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Identification of naturally occurring chemicals from *Artemis. princeps* var. *orientalis*

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ABSTRACT

The GC/MS method was employed for analysis and identification of phytotoxic substances from wormwood plants (*Artemisia princeps* var. *orientalis*). Benzoic acid and 16 phenolic compounds were detected in wormwood leaf water extract. Terpenoids such as n-hexanal, (α+β)-phellandrene, (+)-camphene, phenylethylalcohol, benzaldehyde, α-pipene, β-pinene, 3-carene, α-terpinene, *p*-cymene, D-limonene, cineole, phenylacetaldehyde, γ-terpinene, *trans*-2-octanal, (−)-thujone, camphor, terpinen-4-ol, geranyl acetate, carveol, iso-phorone, nerol, iso-bornyl acetate, eugenol, β-caryophyllene, β-myrcene, α-humulene, cadinene, terpinolene, linalyl formate, *trans*-caryophyllene, furfural, acetoin, dipentene, geraniol and cedrol were identified from wormwood essential oil.

Key words : *Artemisia princeps* var. *orientalis*, phytotoxic substances, water extract, wormwood essential oil

INTRODUCTION

Different parts of plants have been bioassayed and inhibitors have found in them (11). Chemicals from leaves of *Artemisia princeps* var. *orien* suppressed germination and seedling growth of selected plants (13, 21). Most kn allelopathic compounds are secondary plant metabolites viz., phenolic a flavonoids, terpenoids, steroids, alkaloids and organic cyanides (5, 9, 14, 15, The chemical composition of essential oil of *Artemisia* has been investig (2, 8, 18). The objective of the present study was to determinewhich of chemical components of *Artemisia princeps* var. *orientalis* exhibited allelopa effects (21).

MATERIALS AND METHODS

Water-soluble Substances

The extraction of phenolic compounds from wormwood plants was car out by the method of Kil and Yim (13). A gas chromatograph (Hewlett Pacl

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5890, U. S. A.) using J & W fused silica capillary and methylsilicone banded column (B-1; 60 m × 0.25 mm i. d) was employed for gas chromatography. Oven temperature range was 100–300°C at 4°C/min. The head pressure of column was 30 psi and split ratio was 1 : 50. The integrator was a Hewlett Packard 3392 A and the volume of sample injected was 0.5–1.0 μl. Identification of each peak was made by comparing the retention times of the peaks with those of commercial compounds obtained from Sigma and Aldrich chemical companies.

Volatile Substances

Karlsruker's apparatus was used to prepare volatile substances from wormwood plants. The volatile substances were analysed by gas chromatography (Hewlett-Packard 5890) using SE-54 column (50 m × 0.33 μm × 0.2 mm i. d.). Temperature was programmed from 45°C (5 min) to 300°C (3 min) at 4°C/min. Carrier gas was helium, flow rate 0.5 ml/min, with FID. Injector temperature was 250°C. Split ratio was 1 : 10 and head pressure was 34 psi. Injection volume of all samples was 0.20 μl. Identification of each peak was made by the comparison of retention times and mass spectra of the peaks with those of commercial compounds obtained from Sigma, Aldrich and Fluka chemical companies.

RESULTS AND DISCUSSION

Water-soluble Phytotoxins

Phytotoxic chemicals present in wormwood leaf extract were identified by gas chromatography. The identity of these compounds was confirmed by comparing the retention time of the standards. Phenolic acids such as salicylic, gentisic, caffeic acid and protocatechuic acid were the major chemicals identified in wormwood leaf extract (Fig. 1).

Volatile Phytotoxins

Figures 2, 3 and 4 show the chromatograms of wormwood essential oil extracted from leaf, root and flower, respectively. The identification of each component was made by comparison of the retention time and mass spectra with those of commercial authentic samples.

