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Original Research Article

## Influence of growth stage on essential oil content and major chemical constituents of *Artemisia pallens* Bess

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### ABSTRACT

*Davana* (*Artemisia pallens* Bess), belonging to Asteraceae family, is an important aromatic crop of South India. The field experiment was laid out in randomized randomized complete block design (RCBD) with 6 replication during winter season 2018-2019. The 35 days old seedling were transplanting to the main field with spacing of 30 cm × 30 cm in order to investigate the influence of different growth stages on the essential oil content and major chemical composition of *davana*. The essential oil content found to be varied from vegetative stage (0.12%) to full blooming stage (0.25%). The major component davanone was found to be higher at before anthesis followed by anthesis stage (full blooming stage) and gradually decreased from early initiation of seed set stage to seed maturing stage. However, the amounts of other major constituent of *davana* oil like (*E*)-ethyl cinnamate and bicyclo germacrene increased from the vegetative stage to the seed maturing stage. Significant interaction effect observed between chemical content and different stages. Based on the experimental results full blooming stage is ideal for harvesting and it was also confirmed with the normal practice of farmers harvesting the crop.

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## 1. Introduction

*Artemisia pallens* Bess. popularly known as *davana*, belonging to family Asteraceae, is native to India and has gained considerable industrial importance for its fragrant and pleasant sweet aroma. The oil is used in cosmetic, flavouring of cakes, tobacco and high grade perfumery industries (Husain et al., 1988; Mallavarapu et al., 1999; Jeffrey, 2001). Commercially *davana* crop cultivated in South India particularly in the states of Karnataka, Tamil Nadu and Andhra Pradesh appears as a short duration crop from November to March. Production and export trade of *davana* oil India has a monopoly (Jayanthi et al., 2013). *Davana* oil, is a brown viscous liquid with deep mellow, persistent, rich fruity, sweet and balsamic odour notes (Limes and Lamparsky, 1986; Pisana, 1989). *Davana* oil is mainly used in making perfumery and fragrances, its acts as soothing to rough, dry, chapped skin, skin infections and cuts. It has been traditionally used in Indian folk medicine for the treatment of diabetes mellitus, wound healing, immunomodulating, anthelmintic, antipyretic, antiseptic aphrodisiac and mood elevator. This

oil also has mild insect repellent property and is effectively used to reduce the risk of chronic diseases, cardiovascular disorders and cancer (Shreyas et al 2018). Essential oil of *davana* has been isolated and characterized for its volatile constituents and subjected to several investigations by Misra et al., (1991), Catalan et al., (1991) and Mallavarapu et al., (1999). The major constituent of *davana* oil is a sesquiterpene ketone, davanone, which is present in the oil to an extent of 24-67% (Mallavarapu et al., 1999). Even though davanone is odourless, perfumery and flavour industries prefer a high content of davanone. The presence of high amount of davanone may enhance the odour of the oil. Essential oil content and its composition influenced by both biotic (genotypic, morphogenesis) and abiotic (climate conditions) factors affecting plant growth stage at harvest has been studied in many aromatic crops (Pisana, 1989; Khorshidi et al., 2009; Verma et al., 2010; Aksit et al., 2013). Therefore, the aim of the present study was to investigate the influence of different growth stage on oil content and major composition of *davana* crop.

## 2. Experimental

### 2.1. Plant materials

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Freshly harvested davana seeds were collected from farmer's field in the region of Bangalore, Karnataka, India and used for the present study.

## 2.2. Experimental site

The present experiment was conducted at the CSIR-Central Institute of Medicinal and Aromatic Plants, Research centre, GKVK Post, Allalandra, Bengaluru to evaluate the effect of different plant growth stages on essential oil content and constituent.

## 2.3. Cultivation practices

Seed sowing in nursery was completed on 15-09-2018 and 35 days old seedlings were transplanted

to mainfield with spacing of 30 cm × 30 cm on 20-10-2018. The crop was fertilized at the rate of 150:40:40 kg of NPK, respectively (N: Urea, P: Diammonium phosphate and K: Potassium) and the field was irrigated twice a week with normal agricultural practices. Total life span of the crop is around 150-160 day for seed purposes and for oil it may be around 120-125 days. The plants were harvested at four different stages: vegetative stage (70-75 days after transplanting), Flower head initiation stage (100-105 days after transplanting), full blooming stage (115-120 days after transplanting) and seed maturation stage/seed setting stage (135-140 days after transplanting). The general view and photograph of the experimental plot was shown in Fig. 1.



