Influence of Plant Growth Stage on the Essential Oil Content and Composition in Davana (*Artemisia pallens* Wall.)

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The influence of three plant growth stages (full emergence of flower heads, anthesis, and initiation of seed set) on the essential oil content and composition in Davana (*Artemisia pallens* Wall) was investigated over two successive seasons. The essential oil content was found to be higher at the full emergence of flower heads than at anthesis and initiation of seed set stages. The contents of davanone, the major constituent of davana oil, and linalool decreased while those of (*Z*)- and (*E*)-methyl cinnamate, (*E*)-ethyl cinnamate, bicyclogermacrene, davana ether, 2-hydroxyisodavanone, and farnesol increased from flower heads emergence stage to the initiation of seed set stage. These results support the general practice of harvesting the crop at full bloom stage. Five compounds, viz., (*Z*)- and (*E*)-methyl cinnamates, (*Z*)- and (*E*)-ethyl cinnamates, and geranyl acetate, were identified for the first time in davana oil.

Keywords: *Davana; Artemisia pallens Wall.; plant growth stage; essential oil composition; davanone, (Z)- and (E)-methyl cinnamates; (E)-ethyl cinnamate; davana ether*

INTRODUCTION

Artemisia pallens Wall. (Family: Asteraceae), popularly known as davana, is a fragrant herb with a pleasant sweet aroma. It is native to India and is grown in South India particularly in the states of Andhra Pradesh, Karnataka, and Tamil Nadu. The plant is used in garlands and bouquets to which the leaves of davana contribute fresh and sweet aroma. Steam or hydrodistillation of the shade-dried aerial parts of the flowering herb furnishes the essential oil, davana oil, which is rich in fruity, sweet, and balsamic odor notes (Lamparsky and Klimes 1985; Klimes and Lamparsky, 1986). It is a highly praised oil in perfumery and is used in the flavoring of tobacco, cakes, pastries, expensive beverages, and fine perfumes (Lawrence, 1978; Husain et al., 1988). There is a fairly good demand for this oil and India exported approximately 1350 kg of the oil mostly to United States, Europe, and Japan during 1995−96.

The oil of davana has been the subject of several investigations for the isolation and characterization of the volatile constituents of the oil (Simpa and Vanderwal 1968; Naegeli et al., 1970; Baslas, 1971; Thomas and Pitton, 1971; Thomas and Ozainne, 1974; Thomas et al., 1974; Gulati and Khan 1980; Lampasky and Klimes, 1985; Klimes and Lamparsky, 1986; Chandra et al., 1987; Misra et al., 1991; Catalan et al., 1991). The major constituent of davana oil is a sesquiterpene ketone, davanone, which is present in the oil to an extent of 30−65%. Davanone exists in several isomers and eight isomers of this compound were characterized (Lamparsky and Klimes, 1985). Davanone when rigorously purified was found to be practically odorless (Thomas, 1974). However, it is understood that perfumery and flavor industries prefer a high content of davanone (more than 50%). Although davanone is odorless, its presence in large amounts may enhance the odor of the oil. This odorless compound may possibly be acting as a natural fixative and synergist lending much tenacity and smoothness to the flavor of davana oil, similar to the heavy odorless constituents of jasmine absolute playing decisive role in the fragrance of jasmine (Demole, 1982). The odoriferous constituents of the oil were reported to be davana ether (Thomas and Pitton, 1971; Thomas and Dubini, 1974), davanafurans (Thomas and Dubini, 1974a,b), 2-(3-methylbut-2-enyl)-3-methyl-2,5-dihydrofuran and 2-(3-methylbut-2-enyl)-5-(5-cinnamoyloxy-2-oxo-1,5-dimethylhex-3-enyl)-3-methyl 2,5-dihydrofuran (Chandra et al., 1987), and hydroxydihydrorosefuran (Misra et al., 1991). The plant growth stage at harvest has been found to influence oil content and composition in many aromatic plants (Akhila et al., 1984; Chalchat et al., 1994; Gupta, 1996; Dhar and Dhar, 1997; Piccaglia et al., 1997). The present study