Twenty-six components were identified in leaf essential oil, 28 in flower oil and 23 in root oil. The major constituents of the essential oil in leaves and flowers were cineole and camphor, and those in roots were (α+β)-phellandrene and isobornyl acetate.

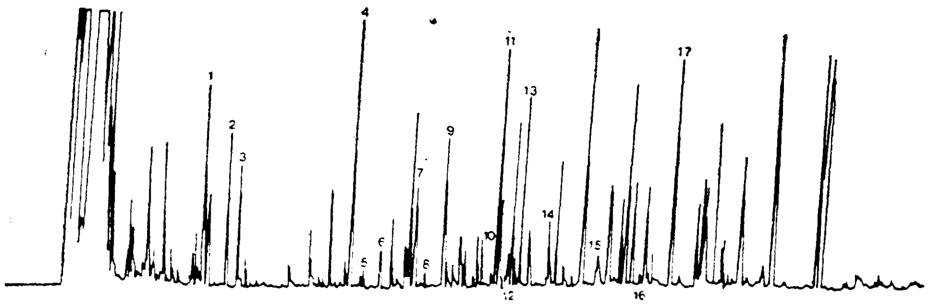

Fig. 1. GC chromatogram of the combined ether extracts of *A. princeps* var. *orientalis* leaf. 1, benzoic acid; 2, phenylacetic acid; 3, catechol; 4, salicylic acid; 5, *t*-cinnamic acid; 6, *m*-hydroxybenzoic acid; 7, *p*-hydroxybenzoic acid; 8, phlorogluicinol; 9, *p*-phenylbenzaldehyde; 10, vanillic acid; 11, gentisic acid; 12, *o*-hydroxycinnamic acid; 13, protocatechuic acid; 14, syringic acid; 15, *p*-coumaric acid; 16, ferulic acid and 17, caffeic acid. Other peaks in the chromatogram did not appear regularly from one analysis to another.

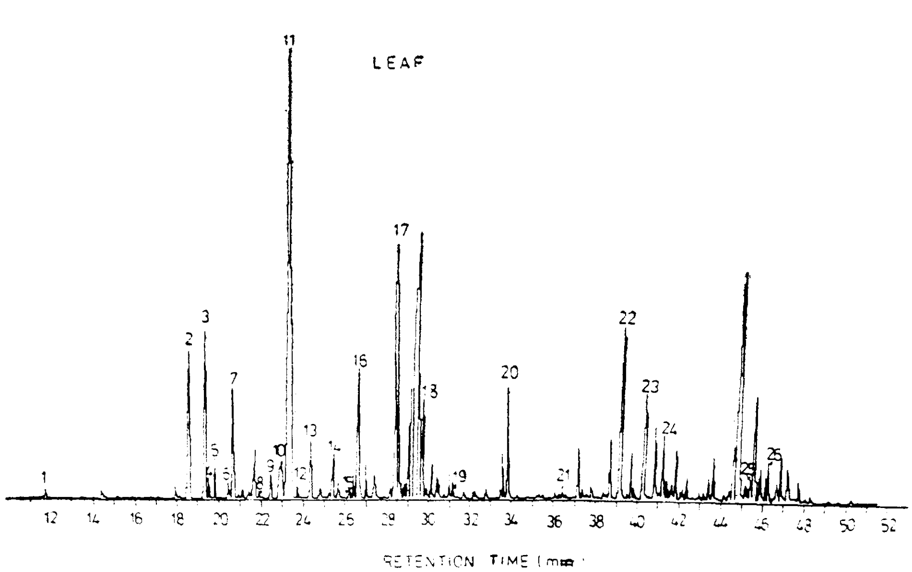

Fig. 2. GC chromatogram of the essential oil of *A. princeps* var. *orientalis* leaf. 1, n-hexanal; 2, (α+β)-phellandrene; 3, (+)-camphene; 4, phenyl ethyl alcohol; 5, benzaldehyde; 6, α-pinene; 7, β-pinene; 8, 3-carene; 9, α-terpinene; 10, *p*-cymene; 11, cineole; 12, phenyl-ethyl acetaldehyde; 13, γ-terpinene; 14, terpinolene; 15, linalyl formate; 16, (−)-thujone; 17, camphor; 18, terpinen-4-ol; 19, carveol; 20, iso-bornyl acetate; 21, eugenol; 22, β-caryophyllene; 23, α-humulene; 24, cadinene; 25, *trans*-caryophyllene and 26, farnesol.

