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Chemical Composition of the Essential Oil of *Withania coagulans*

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The present research reports the chemical composition of the essential oil of *Withania coagulans* extracted by steam distillation and analyzed by GC-MS. The chromatographic analysis of this essential oil showed 20 compounds, representing 90.80 % of the total oil constituents. The oil contains a complex mixture consisting of sesquiterpenes, esters, acids, alkanes and aldehyde. It was dominated by sesquiterpenes (54 %) and esters (21.50 %) while alkanes, acids and aldehydes were only present in small percentage (9.11, 5.5 and 0.32 %, respectively). Thus carophyllene is present with highest percentage and allene is present with lowest percentage.

Key Words: *Withania coagulans*, Essential oils, Solanaceae, Carophyllene.

INTRODUCTION

Essential oils are volatile and fragrant substances of plants. They are obtained from plants through steam distillation or other processes[1]. They may be present in particular secretory parts. These oils generally contain volatile substances, which are terpenes and their oxygenated derivatives usually known as camphors[2,3]. Different methods of extraction of essential oils from plants are adopted according to the nature of material of plant[4]. The Solanaceae are herbs, shrubs, or trees comprising about 85 genera and 2,800 species. There are approximately 27 species, subspecies, varieties, forms and cultivars in *Withania* genus. *Withania coagulans* is a small shrub of the genus Solanaceae, common in the Punjab, Sindh, Afghanistan and Baluchistan. The round capsular fruit is used in the fresh state as an emetic and when dried it is used as a stomachic. It has the peculiar property of coagulating or curdling milk. Its extract possessed hypoglycemic activity in diabetic rats, maintaining glucose level and quick treatment for dyspepsia[5]. The essential oil composition of *Withania coagulans* had not been reported before. The present data revealed for the first time the characteristic composition of the essential oil of this species. The compounds which were determined in this study could be used as important descriptors to characterize the essential oil of *Withania coagulans* fruit and consequently this species itself.

EXPERIMENTAL

The solvent (dichloromethane) used in the experiment was of analytical grade purchased from Merck.

Collection of plant material: The fruits of *Withania coagulans* were collected from Sahiwal district of Pakistan. The plant specimen was identified by Mir Ajab Khan, Department of Botany, Quaid-e-Azam, University, Islamabad, Pakistan. The voucher specimens of the plants used in the present study was kept for record in the Prem Madam Herbarium (Accession No: P.M. 0004) of Lahore College for Women University Lahore Pakistan.

Preparation of material: The fruits of *Withania coagulans* (1500 g) were dried away from the sunlight and grinded to yield 1200 g of powdered sample (80 %).

Essential oil extraction: Extraction of oil from the fruit of *Withania coagulans* was done by steam distillation method.

Steam distillation: 1200 g of powdered *Withania coagulans* fruits were taken in 5 L reaction vessel and attached to a steam generator. A water cool condenser was also attached with reaction vessel. Steam generator produced the steam which passed through the sample condensed and collected with essential oils. The oil was separated by separating funnel. Then added the anhydrous magnesium sulphate and stored at 4 ºC before use. The yield based on fresh weight of the sample was calculated 0.35 g (90.80 %). The obtained essential oil was taken in very small amount about 0.2 µL dissolved in dichloromethane and used for GC-MS analysis.

Analysis of essential oil by gas chromatography and mass spectroscopy: The volatile constituent's analysis was achieved on a Shimadzu GC-MS-QP 2010 with data system Lab Soln. and Company Shimadzu. From the chromatogram various components were identified by comparing the peaks and retention time with standard values.

The DB-5 column of version 2.2 was indirectly coupled to the mass spectrometer. The DB-5 column was 3 cm in length, 0.25 mm id and 0.5 µm in thickness (Agilent Technologies, J and W Scientific Products, Folsom, CA, USA). Carrier gas was helium (BOC) with a flow rate of 1 mL/min and pressure of 122 KPa. Scanning rate was 2 S/decade. Split was 1/10. Injector was split/splitless. GC oven temperature programming was 50 ºC hold for 1 min, raised at 5 ºC/min and hold for 5 min. Injection temperature was 250 ºC. Detector temperature was 280 ºC. Mass spectra was 1.5-1024. Mass range was from 40-300 amu at 1 scan/s. Ion source temperature was 200 ºC. Ion source was EI. The mass spectrometer was operating in the EI-mode at 70 eV.

Component identification: The compounds of the essential oil were identified by comparison of their mass fragmentation pattern and retention time with those of MS library[6]. Spectral Data was given in Table-1.