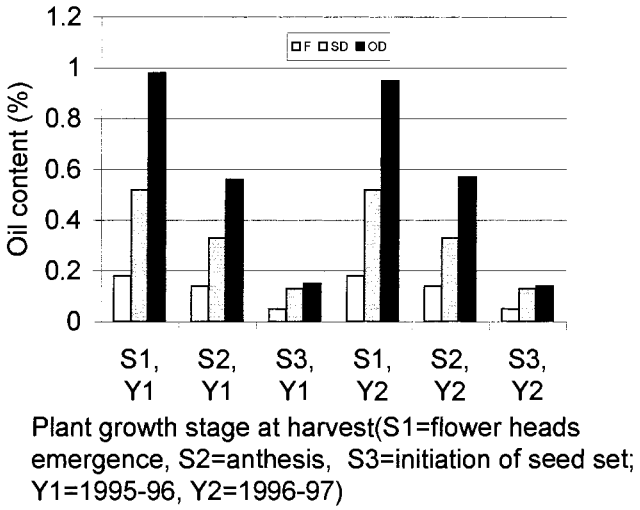

Figure 1. Mean oil content in davana herb at three stages of plant growth (F = on fresh weight basis, SD = on shade dry weight basis, OD = on oven dry weight basis).

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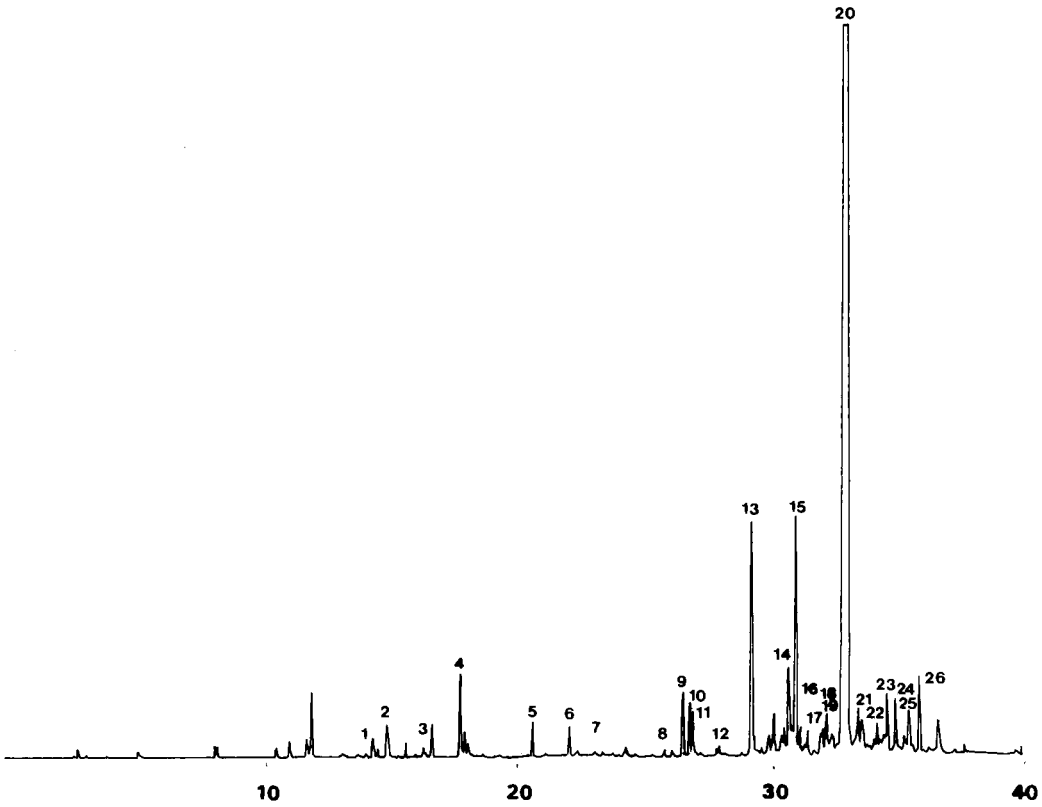

Figure 2. GC profile of davana oil obtained from plants harvested at flower heads emergence stage.

