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Short communication

Analysis of fatty acid composition of *Withania coagulans* fruits by gas chromatography/mass spectrometry

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Abstract

Background and objectives: *Withania coagulans* Dunal (Solanaceae) fruits are recommended to treat asthma, dyspepsia, biliousness, flatulent colic, liver complaints, intestinal infections, strangury, wounds and as diuretic, emetic and sedative agent in Indian traditional system of medicine. The objective of this study was to describe the systematic fatty acid composition of the petroleum ether extract of *W. coagulans* fruits. **Methods:** Petroleum ether extract of *W. coagulans* fruits was prepared by maceration. Components of the *W. coagulans* petroleum ether extract were identified by gas chromatography-mass spectrometry and their concentrations were determined. **Results:** The fixed oil of *W. coagulans* fruits constituted of twenty nine components including unsaturated (52.36%) and saturated (22.15%) fatty acids, alkenes (5.65%), phytosterols (4.39%), fatty alcohols (4.14%), aromatic acid (3.56%), monoterpenes (3.22%), triterpenoids (1.83%) and alkanes (2.7%). Most of the saturated and unsaturated fatty acids were identified as their methyl esters. **Conclusions:** Palmitoleic and 11-eicosenoic acids have been reported for the first time in petroleum ether extract of the *W. coagulans* fruits. The present study has illustrated the chemical nature of *W. coagulans* fruit and described its fatty acids composition.

Keywords: fatty acid, fruits, GC/MS, *Withania coagulans*

Introduction

Withania coagulans Dunal belonging to family Solanaceae, is commonly known as vegetable rennet or Indian cheese maker. It is a small genus of shrub distributed in Pakistan [1], Afghanistan, Iran and northern India including Panjab, Simla, Garhwal and Kumaun [2-3]. Fruits of *W. coagulans* are red or brownish, smooth, globose, enclosed in leathery calyx; pulp is brown having nauseous fruity odour and is used as a coagulant [4]. Pulp and husk of fruits contain an enzyme called withanin, responsible for milk coagulating properties [5-6]. Fruits possess diuretic, emetic and sedative action and are used to treat dyspepsia, liver complaints, flatulent colic, intestinal infections, strangury and wounds [3]. *Withania coagulans* fruit extracts are reported to

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possess anti-inflammatory, anthelmintic, antimicrobial, antifungal, cardiovascular, free radical scavenging, hypoglycaemic, hepatoprotective, hypolipidemic, central nervous system depressant, immunosuppressive and anti-tumour properties [7].

Phytochemically, the fruits contain free amino acids, esterases, alkaloids [4], fatty acids and essential oil (sesquiterpenes) [8-9]. *n*-Nonacosanyl linolenate, *n*-octacosanyl linolenate, *n*-heptacosanyl linolenate, *n*-octatriacont-17-enoic acid, *n*-dotriacont-21-enoic acid, *n*-tetratriacontanoic acid, withacoagulinyl tetraglucoside, capryloyl hexaglucoside, menthyl tetraglucoside, geranilan-10-olyl dihydrocinnamoate and geranilanolyl salicylic glycoside [10-11], coagulanolide [12], 3β-hydroxy-2,3-dihydrowithanolide H, β-sitosterol-*D*-glucoside [13] and 3β-hydroxy-2,3-dihydrowithanolide F [14-16] have been reported from the fruit berries. Owing to the traditional uses of *W. coagulans*, the chemical composition of the petroleum ether extract of the fruits was studied.

Experimental

Plant material

The fresh fruits of *W. coagulans* were purchased from the local market of Delhi, India (2010). The sample was authenticated by Dr. H.B. Singh, Scientist F and Head, Raw Materials, Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India. A voucher specimen of the plant sample was deposited at the Herbarium of NISCAIR with reference number NISCAIR/RHMD/Consult/-10-11/1665/263.

Preparation of extracts

Finely ground powder (100 g) of *W. coagulans* fruits was soaked in petroleum ether (3×250 mL) for eight h and then filtered through a Whatmann filter paper No.1. Sodium sulphate was used to remove traces of moisture in the filtrates. The filtrates were concentrated under reduced pressure using a rotary evaporator at 45 °C to get a yellow color fixed oil. The percentage yield of the petroleum ether extract was 13.48% v/w.