Fig. 1. General crop view of davana (*Artemisia pallens* Bess).

## 2.4. Isolation of essential oil (%)

The 48 hours shade dried upper whole plant material of different stages were subjected to hydro-distillation in Clevenger apparatus for six hours in order to determine the essential oil content in percentage (Clevenger 1928). The oil was dehydrated with anhydrous  $\text{Na}_2\text{SO}_4$  and stored in refrigerator until further use.

## 2.5. Gas chromatography analysis

The GC analysis of the essential oils samples were performed on a Varian CP-3800 model gas chromatograph with Galaxy software system equipped with flame ionization detector (FID) and an electronic integrator. Separation of the compounds was achieved employing a Varian CP-Sil 5CB capillary column (ID: 50 m X 0.25 mm; film thickness 0.25  $\mu\text{m}$ ). Nitrogen was used as a carrier gas at the constant flow rate of 0.4 mL/min. The column temperature was programmed from 60 °C (held for 2 min) to 220 °C (held temperature

were set at 250 °C and 300 °C, respectively). The relevant 0.2  $\mu\text{L}$  samples were injected for 6 min at a rate of 5 °C/min then at an elevated temperature of 245 °C applying a rate of 5 °C/min. The injector and detector tem:100:20 split ratio. Retention indices were generated with a standard solution of *n*-alkanes ( $\text{C}_6$ - $\text{C}_{19}$ ). The composition was reported as a relative percentage of the total peak are without FID response factor correction (Mallavarapu et al., 1999; Sastry et al., 2015).

## 2.6. Identification and quantification of compounds

Chemical compounds identification was done by comparison of their Kovats retention indices from GC FID peaks relative to  $\text{C}_6$ - $\text{C}_{19}$  alkanes as per the their elution order on varian CP-SIL 5CB column and with those reported for compounds in the literature.

## 2.7. Statistical analysis

Data were subjected to a factorial RCBD analysis of

variance (ANOVA). Statistical analysis was carried out using R statistical software version 3.3.2 (RCore Team, 2016). Graph Pad prism, version 6.0 (Graph Pad Software, San Diego, CA) was used to plot different graphs.

### 3. Results and Discussion

#### 3.1. Analysis of variance

The analysis of variance for different chemical constituents at four phenological stages ( $p < 0.05$ ) showed significant difference among the essential oil content and composition (Table 1).

Factorial randomised complete block design was used to assess the interaction effect of phenological stages on major chemical components. Analytical results showed significant interaction effect among growth stages and oil content and chemical composition (Table 2).

#### 3.2. Essential oil content

Mean values of essential oil content obtained from different stages are given in Fig. 2. Essential oil content increased progressively from vegetative stage to full blooming stage and content was reduced in seed maturation stage.

The highest content of essential oil was obtained in full blooming stage (0.24%), while, the lowest value was obtained from vegetative and early seed bud stages (0.15%), respectively. It is evident from the data that plant growth stages influenced the oil content and full blooming stage is ideal for higher recovery of oil. Thus, crop development stages at time of harvesting has major effects on oil content. Similar kind of results reported in rosemary in which the effect of phenological stage on essential oil content and composition were

investigated and full flowering time was the most suitable time for oil content and composition (Kianoush et al., 2017) and in davana (Mallavarapu et al., 1999). One of the assumption is that during flowering time increased synthesis of the essential oils and crop plant water content will decrease with maturation (Arif Sanli et al., 2016).