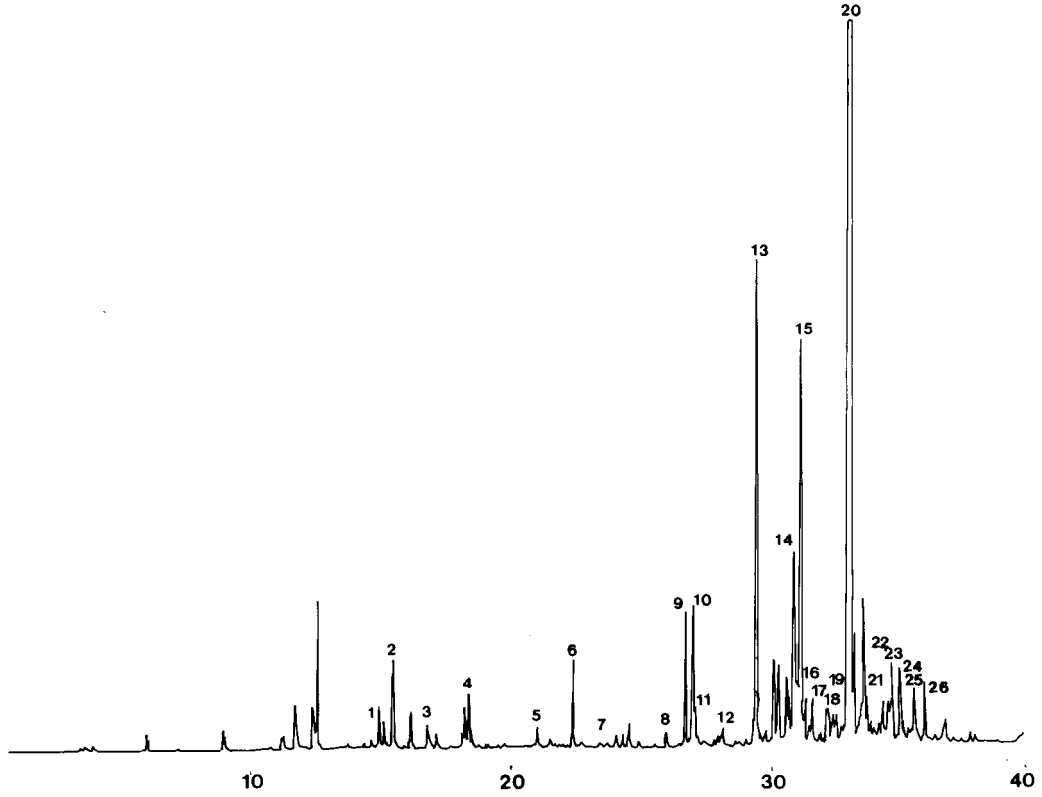

Figure 3. GC profile of davana oil obtained from plants harvested at anthesis stage.

was taken up to determine the influence of plant growth stage on oil content and composition in davana.

MATERIALS AND METHODS

Plant Material. Seeds of davana procured from the local market were used for raising the plants. The experiments on the influence of harvesting stage on the essential oil content and composition in davana were conducted in the farm of the institute during 1995−96 and 1996−97. During the year 1995−96, seeds were sown in the nursery on December 1, 1995. Seedlings were transplanted in the field at a plant spacing of 30 × 30 cm on December 31, 1995. The plants were fertilized at the rate of 150:40:40 kg of N, P_2O_5, and K_2O, respectively, per hectare and irrigated twice a week. The plants were

