Comparison of chemical composition of *Aerva javanica* seed essential oils obtained by different extraction methods

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Abstract: *Aerva javanica* (Burm.f.) Juss. ex Schult. seed essential oils were obtained by hydrodistillation (HD) and dry steam distillation (SD) extracting methods and analyzed by using gas chromatography-mass spectrometry(GC-MS). Twenty and eighteen components representing 90.5% and 95.6% of the seed essential oil were identified, using hydrodistillation and dry steam distillation, respectively. The major constituent identified from seed essential oil obtained by HD were heptacosane (25.4%), 3-allyl-6-methoxyphenol (14.1%), pentacosane (12.1%), 6,10,14-trimethyl-2-pentadecanone (7.9%), nonacosane (7.1%), tricosane (3.6%), α-farnesene (3.5%), dodecanal (2.7%) and octacosane (2.1%). Whereas the major constituent identified from seed essential oil obtained by SD were heptacosane (41.4%), pentacosane (21.2%), nonacosane (14.8%), tricosane (6.3%), octacosane (4.2%) and tetracosane (3.0%).

Keywords: *Aerva javanica*, Amaranthaceae, chemical composition, GC-MS.

INTRODUCTION

Genus *Aerva* belonging to the family Amaranthaceae, distributed in warm parts of Africa and Asia. *Aerva* genus is represented by 20 species in Pakistan and India (Sharif *et al*., 2011). Various species of *Aerva* possess analgesic, anti microbial, anti-inflammatory activities and used as a valuable medicine for sore throat, cough, wounds and indigestion (Imran *et al*., 2009).

Aerva javanica (Burm.f.) Juss. ex Schult. locally called anti-Booh is a herb and widely distributed in various parts of the world. In traditional, herb is used as diabetic, diuretic, demulcent and kidney stones (Qureshi and Bhatti, 2009). Powder of *A. javanica* is applied externally to ulcers in domestic animals. Seeds are used to relieve headache (Reddy and Reddy, 2009).

Flowers and roots of *A. javanica* possess medicinal properties against kidney problems and rheumatism. Leaves of *A. javanica* are used externally to heal the wounds and inflammation of joints, leaves also used for fodder to goats. Decoction of plant is used as a gargle for toothache (Qureshi and Bhatti, 2009) and to remove swelling. *Aerva javanica* shows antihyperglycaemic (Reddy and Reddy, 2009), cytogenetical (Soliman, 2006), cytotoxic (Al-Fatimi *et al*., 2007) and antiplasmodial activities. Carbohydrates, steroids, triterpenoids and flavonoids has been reported earlier from *A. javanica* (Ahmed-el *et al*., 2010).

Our previous work on the essential oil of *Aerva javanica* leaves and stems show that leaves contain hentriacontane (21.48%), nonacosane (20.59%), heptacosane (19.78%), pentacosane (5.58%), octacosane (3.47%), triacontane (2. 81%) and hexacosane (2.04%). Whereas the essential oil of stems contain nonacosane (23.26%), heptacosane (22.48%), hentriacontane (18.32%), octacosane (3.42%), triacontane (2.24%) and squalene (2.07%) (Samejo *et al*., 2012).

Present work reports the comparison of chemical composition of essential oil of *Aerva javanica* seed evaluated by hydrodistillation and dry steam distillation for first time and the comparison of the components of the steam distilled oil with the hydrodistilled methods.

MATERIALS AND METHODS

Plant material

The seed of the *Aerva javanica* plant was collected from Village Mehendri-Jo-Par (longitude: N 25˚34' 2'' and latitude: E 70˚ 11' 20''), District Umerkot, Sindh Province of Pakistan. A voucher specimen (15174) of plant was deposited at the herbarium of Institute of Plant Sciences, University of Sindh Jamshoro (Pakistan). The plant was identified by a Taxonomist of the same institution. The plant material was air dried under normal conditions and ventilated place until reaching a constant weight, then crushed to obtain a homogeneous powder to increase the efficiency of its essential oils.