#### 3.3. Major essential chemical composition

All essential oils samples were analyzed with GC/FID. In total, 140-145 individual constituents were identified representing 99.9-100% of the total essential oil profile. The detailed chemical analysis of the essential oils of the different harvesting stages showed that the major constituents were davanone (39.29%-45.893%), (*E*)-ethyl cinnamate (5.306%-7.143%) and bicyclo germacrene (3.133-5.889%) accounting for more than the 60 percent of total identified compounds. The quantitative and qualitative composition of the essential oils obtained from pallens are given in Fig. 3 and supplementary Table. Whereas, representative GC-FID chromatogram of four stages was reported in Fig. 4. As shown, significant differences and two-way interactions were detected in the qualitative and quantitative composition of the oils obtained from the various stages. In the present study, davanone content was low in vegetative stage (39.219%) as compared to flower head emergence stage (45.202%) and full blooming stage (45.893%). However, decreasing trend was observed once the crop started to form seed (42.53%). (*E*)-Ethyl cinnamate concentration was high in full blooming stage (7.143%) followed by seed maturation (5.632%), vegetative stage (5.369%) and flower initiation stage (5.306%) respectively. Furthermore, bicyclo germacrene content was high in vegetative stage (5.889%) and content was decreased gradually from flower heads emergence (4.679%) to the

**Table 1**

Analysis of variance for different stages of harvest in davana during 2018.

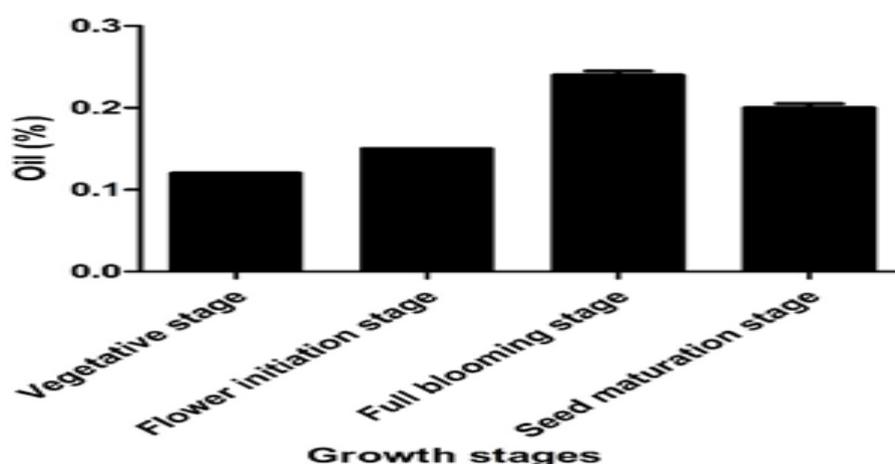
S.V	DF	MSS		
		Ethyl Cinnamate (%)	Bicyclo Germacrene (%)	Davanone (%)
Replication	5	0.140	2.22W9	54.586
Treatments	3	4.4918*	7.6440*	55.1099*
Error	15	0.903	1.453	14.404
Total	23	1.205	2.429	28.449
CD (5%)		0.499	0.633	1.993

SV: Sources of Variation, DF: Degrees of Freedom, MSS: Mean Sum of square; Significance level: "\*\*\*" =  $p \leq 0.01$ , "\*\*" =  $p \leq 0.05$ .

**Table 2**

Factorial analysis of variance for different stages of harvest in davana during 2018.

S.V	DF	MSS	CD at 5%
Replication	5	17.055	
Treatments	11	2121.3961**	
1. Chemical	2	11566.8102**	0.338
2. Stage	3	13.584	0.451
3. Chemical × Stages	6	26.8308**	1.353
Error	55	8.198	

Significance level: "\*\*\*" =  $p \leq 0.01$ , "\*\*" =  $p \leq 0.05$ ." CD: Critical difference.**Fig. 2.** Oil content distilled at different stages of plant growth expressed in percentage.

full blooming stage (3.133%). However, its content increased in seed maturation stage.