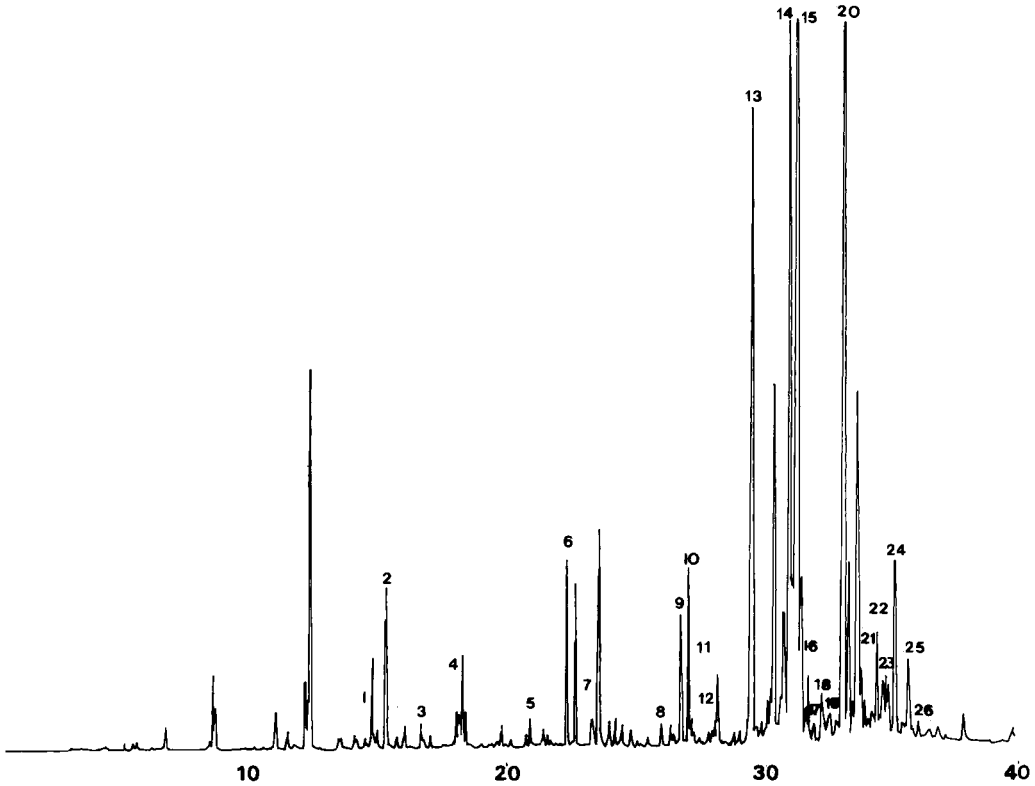

Figure 4. GC profile of davana oil obtained from plants harvested at initiation of seed set stage.

Table 1. The Composition (Expressed as Relative Peak Area Percentage) of Davana Oil Distilled at Different Stages of Plant Growth during 1995−96 (A) and 1996−97 (B) Seasons

		area percentage						
		flowerhead emergence		anthesis		initiation of seed set		method of
compound	RI	A	B	A	B	A	B	identification[a]
1. myrcene	984	0.1	0.1	0.1	0.1	0.2	0.1	RI, PE, MS
2. *p*-cymene	1012	0.7	0.2	0.9	1.1	1.5	1.5	RI, PE, MS
3. γ-terpinene	1050	0.1	0.3	0.2	0.5	0.4	0.4	RI, PE, MS
4. linalool	1086	1.4	1.5	0.8	0.7	0.1	0.2	RI, PE, MS
5. terpinen-4-ol	1166	0.6	0.5	0.3	0.4	0.4	0.3	RI, PE, MS
6. nordavanone	1207	0.4	0.5	0.8	1.1	1.7	1.8	RI, PE, MS
7. geraniol	1238	tr	0.1	0.1	0.1	0.3	0.4	RI, PE, MS
8. (*Z*)-methylcinnamate	1321	0.1	0.1	0.2	0.3	0.3	0.3	RI, MS
9. (*Z*)-ethylcinnamate	1346	0.9	1.1	1.0	1.8	1.6	1.5	RI, MS
10. (*E*)-methylcinnamate	1354	0.4	0.9	1.3	1.6	1.8	1.8	RI, PE, MS
11. geranyl acetate	1359	0.7	0.7	0.5	0.5	0.2	0.2	RI, PE, MS
12. davana furan	1389	0.1	0.1	0.1	0.1	0.3	0.2	MS
13. (*E*)-ethylcinnamate	1437	3.1	4.8	5.3	7.4	9.3	9.4	RI, PE, MS
14. davana ether	1487	0.5	1.3	2.5	3.0	7.3	8.4	RI, MS
15. bicyclogermacrene	1497	4.2	2.0	5.4	4.7	13.0	11.1	RI, MS
16. δ-cadinene	1514	0.4	0.4	0.4	0.5	0.8	0.7	RI, MS
17. artemone	1536	0.5	0.5	0.7	0.7	0.9	0.8	RI, MS
18. artedouglasia oxide	1542	0.4	0.6	0.4	0.2	0.2	0.1	RI, MS
19. (*E*)-nerolidol	1548	0.6	0.3	0.1	0.2	0.2	0.4	RI, MS
20. davanone	1567	67.0	67.1	60.2	53.7	22.9	23.8	RI, MS
21. davanol isomer	1591	0.5	0.5	0.7	0.4	0.6	0.6	RI, MS
22. T-cadinol	1615	0.5	0.2	1.1	0.4	1.2	1.1	RI, MS
23. β-eudesmol	1631	1.0	1.0	1.2	0.8	0.7	0.5	RI, MS
24. 2-hydroxyisodavanone	1644	1.2	0.8	1.2	0.9	2.1	2.0	RI, MS
25. farnesol	1664	0.9	1.0	1.4	1.4	2.4	1.9	RI, MS
26. β-santalol	1678	0.8	2.3	0.5	1.1	0.2	0.4	RI, MS

[a] Abbreviations: RI, retention index on BP-1 column; MS, mass spectra; PE, peak enrichment on co-injection with authentic compound.

harvested when they reached the following stages: (i) full emergence of flower heads, (ii) anthesis, and (iii) initiation of seed set. The harvesting dates corresponding to these three stages were March 26, April 19, and May 10, 1996, respectively. During the year 1996−97, seeds were sown in the nursery on October 24, 1996 and seedlings were transplanted in the field on December 4, 1996. Plants were harvested at the three stages mentioned above on March 2, April 21, and May 12, 1997.