Fatty acid methyl ester (FAME) preparation

A one step extraction-methylation procedure according to Browse *et al.* was applied to the petroleum ether extract with slight modifications [17]. The extract (1 g) was mixed with 3 mL of the methanol H_2SO_4 which was prepared by diluting a 3 M solution of H_2SO_4 to 1 M with methanol. After cooling, 0.3 mL of hexane and 1 mL of 0.9% NaCl were added and the fatty acid methyl esters (FAMEs) were vigorously extracted by shaking. The sample was then centrifuged (1000×g×30 s) and the hexane layer was used for the fatty acids analysis.

GC/MS analysis

The analysis of the fixed oil from the petroleum ether extract was carried out on a GC/MS system (Shimadzu QP-2010) with HP-20M column (50 m×0.32 mm×0.30 µm). Helium was used as the carrier gas with a flow rate of 1.21 mL/min. The temperature of oven was 80 ºC for 1 min and subsequently controlled isothermally for 2 min. Injector port: 270 ºC, detector: 280 ºC, split ratio 1:50, injected volume of the sample: 1 µL. The recording was executed at 70 eV, scan duration 1.5 s; mass range 40-750 amu. Software implemented to handle mass spectra and chromatograph was a Chem station.

Identification of components

All constituents were identified by comparison of their retention indices (RI) either with those of standard compounds available in author's library or with those of literature in close agreement to RI [18-20]. Further identification of components was carried out by comparison of mass spectra and their fragmentation patterns obtained by GC/MS analysis with those stored in the spectrometer database of NBS 54 K.L, WILEY8 libraries and published literature. Retention indices of the components were determined relative to the retention times of a series of *n*-

alkanes relative to C_9-C_{20} on HPS and HP-20M columns.

Results and Discussion

The fixed oil (13.48% v/w) from the petroleum ether extract of the fruits constituted of twenty nine components including alkanes, alkenes, fatty alcohols, aromatic acids, monoterpenes, phytosterols and saturated and unsaturated fatty acids (table 1). Among these, four unsaturated fatty acids (52.36%) constituted maximum percentage of the fixed oil and all were identified. The major unsaturated fatty acid was linoleic acid (42.05%) followed by oleic (6.97%), palmitoleic (2.29%) and 11-eicosenoic (1.05%) acids. Nine saturated fatty acids were determined to constitute 22.15% of the fixed oil. Stearic acid (6.67%) was the dominant saturated fatty acid followed by palmitic (3.45%), docosanoic (3.10%), octanoic (2.32%), arachidic (2.17%), myristic (1.84%) and pentadecanoic (1.20%) acids with minor percentages of lauric (0.95%) and capric (0.45%) acids. Most of the saturated and unsaturated fatty acids were identified as their methyl ester (ME).

Three alkane hydrocarbons (2.7%) included *n*-tetradecane (1.12%), *n*-heneicosane (0.87%) and *n*-tetracontane (0.71%) together with four alkene hydrocarbons (5.65%), *viz.*; 1-nonadecene (1.78%), 1-tetradecene (1.50%), 1-heptadecene (1.47%) and 1-dodecene (0.90%) were characterized. An aromatic dicarboxylic acid, phthalic acid (3.56%) and two monoterpenes as thiogeraniol (2.06%) and linalool (1.16%) were determined in considerable amounts in association with two fatty alcohols *viz.*, 2-(9,12-octadecadienyloxy) ethanol (2.54%) and 1-eicosanol (1.60%). The phytosterols occurring in small amounts were characterized as β-sitosterol (3.74%) and 3β-stigmasta-5,24(28)-dien-3-ol (0.65%), whereas a tetracyclic triterpenoid, 24-methyl-9,19-cyclolanost-25-en-3-ol (0.97%) and a pentacyclic triterpenoid, lupeol (0.86%) were determined positively (table 1). All the identified chemical components in fixed oil with their retention index (RI), molecular formula, molecular weight and relative composition (RC) have been presented in table 1.