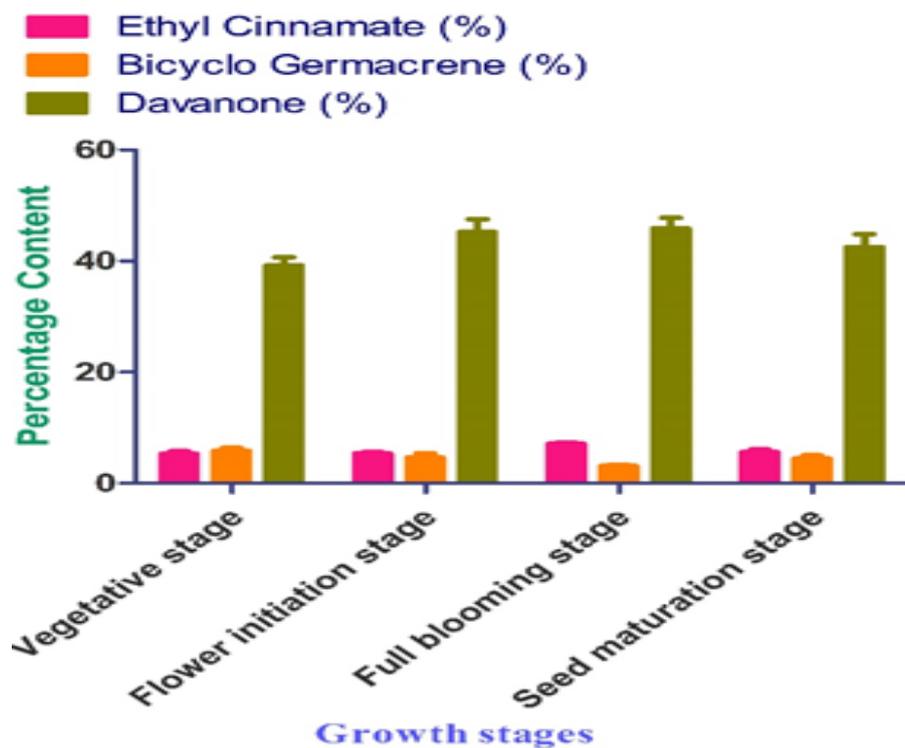
The chemical composition varied due to many factors such as growth stages, genetic, external factors like climatic conditions and extraction methods as well as the analytical conditions (Kim and Lee, 2004; Anwar, 2009; Aliniaiefard et al., 2010). Our findings suggest that full blooming stage is ideal for higher recovery of chemical constituents. The enzymes are necessary to activate the biosynthesis of essential oil components however, these enzymes are not active during the vegetative stage. Thus, the harvest stage is one of the most important factors affecting essential oil quality (Rodrigues et al 2013). Our findings are in line with previous studies of davana (Mallavarapu et al., 1999) and other aromatic crops like in peppermint (Rohloff et al. 2005), in *Satureja rechingeri* (Sefidkon et al. 2007) and in *Origanum monites* (Kizil et al. 2008). Golparvar et

al. (2011) reported that 50% blooming stage of *Thymus vulgaris* L. is the best harvesting times for obtaining the highest essential oil and thymol content.

On the basis of the present results, harvesting of davana crop at full blooming stage for distillation yielded more essential oil and also provides an insight into the chemical composition.

### 3.4. Correlation analysis

Phenotypic correlation was calculated between major chemical constituents. They had a significant correlation coefficient among major chemical constituents (Table 3). Davanone and ethyl cinnamate contents showed positive correlation to oil yield, and bicyclo germacrene had a highly significant negative correlation to the essential oil content. Bicyclo germacrene had significant negative correlation with davanone and ethyl cinnamate.



**Fig. 3.** The composition of davana oil distilled at different stages of plant growth.

The strong positive correlation between oil content and davanone and ethyl cinnamate predicts an increase in oil content may lead to increase of davanone and ethyl cinnamate. The presence of positive correlation values suggest that both variables benefit from each other and they are influenced by the same set of environmental variation. In turn, negative values indicate that the environment favours one variable over the other (Cruz 2005).

#### 4. Concluding remarks

In conclusion, essential oil content and composition of *Davana* (*Artemisia pallens* Bess) has been varied in different stages of plant growth. Overall, research results indicated that higher content of essential oil was obtained in crop harvested at full blooming stage and also ideal for keeping up the quality essential oil. For all four developmental stages (vegetative stage, early flowering stage, full blooming stage and seed set stage) davanone, (*E*)-ethyl cinnamate and bicyclo germacrene constituted the major part of the essential oil.