Isolation of Essential Oil. During both years triplicate plant samples of 500 g each of the fresh herb were dried in shade for 3 days before they were subjected to hydrodistillation. The shade-dried aerial parts of davana plants were hydrodistilled in a Clevenger-type apparatus for 8 h. The oil samples were collected, dried over anhydrous Na_2SO_4, and stored at 5 °C in the refrigerator until analyzed. The oil contents were calculated on fresh, shade, and oven dry weight bases.

GC and GC/MS Analyses. The GC analyses of the oil samples were performed on a Perkin-Elmer gas chromatograph 8500 equipped with flame ionization detector (FID) and a GP-100 printer plotter using a fused silica capillary column, BP-1 (Scientific Glass Engineering Pty. Ltd., Australia), coated with methyl polysiloxane (25 m × 0.5 i.d., 0.25 μm film thickness) as stationary phase. Nitrogen was used as carrier gas at 10 psi inlet pressure. Temperature programming was performed from 60 to 220 °C at 5 °C/min, and the final temperature was held for 10 min. Samples were injected by splitting; the split ratio being 1:80. Injector and detector temperatures were 250 and 300 °C, respectively.

GC/MS analysis of davana oil was performed on Hewlett-Packard instrument model 5989 interfaced with HP 5990B and equipped with HP gas chromatograph 5890 Series II. GC conditions: Fused silica capillary column HP-5 (Hewlett-Packard, USA; 25m × 0.25 mm id); helium was used as carrier gas. Temperature program: From 50 to 250 °C at 10 °C/min, with an initial hold time of 5 min. MS conditions: EI mode 70 eV. Ion source temperature: 250 °C.

Identification and Quantification of Compounds. Component identification was done by comparison of their Kovats retention indices (RI relative to C8−C21 alkanes) with those reported for compounds in the literature (Jennings and Shibamoto, 1980; Ramaswamy et al., 1988; Davies 1990; Weyrstahl et al., 1997), by peak enrichment on co-injection with standards wherever possible and by comparison of mass spectra of peaks with those of compounds reported in the literature (Jennings and Shibamoto, 1980; Adams, 1988; Ramaswamy et al., 1988). The relative amounts of individual compounds were computed from peak areas without FID response factor correction.

RESULTS AND DISCUSSION

The content of essential oil as well as its composition (specially davanone) did not vary much from year to year. However, the plant growth stage at harvest was found to affect essential oil content as well as composition (Figures 1−4; Table 1). The oil content decreased from full emergence of flower heads to the initiation of seed set stage, at which it was lowest. Similarly, the content of davanone, the major constituent of davana oil, decreased drastically from the flower head emergence stage (67%) to the initiation of seed set stage (22.9−23.8%). Linalool also showed a similar trend (from 1.35−1.46% to 0.67−0.81%). On the other hand, the contents of (*Z*)- and (*E*)- methyl cinnamates, and (*E*)-ethyl cinnamate, bicyclogermacrene, farnesol, and davana ether increased gradually from flower heads emergence to the initiation of seed set stage. These changes suggest transformation of compounds from one to another with the changes in plant growth stage.

In the present study, nerol and geraniol were found as minor constituents in davana oil. These results are at variance with those of Misra et al. (1991), who reported nerol and geraniol in concentrations of 10 and 5%, respectively, in davana oil. (*Z*)- and (*E*)- methyl cinnamates, (*Z*)- and (*E*)-ethyl cinnamates, and geranyl acetate which are known to possess sweet balsamic odor (Bedoukian, 1967) were found for the first time in davana oil.

Among the reported odoriferous compounds of davana oil, davana ether and davana furan were found in higher amounts at initiation of seed set stage. However, as at this stage the davanone content (23%) was lower than that preferred (≥50%) by the user industry and also as the oil content was very low; herefor it would be neither economical nor of any utility to harvest the crop at this stage. The high contents of essential oil and davanone, and moderate amounts of davana ether in the oil of plants harvested at flower heads emergence and anthesis stages support the general practice of harvesting the crop at full bloom stage. At this stage, since davana is a cross-pollinated crop, plants can be found in various stages from flower heads emergence to anthesis.

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