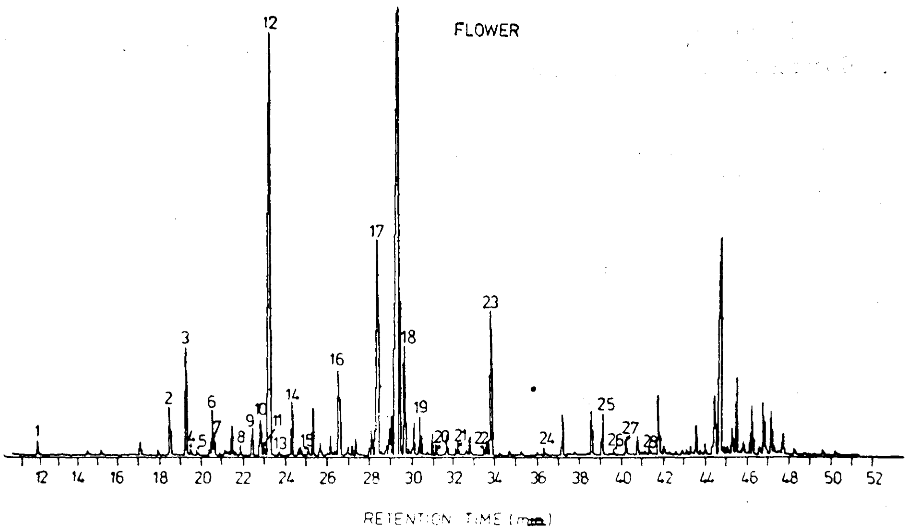

Fig. 3. GC chromatogram of the essential oil of *A. princeps* var. *orientalis* flower. 1, n–hexanal; 2, (α+β)–phellandrene; 3, (+)–camphene; 4, phenyl ethyl alcohol; 5, benzaldehyde; 6, α–pinene; 7, β–pinene; 8, 3-carene; 9, α-terpinene; 10, *p*–cymene; 11, D–limonene; 12, cineole; 13, phenyl acetaldehyde; 14, γ–terpinene; 15, *trans*–2–octanal; 16, (—)-thujone; 17, camphor; 18, terpinen–4–ol; 19, geranyl acetate; 20, carveol; 21, iso–phorone; 22, nerol; 23, iso-bornyl acetate; 24, eugenol; 25, β–caryophyllene; 26, β-myrcene; 27, α–humelene and 28, cadinene.