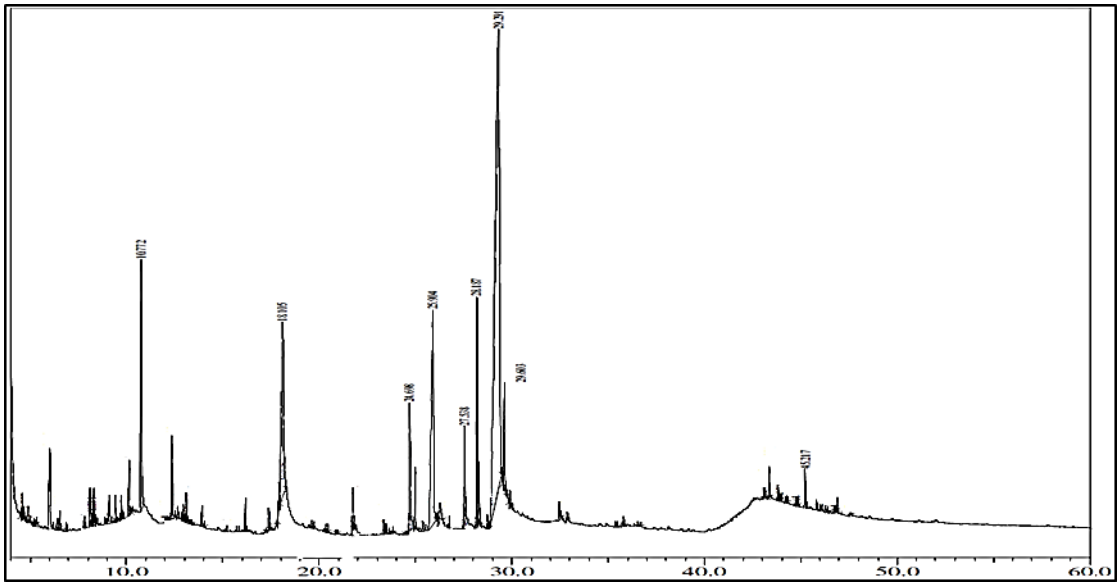

Figure 1. GC spectrum of the petroleum ether extract of the *Withania coagulans* fruits

Table 1. Chemical composition of petroleum ether extract of fruits of *Withania coagulans*.

No.	Components	RI	Molecular formula	Molecular weight	RC (%)
1.	Linalool	1098	$C_{10}H_{18}O$	154	1.16
2.	Octanoic acid	1173	$C_8H_{16}O_2$	144	2.32
3.	1-Dodecene	1219	$C_{12}H_{24}$	168	0.90
4.	Capric acid	1380	$C_{10}H_{20}O_2$	172	0.45
5.	1-Tetradecene	1403	$C_{14}H_{28}$	196	1.50
6.	*n*-Tetradecane	1421	$C_{14}H_{30}$	198	1.12
7.	Lauric acid, ME	1558	$C_{12}H_{24}O_2$	200	0.95
8.	1-Heptadecene	1701	$C_{17}H_{34}$	238	1.47
9.	Myristic acid, ME	1769	$C_{14}H_{28}O_2$	228	1.84
10.	Thiogeraniol	1850	$C_{10}H_{18}S$	170	2.06
11.	Pentadecanoic acid, ME	1854	$C_{15}H_{30}O_2$	242	1.20
12.	1-Nonadecene	1917	$C_{19}H_{38}$	266	1.78
13.	Phthalic acid	1920	$C_{12}H_{14}O_4$	222	3.56
14.	Palmitoleic acid, ME	1976	$C_{16}H_{30}O_2$	254	2.29
15.	Palmitic acid, ME	1978	$C_{17}H_{34}O_2$	270	3.45
16.	Linoleic acid, ME	2083	$C_{19}H_{34}O_2$	294	42.05
17.	*n*-Heneicosane	2109	$C_{21}H_{44}$	296	0.87
18.	Stearic acid, ME	2167	$C_{19}H_{38}O_2$	298	6.67
19.	1-Eicosanol	2288	$C_{20}H_{42}O$	298	1.60
20.	11-Eicosenoic acid, ME	2318	$C_{21}H_{40}O_2$	324	1.05
21.	Ethanol, 2-(9,12-octadecadienyloxy)	2344	$C_{20}H_{38}O_2$	310	2.54
22.	Arachidic acid, ME	2366	$C_{20}H_{40}O_2$	312	2.17
23.	Oleic acid, ME	2453	$C_{19}H_{36}O_2$	296	6.97
24.	Docosanoic acid, ME	2475	$C_{23}H_{46}O_2$	354	3.10
25.	3β-Stigmasta-5,24(28)-dien-3-ol	2780	$C_{29}H_{48}O$	412	0.65
26.	Lupeol	2834	$C_{30}H_{50}O$	426	0.86
27.	24-Methyl-9,19-cyclolanost-25-en-3-ol	2848	$C_{31}H_{52}O$	440	0.97
28.	β-Sitosterol	3408	$C_{29}H_{50}O$	414	3.74
29.	*n*-Tetracontane	4395	$C_{40}H_{82}$	562	0.71

RI: Kovats/retention index; RC: relative composition; ME: methyl ester

Palmitoleic and 11-eicosenoic acids have been reported for the first time in fixed oil of *W. coagulans* fruits. According to Yang *et al.* hyperglycemia and hypertriglyceridemia can be improved by palmitoleic acid through increasing insulin sensitivity [21]. Matthan *et al.* studied the effects of dietary palmitoleic acid on plasma lipoprotein profile and aortic cholesterol accumulation that were similar to unsaturated fatty acids [22].