#### Conflict of interest

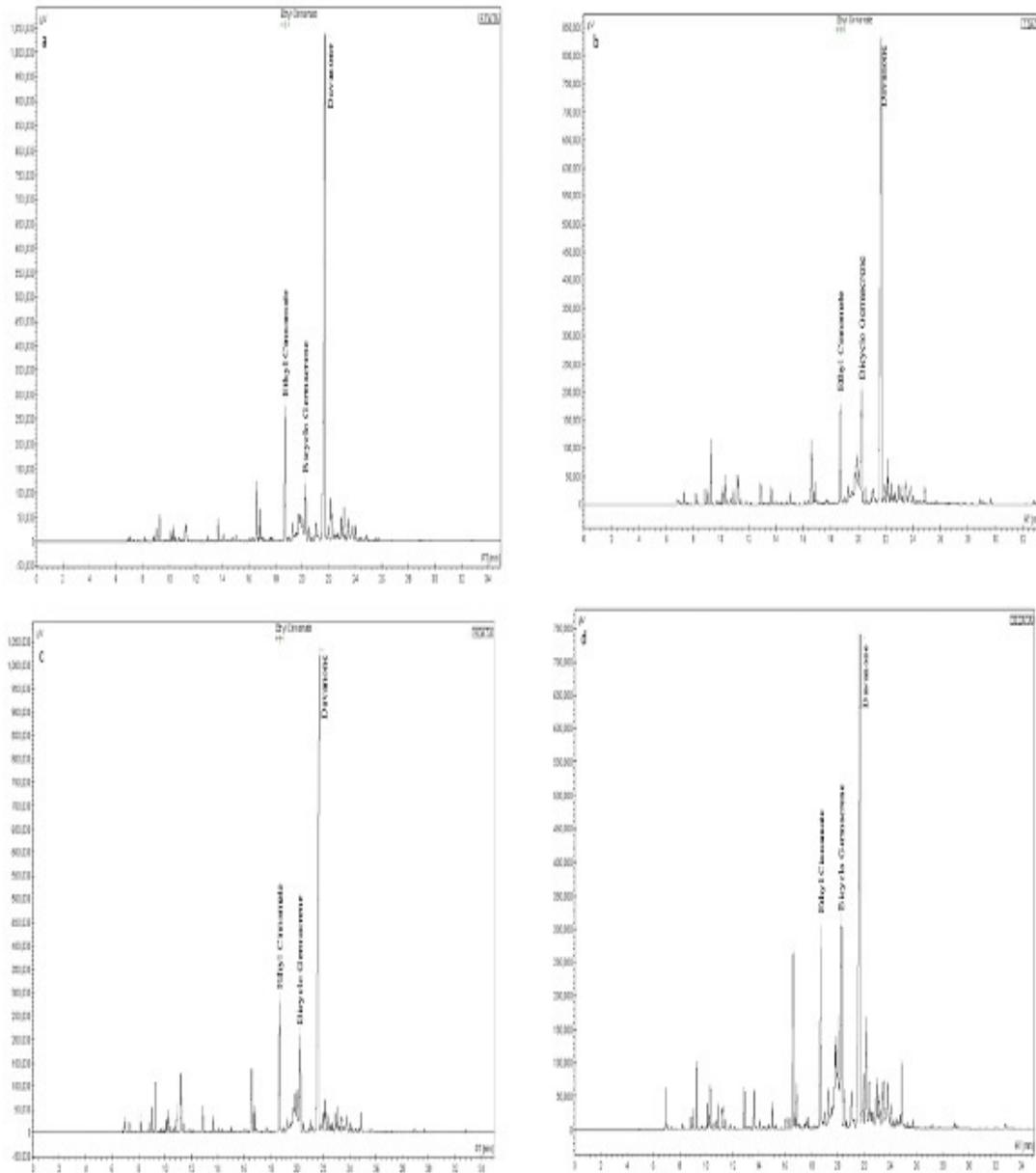
The authors declare that there is no conflict of interest.

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**Fig. 4.** GC profile of davana oil obtained from plants harvested at different stages of crop growth (a: Flower head initiation stage, b: Full blooming, c: seed setting stage, d: Vegetative stage).

**Table 3**  
Phenotypic correlation coefficient among major chemical components and oil content

Traits	Ethyl cinnamate (%)	Bicyclo germacrene (%)	Davanone (%)	Oil (%)
Ethyl cinnamate (%)	1			
Bicyclo germacrene (%)	-0.890**	1		
Davanone (%)	0.614**	-0.862**	1	
Oil (%)	0.873**	-0.948**	0.677**	1

Significance level: \*\*p<0.01, \*\*\*p<0.005.

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