%	100%	100%	100%
thujone mixture	68%	100%	100%	100%
borneol	0	0	0	0

parts of India (10). The oil investigated in this study is clearly the "South Indian" α-thujone chemotype, although the levels of 1,8-cineole, borneol, terpinen-4-ol and β-eudesmol were higher than those reported previously. Since these compounds are associated with the "North Indian" chemotype, this suggests a degree of continuous variation between the two chemotypes.

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