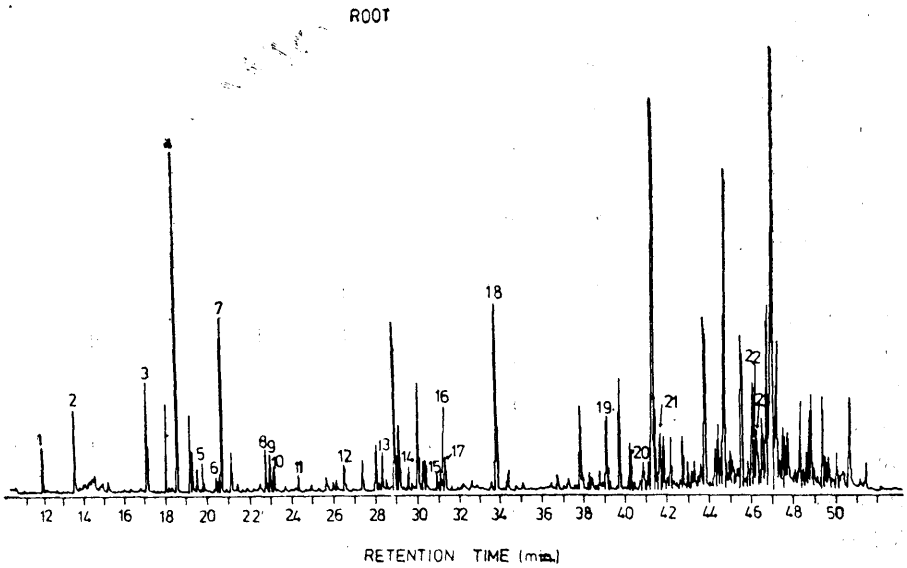

Fig. 4. GC chromatogram of the essential oil of *A. princeps* var. *orientalis* root. 1, n–hexanal; 2, furfural: 3, acetoin; 4, (α+β)–phellandrene; 5, bezaldehyde; 6, α–pinene; 7, β-pinene; 8, *p*–cymene; 9, dipentene; 10, cineole; 11, γ–terpinene; 12 (—)-thujone; 13, camphor; 14, terpinen–4 ol; 15, geranyl acetate; 16, carveol; 17, geraniol; 18, iso-bornyl acetate; 19, β-caryophyllene; 20, α–humulene; 21, cadinene; 22, trans-caryophyllene and 23, cedrol.

Various components of essential oil from several species of *Artemisia* h been documented in the literature (Table 1).

TABLE 1. Volatile compounds in different *Artemisia* species

Compound	*Artemisia princeps* var. *orientalis**			*A. g.*(2)	*A. r.*(2)	*A. p.*(2)	*A. h.*(8)	*A. c.*(
	Leaf	Flower	Root					
1	2	3	4	5	6	7	8	9
n–Hexanal	+		+					
Benzaldehyde	+		+					
α–Pinene	+		+	+	+		+	
3–Carene	+	+						
α+β–Phellandrene	+			+				
α–Terpinene	+	+		+		+	+	
(—)–Thujone	+		+					
Terpinene–4–ol	+	+		+	+	+	+	
Camphor	+	+		+			+	+
Carveol	+	+	+					
Cineole	+	+	+	+	+	+	+	+
Linalyl acetate	+							
Bornyl acetate	+				+		+	
iso–Bornyl acetate	+	+	+					
Eugenol	+	+		+				
β–Caryophyllene	+	+	+	+				
Geranyl acetate	+	+	+					
trans–Caryophyllene	+		+				+	+
Farnesol	+			+				
(+)–Camphene		+		+	+	+	+	
Nerol		+						
Cadinene		+	+					
β–Pinene			+	+	+		+	+
Cedrol								
D–Limonene		+			+			
α–Thujene				+				
Myrcene				+	+			
β–Cymene				+	+	+	+	+
δ–Terpinene				+				

Con

Table 1 contd.

1	2	3	4	5	6	7	8	9
Terpinolene				+			+	
trans-Pinocarveol				+				
Pinocarvone				+				
Benzyl acetate				+				
α-Terpineol				+			+	
Myrtenol				+		+		
Phellandral				+				
cis-Piperitol				+				
Cuminic aldehyde				+				
p-Isopropil-phenol				+				
p-Menth-2-en-7-ol				+				
1,5-p-Menthadien-7-ol				+				
Carvacrol				+				
Benzyl butyrate				+				
1-p-Menthen-9-ol				+				
α-Copaene				+				
Methyleugenol				+				
3,7-Dimethyloctyl acetate				+	+			
β-Sesquiphellandrene				+				
β-Cubebene				+				
Sabinyl propionate				+				
β-Farnesene				+				
Sabinyl acetate				+				
1-Dodecanol				+				
Nerolidol				+				
Chamazulene				+				
iso-Butyl phthalate				+	+			
Hexadecanoic acid				+	+			
Phytol				+	+			
2-Methyl-butyl-2-methyl-butyrate					+			
Borneol					+		+	+
Citronellol					+			
α-Terpinyl acetate					+	+		
Citronellyl acetate					+			

Contd.