Withania coagulans fruits are the major sources of polyunsaturated omega-6 fatty acid, linoleic acid (42.05%). Linoleic acid is an essential fatty acid and critical for human survival. Linoleic acid and its metabolites may function as anti-hypertensives, anti-atherosclerotic molecules, nitric oxide enhancers, endogenous angiotensin converting enzyme and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor [23-24]. Horrobin & Ziboh (1997) reported that the high consumptions of linoleic acid protect against the cancer development, possibly through the generation of 13-hydroxyoctadecadienoic acid from linoleic acid as it prevents cell adhesion to endothelial cells and can inhibit cancer metastasis [25]. However, β-sitosterol has been reported previously in high percentage in the seeds [15]. Earlier finding has reported fixed oil (10.45%) from the seeds constituting six components only, four saturated fatty acids including lauric (0.07%), myristic (0.09%), palmitic (17.06%) and stearic (1.10%) acids and two unsaturated fatty acids *viz.*, oleic (19.56%) and linoleic (61.19%) acids [8]. Palmitic, linolelaidic and stearic acids and their respective methyl esters were found in *n*-hexane fraction of *W. coagulans* twigs [26]. An aromatic dicarboxylic acid,

phthalic acid (3.56%) has been detected in petroleum ether extract of the *W. coagulans* fruit (table 1). The phthalic acid esters, di-*n*-butyl phthalate and di-(2-ethylhexyl) phthalate are plasticizers reported to cause developmental and reproductive toxicities. Phthalates have been found to cause various toxicity profiles in the liver, kidneys, thyroid and testes. Moreover, they are presented as hormonally-active agents, as they may interfere with the endocrine system in human [27]. On the other hand, phthalates may decompose to phthalic acid which contaminate the plants and may be identified in their extract.

The present work described the fatty acid composition of petroleum ether extract from *W. coagulans* fruits. The yield of fixed oil (13.48% v/w) and number of components (29) were found to be higher while percentage of linoleic acid (42.05%) was less than earlier report [8]. *Withania coagulans* fruits are the source of a monounsaturated fatty acid, palmitoleic acid that improves hepatic lipid metabolism and possesses anti-hyperglycemic effect. The fruits are rich in linoleic acid (42.05%) and the high consumptions of linoleic acid protect against cancer development and may acts as cardio-protective, anti-atherosclerotic and nitric oxide enhancer. The fruit oil of *W. coagulans* has the potential to be exploited as a source for mono and polyunsaturated fatty acids.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

References

[1] Ali N, Ahmad B, Bashir S, Shah J, Azam S, Ahmad M. Calcium channel blocking activities of *Withania coagulans*. *Afr J Pharm Pharmacol*. 2009; 3(9): 439-442.

[2] Hoda Q, Ahmad S, Akhtar M, Najmi AK, Pillai KK, Ahmad SJ. Antihyperglycaemic and antihyperlipidaemic effect of poly-constituents, in aqueous and chloroform extracts, of *Withania coagulans* Dunal in experimental type 2 diabetes mellitus in rats. *Hum Exp Toxicol*. 2010; 29(8): 653-658.

[3] Kirtikar KR, Basu BD. *Indian medicinal plants*. 2nd ed. Dehradun: International Book Distributors, 1999.

[4] Council of Scientific & Industrial Research, Publications & Information Directorate. *The wealth of India: a dictionary of Indian raw materials and industrial products*, (Sp-W). New Delhi: Council of Scientific & Industrial Research, 2005.

[5] Jaiswal D, Rai PK, Watal G. Antidiabetic effect of *Withania coagulans* in experimental Rats. *Indian J Clin Biochem*. 2009; 24(1): 88-93.

[6] Hemalatha S, Kumar R, Kumar M. *Withania coagulans* Dunal: a review. *Pharmacogn Rev*. 2008; 2(4): 351-358.