TABLE-1
COMPOUNDS OF *Withinia coagulans* IDENTIFIED BY GC-MS

S. No.	Name of compound	R:T	m.f.	m.w.	%	m/z/
1	Cyclohexane	2:300	C_6H_{12}	84	0.21	84 (M^+ C_6H_{12}), 83 (M^+ C_6H_{11}), 56 (M^+ C_4H_9), 55, 41
2	Borane carbonyl	2:310	CH_3BO	42	0.22	42 (M^+ CH_3BO), 40 (M^+ CHBO)
3	3-Methyl, hexane	2:350	C_7H_{16}	100	3.20	100 (M^+ C_7H_{16}), 85 (M^+ C_6H_{13}), 71, 57, 43, 41.
4	Heptane	2:550	C_7H_{16}	100	1.20	100 (M^+ C_7H_{16}), 85 (M^+ C_6H_{13}), 71 (M^+ C_5H_{11}), 57, 43, 41
5	Hexanoic acid	2:700	$C_6H_{12}O_2$	116	2.00	116 (M^+ $C_6H_{12}O2$), 99 (M^+ $C_6H_{11}O$), 87 (M^+ $C_5H_{11}O$), 73 (M^+ C_4H_9O), 60 (M^+ C_3H_8O), 40
6	Nonanoic acid	2:710	$C_9H_{18}O_2$	158	3.50	158 (M^+ $C_9H_{18}O_2$), 129 (M^+ $C_8H_{17}O$), 115 (M^+ $C_7H_{15}O$), 98 (M^+ $C_6H_{10}O$), 87, 73, 60, 43, 41
7	Longifolene	12:15	$C_{15}H_{24}$	204	12.0	204 (M^+ $C_{15}H_{24}$), 189 (M^+ $C_{14}H_{21}$), 161 (M^+ $C_{11}H_{14}$), 147, 135, 119, 107, 94, 91, 79, 67, 55, 41
8	Allene	13:32	C_3H_4	40	0.20	42 (M^+ C_3H_4), 40 (M^+ C_3H_2)
9	δ-Cadinene	14:51	$C_{15}H_{24}$	204	11.7	204 (M^+ $C_{15}H_{24}$) 189 (M^+ $C_{14}H_{21}$), 161 (M^+ $C_{11}H_{14}$) 134, 119, 105, 91, 81, 69, 55, 41
10	Caryophyllene	21:20	$C_{15}H_{24}$	204	15.0	204 (M^+ $C_{15}H_{24}$), 189 (M^+ $C_{14}H_{21}$), 161 (M^+ $C_{11}H_{14}$), 148, 133, 120, 107, 93
11	4-*tert*-Butyltoluene	21:25	$C_{11}H_{16}$	148	0.47	148 (M^+ $C_{11}H_{16}$), 133 (M^+ $C_{10}H_{13}$), 115 (M^+ C_9H_7), 105, 91, 77, 65, 51, 41
12	3-Carene	22:17	$C_{10}H_{16}$	136	11.3	136 (M^+ $C_{10}H_{16}$), 121 (M^+ $C_{10}H_{13}$), 105 (M^+ $C_{10}H_9$), 93, 79, 67, 43, 41
13	Methyl ester of hexadeca-noic acid,	26:21	$C_{17}H_{34}O_2$	270	2.50	270 (M^+, $C_{17}H_{34}O_2$), 239 (M+31), 227 (M+43), 213, 199, 185, 171, 157, 143, 129, 115, 101, 87, 74, 69, 41
14	Methyl ester of nonadeca-noic acid	26:22	$C_{20}H_{40}O_2$	312	2.00	270 (M^+, $C_{17}H_{34}O_2$), 239 (M+31), 227 (M+43), 213, 199, 185, 171, 157, 143, 129, 115, 101, 87, 74, 69, 41
15	Ar-Tumerone	27:37	$C_{15}H_{20}O$	216	3.00	216 (M^+ $C_{15}H_{20}O$), 201 (M^+ $C_{15}H_{17}O$), 183 (M^+ $C_{15}H_{15}$), 173 (M^+ $C_{15}H_5$), 159, 145, 132, 119, 105, 91, 83, 65, 55, 39.
16	Methyl ester 8,11-octadeca-dienoic acid	30:36	$C_{19}H_{34}O_2$	294	7.00	294 (M^+ $C_{19}H_{34}O_2$), 263 (M^+ $C_{18}H_{31}O$), 220 (M^+ $C_{14}H_{27}O$), 164 (M^+ $C_{11}H_{23}O$), 150, 135, 123, 109, 95, 81, 67, 55, 41
17	Ethyl ester of linoleic acid	30:37	$C_{20}H_{36}O_2$	308	8.00	308 (M^+ $C_{20}H_{36}O_2$), 263 (M^+ $C_{18}H_{31}O$), 178 (M^+ $C_{13}H_{22}O$), 164 (M^+ $C_4H_{20}O$), 150 (M^+ $C_3H_{18}O$), 136 (M^+ $C_2H_{16}O$), 123, 109, 95, 81, 67, 55, 41
18	Methyl ester 9-octadece-noic acid (Z)	30:55	$C_{19}H_{36}O_2$	296	3.00	296 (M^+ $C_{19}H_{36}O_2$), 264 (M^+ $C_{18}H_{32}O$), 222 (M^+ $C_{15}H_{26}O$), 180, 166, 137, 123, 97, 74, 69, 55, 41
19	Acetaldehyde	31:37	C_2H_4O	44	0.32	44 (M^+ C_2H_4O)
20	5-Dimethyl 2-Undecene-2	46:55	$C_{13}H_{26}$	182	4.50	182 (M^+ $C_{13}H_{26}$), 154 (M_+ $C_{12}H_{22}$), 140 (M^+ $C_{11}H_{20}$), 126, 111, 97, 70, 57, 43, 41