Isolation of the essential oils

Hydrodistillation procedure

100 g of powdered seed samples was subjected to

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hydrodistillation (Zahid *et al*., 2010, Hattab *et al*., 2007) for 3h. The oils were separated from water by using n-hexane (HPLC grade) that was dried over analytical reagent grade anhydrous sodium sulfate and then stored in a refrigerator prior to analysis. The yield (w/w) was 0.1% of a pale yellow essential oil with a characteristic odor.

Dry steam distillation

SD is the vertical steam distillation unit in which the plant material was taken in biomass flask (ground glass joints on bottom and top) and allow the steam (at atmospheric pressure) to contact the biomass. 100 g of powdered seed samples was subjected to dry steam distillation (Kokoska *et al*., 2008) for 3 h. The oils were separated from water by using n-hexane (HPLC grade) that was dried over analytical reagent grade anhydrous sodium sulfate and then stored in a refrigerator prior to analysis. The yield (w/w) was 0.11% of a pale yellow essential oil with a characteristic odor.

Gas chromatography-mass spectrometry

Hydro- and dry steam distilled oils of *Aerva javanica* seeds were perform on an Agilent 6890 N GC coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent auto sampler 7683-B injector. HP-5MS column was used for the analysis with dimensions of 30 m ×0.25 mm i.d., film thickness 0.25 µm. The temperature of an oven was kept at 90°C for 2 min, then programmed from 90°C to 190°C at a rate of 4°C/min, kept for 1 min and then increased up to 280°C at 10°C/min (4 min hold). Helium gas was used as a carrier gas (1.5 mL/min, split ratio, 1:20). 1.0 µL sample was injected; using split mode For MS detection, electron ionization (EI) mode with 70 eV (ionization energy) was used. Injector and MS transfer line temperatures were set at 220 and 290°C, respectively (Samejo *et al*., 2012, (Samejo *et al*., 2012a, (Samejo *et al*., 2012b).

Identification of components

The unknown compounds were identified by comparison of their RI (retention indices) with those reported in literature and National NIST mass spectral library with a resemblance percentage above 90%. RI of components was calculated by Kovats method using normal alkanes (C_6-C_{33}). The Percentage composition of the volatile oils was computed from peak areas of Gas Chromatography (GC).

RESULTS

Aerva javanica seed essential oil was obtained by HD and SD. The yield of essential oil extracted by SD technique was higher than HD technique. Altogether, thirty eight substances were identified by GC-MS using HP-5MS column. The identities of the extracted essential oils from both methods are listed in table1. The chromatograms of the analyzed samples (HD and SD) are depicted in figs. 1(a) and 1(b).

Table 1: Comparative chemical composition of *Aerva javanica* oil obtained by hydrodistillation and dry steam distillation

S. No.	Compounds	RI [a]	HD (%[b])	SD (%[b])
1	3-Allyl-6-methoxyphenol	1362	14.1	-
2	Dodecanal	1412	2.7	-
3	*(E)*-6,10-Dimethyl-5,9-undecadien-2-one	1454	1.3	-
4	*trans*-β-Ionone	1486	1.2	-
5	*α*-Farnesene	1507	3.5	0.3
6	*β*-Panasinsene	1519	1.5	-
7	Tetradecanal	1614	1.8	0.2
8	Heptadecane	1700	-	0.1
9	Hexadecanal	1811	1.5	-
10	6,10,14-Trimethyl-2-pentadecanone	1842	7.9	1.0
11	Nonadecane	1900	0.7	0.2
12	Hexadecanoic acid	1964	-	0.2
13	Eicosane	2000	0.3	0.1
14	Octadecanal	2032	0.9	0.6
15	Heneicosane	2100	-	0.7
16	Docosane	2200	0.4	0.4
17	Tricosane	2300	3.6	6.3
18	Tetracosane	2400	1.5	3.0
19	Pentacosane	2500	12.1	21.2
20	Heptacosane	2700	25.4	41.4
21	Octacosane	2800	2.1	4.2
22	Squalene	2847	0.9	0.4
23	Nonacosane	2900	7.1	14.8
24	Triacontane	3300	-	0.5
	Oil Yield		90.5%	95.6%

[a]**RI**: Retention indices relative to *n*-alkanes (C_6-C_{33}) on HP-5MS,[b]%: Calculated from peak areas of gas chromatography.