[7] Gupta V, Keshari BB. *Withania coagulans* Dunal. (Paneerdoda): a review. *Int J Ayu Herb Med*. 2013; 3(5): 1330-1336.

[8] Sattar A, Ghani MY, Khan SA. Fatty acids of indigenous resources for possible industrial applications PartXV. Fatty acid composition of the seeds oil of *Withania coagulans* and *Withania somnifera*. *Pak J Sci Ind Res*. 1988; 31(2): 139-141.

[9] Bakhtawar S, Mughal T, Naeem I. Chemical composition of the essential oil of *Withania coagulans*. *Asian J Chem*. 2010; 22(1): 122-126.

[10] Ali A, Jameel M, Ali M. New withanolide, acyl and menthyl glucosides from fruits of *Withania coagulans* Dunal. *Acta Pol Pharm Drug Res*. 2014; 71(3): 423-430.

[11] Ali A, Jameel M, Ali M. New fatty acid, aromatic ester and monoterpenic benzyl glucoside from the fruits of *Withania coagulans* Dunal. *Nat Prod Res*. 2015; 29(14): 1307-1314.

[12] Maurya R, Akanksha, Jayendra, Singh AB, Srivastava AK. Coagulanolide, a withanolide from *Withania coagulans* fruits and antihyperglycemic activity. *Bioorg Med Chem Lett*. 2008; 18(24): 6534-6537.
[13] Ramaiah PA, Lavie D, Budhiraja RD, Sudhir S, Garg KN. Spectroscopic studies on a withanolide from *Withania coagulans*. *Phytochemistry*. 1984; 23(1): 143-149.
[14] Budhiraja RD, Sudhir S, Garg KN. Cardiovascular effects of a withanolide from *Withania coagulans* Dunal fruits. *Indian J Physiol Pharmacol*. 1983; 27(2): 129-134.
[15] Velde VV, Lavie D, Budhiraja RD, Sudhir S, Garg KN. Potential biogenetic precursors of withanolides from *Withania coagulans*. *Phytochemistry*. 1983; 22(10): 2253-2257.
[16] Maurya R, Akanksha, Jayendra. Chemistry and pharmacology of *Withania coagulans*: an Ayurvedic remedy. *J Pharm Pharmacol*. 2010; 62(2): 153-160.
[17] Browse J, McCourt PJ, Somerville CR. Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue. *Anal Biochem*. 1986; 152(1): 141-145.
[18] Babushok VI, Linstrom PJ, Zenkevich IG. Retention indices for frequently reported compounds of plant essential oils. *J Phys Chem Ref Data*. 2011; 40(4): 1-46.
[19] Ali M. *Techniques in terpenoid identification*. 1st ed. Delhi: Birla Publication, 2001.
[20] McLaerty FW. *Registry of mass spectral data*. New York: Wiley, 1989.
[21] Yang ZH, Miyahara H, Hatanaka A. Chronic administration of palmitoleic acid reduces insulin resistance and hepatic lipid accumulation in KK-Ay mice with genetic type 2 diabetes. *Lipids Health Dis*. 2011; 120(10): 1-8.
[22] Matthan NR, Dillard A, Lecker JL, Blanche I, Lichtenstein AH. Effects of dietary palmitoleic acid on plasma lipoprotein profile and aortic cholesterol accumulation are similar to those of other unsaturated fatty acids in the F1B golden syrian hamster. *J Nutr*. 2009; 139(2): 215-221.
[23] Das UN. Essential fatty acids - a review. *Curr Pharm Biotechnol*. 2006; 7(6): 467-482.
[24] Das UN. Biological significance of essential fatty acids. *J Assoc Physicians India*. 2006; 54(4): 309-319.
[25] Horrobin DF, Ziboh VA. The importance of linoleic acid metabolites in cancer metastasis and in the synthesis and actions of 13-HODE. *Adv Exp Med Biol*. 1997; 433: 291-294.
[26] Ali ST, Ali SK, Baloch MN, Wahab A, Subhan SA. GC/MS based phytochemical analysis and antimicrobial screening of non-polar fraction of *Withania coagulans*. *Int J Curr Res*. 2015; 7(4): 14361-14363.
[27] Saeidnia S, Abdollahi M. Are medicinal plants polluted with phthalates? *Daru J Pharm Sci*. 2013; Article ID PMC3671212.