RESULTS AND DISCUSSION

Identification of compounds by GC-MS analysis

General procedure: The essential oil isolated by steam distillation from the fruits of *Withania coagulans* had a light yellow colour and a pungent odour at room temperature. The weight of the oil obtained from fruits of *Withania coagulans* was 0.35 g (90.80 %). The GC/MS proved to be a reasonably suitable method to identify a number of volatile substances from fruits of *Withania coagulans*. For the separation of volatile compounds capillary column DB-5 was selected which was more suitable for esters and less polar substances. Helium was used as carrier gas. All the fractions were investigated by GC/MS analytically. For the identification of known compounds a computer version of the extended EPA/NIH Mass spectral data base with 174948 EI mass spectra was available[6].

Fatty acid esters: In fatty acid esters the characteristic fragment was $R\text{-}C{=}O^+$ which is formed due to cleavage. A series of $[(CH_2)_nCOOR]^+$ ions formed through Mclafferty rearrangement were found in greater abundance. Methyl ester of fatty acids gave most abundant peak at m/z 74 due to Mclafferty's rearrangement. The essential oil of *Withania coagulans* when analyzed by GC/ MS under a temperature programme (100-280 at 4 ºC/min.) gave a chromatogram which showed 5 molecular ion peaks corresponding to methyl esters and ethyl esters, 8,11-octadecanoic acid, methyl ester, hexadecanoic acid, methyl ester, 9-octadecanoic acid, methyl ester, nonadecanoic acid, methyl ester and linoleic acid, ethyl ester.

Alkanes: The essential oil of *Withania coagulans* when analyzed by GC/MS under a temperature programme (100-280 ºC at 4 ºC/min) gave a chromatogram which showed three ion peaks corresponding to alkanes such as cyclohexane, heptane and 3-methyl hexane. Alkanes gave most abundant peak at m/z 43 due to Mclafferty's rearrangement.

Terpenes: GC/MS analysis of essential oil of *Withania coagulans* gave a chromatogram which showed 5 ion peaks corresponding to terpenes such as longifolene, δ-cadinene, caryophyllene, 3-carene and ar-tumerone. The molecular ion peak at m/z 204 corresponded to molecular formula $C_{15}H_{24}$ characteristic of sesquiterpenes such as longifolene, δ-cadinene and caryophyllene.

Acids: GC/MS analysis of essential oil of *Withania coagulans* gave a chromatogram which showed two ion peaks corresponding to acids such as hexanoic acid and nonanoic acid. Acids gave most abundant peak at m/z 60 due to Mclafferty's rearrangement.

Conclusion

The chromatographic analysis of this essential oil showed 20 compounds, representing 90.80 % of the total oil constituents. The oil contained a complex mixture consisting of sesquiterpenes, esters, acids, alkanes and aldehyde. It was dominated by sesquiterpenes (54 %) and esters (21.50 %) while alkanes, acids and aldehydes were only present in small percentage 9.11, 5.5 and 0.32 %, respectively. The major

components in the oil detected were caryophyllene (15 %), longifolene (12 %), δ-cadinene (11.7 %), 3-carene (12 %), linoleic acid, ethyl ester (8.0 %), 8,11-octadecadienoic acid, methyl ester (7.0 %), 2-undecene-2,5-dimethyl (4.5 %), 9-octadecenoic acid (Z), methyl ester (3.0 %), nonanoic acid (3.5 %). These compounds were reported for the first time in the essential oil of *Withania coagulans* fruits, hence it could be used as an important descriptor to characterize the essential oil of *Withania coagulans* fruits and consequently this species itself. Since the essential oil composition of the studied plant has not been reported before, the present data revealed for the first time the characteristic composition of the essential oil of this species.

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