Table 1 contd.

1	2	3	4	5	6	7	8
β–Elemene					+	+	
α–Guaience					+		+
β–Charmigrene					+		
Artemone					+	+	
Citronellyli–valerate					+		
Linalyl 3–methyl–butyrate					+		
Davanone					+	+	
Guaiol					+		
Bazzaneol					+		
Tetradecanoic acid					+		
4,6,10–Trimethyl–2–pentadecanone					+		
1–Hexadecanol					+		
Pentadecanoic acid					+		
1–Otadecanol					+		
Linoleic acid					+		
2–Methyl–1–butanol						+	
cis–3–Hexenyl butyrate						+	
trans–3–Hexenyl butyrate						+	
2–Methylbuty–2–methylbutyrate						+	
n–Amyl–3–methyl butyrate						+	
trans–Pinocarreol						+	
δ–Terpinol						+	
α–Terpinol						+	
Myrtenol						+	
iso–Amyl tigliate						+	
trans–β–Farnesene						+	
β–Phenylethyl–2–methylbutylate						+	
β–Phenylethyl–n–valerate						+	
β–Phenylethyl tigliate						+	
Hdroxy davanone						+	
iso–Butyl Phthalate						+	
p–Cymen–8–ol							+
Piperitol							+
Cumin aldehyde							+

Table 1 contd.

1	2	3	4	5	6	7	8	9
Terpenylacetate							+	
Piperitone							+	
Thymol							+	
trans-Sabinenehydrate							+	
Bornyl propionate							+	
Chrysanthenone							+	
β-Elemenene							+	
δ-Cadinen							+	
α-Humulene							+	
α-Cedrene							+	
α-Guainene							+	
β-Guainene							+	
Curcumene							+	
Artemisia Ketone								+
iso-Thujone								+

*Present study; *A.g.—Artemisia glabella, A. r.—A. rupestris, A. p.—A. persica, A. h.—A. herba-alba, A. c.—A. californica.*

Inhibition of seed germination and seedling elongation by wormwood plants in the absence of physical competition shows that the inhibition is due to allelochemicals of the plants (21) The identification of benzoic acid, phenylacetic, salicylic, *t*-cinnamic, *m*-hydroxybenzoic, *p*-hydroxybenzoic, vanillic, gentisic, α-hydroxycinnamic, protocatechuic, syringic, *p*-coumaric, ferulic, caffeic acid, catechol, phloroglucinol and *p*-benzaldehyde by GC in wormwood leaf also supports its allelopathic potential. There is considerable evidence that allelopathy is probably due to complex interactions of all compounds present (1, 3, 4, 6, 7, 12, 16, 20).

Halligan (10) reported that the volatile toxins from *A. californica* were monoterpenoids, sesquiterpenoids and sesquiterpene hydrocarbons, the major compounds being 1, 8-cineole, artemisia ketone, α-thujone, camphor. The essential oils were fractionated from ***A. abrotanum*** leaves by dry column chromatography and about 40 different chemicals including 1, 8-cineole were identified by gas chromatography and mass spectrometry (17). The steam-distilled essential oil was analysed from the aerial parts of three Asiatic species of ***Artemisia*** by gas chromatography and mass spectrometry and identified about 100 compounds; davanone, 1-hexadecanol and 1, 8-cineole being the main components of ***A. glabella, A.***

rupestris and *A persica*, respectively (2). Commonly occurring chemicals am constituents of essential oil of the three Asiatic *Artemisia* spp. were α-pinene pinene, α-terpinene, terpinen-4-ol, 1, 8-cineole and (+)-camphene.

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