Components were detected in the hydrodistilled seed oil of *Aerva javanica* representing 90.5% of the total oil. Major constituents were heptacosane (25.4%), 3-allyl-6-methoxyphenol (14.1%), pentacosane (12.1%), 6,10,14-trimethyl-2-pentadecanone (7.9%), nonacosane (7.1%), tricosane (3.6%), α-farnesene (3.5%), dodecanal (2.7%) and octacosane (2.1%). Whereas the dry steam distilled oil of the plant contained eighteen compounds (95.6%) with heptacosane (41.4%), pentacosane (21.2%), nonacosane (14.8%), tricosane (6.3%), octacosane (4.2%) and tetracosane (3.0%) as the main constituents.

Chemical class allocation of essential oil constituents are summarized in table 2. The constituents were divided into four classes, which were terpenoids (oxygenated monoterpenes, triterpene hydrocarbons, and sesquiterpenes hydro carbon), phenolic compounds, hydrocarbons and others.

DISCUSSION

Phenolic compounds (3-allyl-6-methoxyphenol) and terpenoids show the antimicrobial activity and mostly sesqui and monoterpenes are active against fungi and bacteria (Vijayakumar *et al*., 2012).

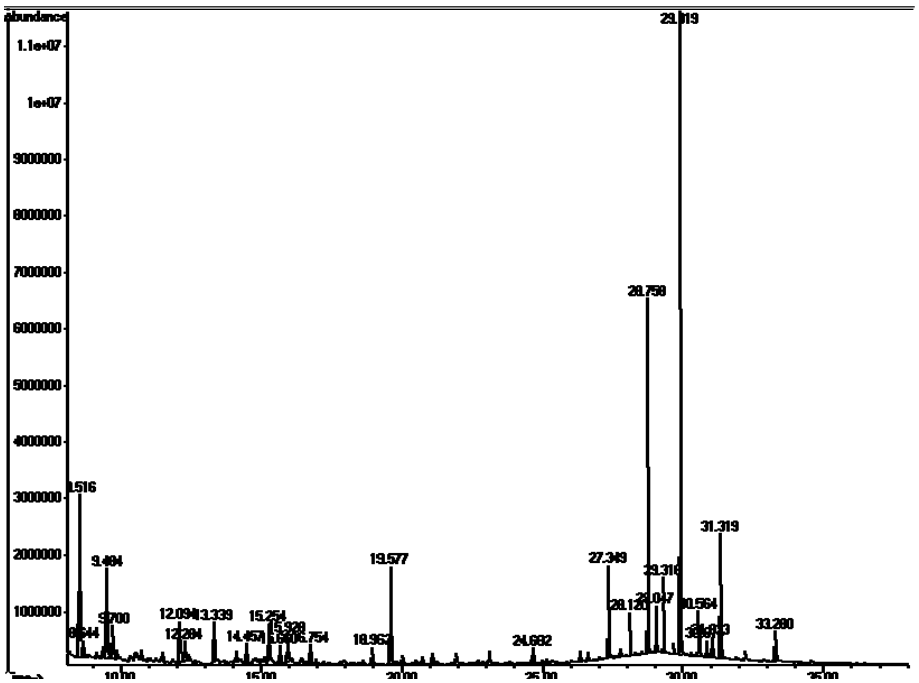

Fig. 1(a): Gas Chromatogram of the seed essential oil of *Aerva javanica* obtained by HD

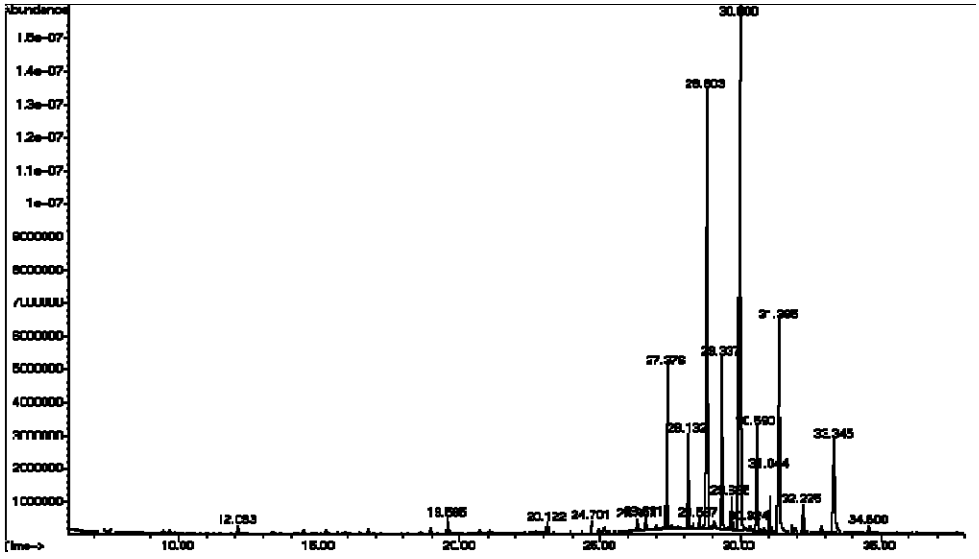

Fig.1 (b): Gas Chromatogram of the seed essential oil of *Aerva javanica* obtained by SD

The techniques for the extraction of volatile oils have also been reported to cause variations in the volatile compounds of samples (Hattab *et al*., 2007). According to our results there were some similarities and differences in chemical composition of the seeds essential oil of *Aerva javanica* obtained by HD and SD methods. A farnesene, tetradecanal, 6,10,14-trimethyl-2-pentadecanone, nonadecane, octadecanal, tetracosane, nonacosane, eicosane, tricosane, docosane, heptacosane, octacosane, pentacosane and squalene were common constituents of essential oils using HD as well as SD extraction methods. Some of the essential oil constituents, 3-allyl-6-methoxyphenol, dodecanal, *(E)*-6,10-dimethyl-5,9-undecadien-2-one, *trans-β*-ionone, *β*-panasinsene and hexadecanal have been obtained only with the HD method. On the contrary, heptadecane, hexadecanoic acid, heneicosane and triacontane were only extracted by SD method. Sesquiterpenes hydrocarbons and triterpenes hydrocar-bons were higher in hydrodistilled oil than dry steam distilled oil, where as oxygenated monoterpenes were absent in dry steam distilled oil.

Table 2: The chemical class distribution in the essential oils of *Aerva javanica*

Compounds	HD		SD	
	(%)	NC*	(%)	NC*
Terpenoids				
Oxygenated monoterpenes	2.5	2	-	-
Triterpenes hydrocarbon	0.9	1	0.4	1
Sesquiterpenes hydrocarbon	5.0	2	0.3	1
Phenolic compounds	14.1	1	-	-
Hydrocarbons	53.2	09	92.9	12
Others	14.8	5	2.0	4
Total	90.5	20	95.6	18

*=Number of compounds

Extracts obtained by SD revealed quantitative differences in their composition when compared with HD. For example, SD extract contains higher amounts of hydrocarbons (92.9 vs. 53.2%), and lower amounts of sesquiterpenes hydrocarbon (0.3 vs. 5.0%), triterpenes hydrocarbon (0.4 vs. 0.9%), oxygenated monoterpenes (00 vs. 2.5%) and phenolic compounds (00 vs. 14.1%). It can be concluded that SD is good method for extracting hydrocarbons from plants.

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