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RESEARCH ARTICLE

GC-MS BASED PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL SCREENING OF NON-POLAR FRACTION OF *WITHANIA COAGULANS*

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ABSTRACT

Withania coagulans, a medicinal plant traditionally use for the treatment of a variety y of human ailments particularly in diabetic. Investigation of the phytochemical composition and antimicrobial activity of non-polar fraction of ethanol extract of *Withania coagulant* twigs is discuss in present report. The phytochemical constituents of non-polar fraction of ethanol extract of twigs were studied through gas chromatography-mass spectrometry (GC/GCMS). A total of eleven compounds were identified which include sterols, fatty acid and their methyl esters. The Kovat's Retention Indices were also used to support the identification of these compounds. Significant antimicrobial activity of the n-hexane fraction has been observed against several bacterial strains. The distinctive chemical composition of *W. coagulans* twigs along with substantial antimicrobial activity not only authenticate their traditional medicinal uses but also indicate their potential as a source of natural antimicrobial compounds.

INTRODUCTION

Withania coagulans, one of the famous traditional medicinal plant; commonly known as Asgandha, widely distributed in tropical to subtropical region, in Pakistan found in Karachi, Sindh, Kohat, Punjab and Baluchistan (Chadha, 1976; Sharma, 2004). The great importance owning herb is mainly used for the treatment of diabetes. Its fruits are reported to sedative, diuretic, emetic, dyspepsia and intestinal infections (Krishnamurthi, 1969; Brown. 1995; Dymock *et al.*, 1972; Maury and Akanksha, 2010). Phytochemical studies on this plant have demonstrated the presence of a number of phytochemicals which include with anolide. The prime focus of the present study is the identification of phytochemicals in non-polar fraction of ethanol extract from twigs of *Withania coagulans* and the therapeutic value of this fraction. It may be noted that prior to this report the identification of compounds of the non-polar fraction of the ethanol extract of twigs has not been reported.

Experimental

General

The Shimadzu GC-17 gas chromatograph with flame ionization detector (GC-FID), equipped with less-polar capillary column SPB-5 (45m x 0.53mm ID with 0.50um film thickness of 5% phenyl and 95% methyl silicone), hooked with Shimadzu workstation Class GC-10 used for GC-FID analysis. The nitrogen is used as carrier and make-up gas with the flow rate 1.4 and 40 $mLmin^{-1}$ respectively. The split injector with a splitting ratio of 1:30was set at 250°C and the FID was set at 270°C. The analysis was performed with an initial temperature 60°C for 1min, and then ramped at a rate of 8°C/min to a final temperature 240°C with holding time 30min. Kovats retention indices were also calculated for identified phytochemicals. The gas chromatography electron impact mass spectrometer (GC-EIMS) studies was performed on a Hewlett-Packard 5890 gas chromatograph, equipped with HP-5 (25m x 0.22mmID and 0.25um film thickness, an equivalent of SPB-5) was joint with a Jeol, JMS-HX 110 mass spectrometer operating in EI mode. The temperature of ion source was set at 250°C and the energy of bombardment electron was set at 70 eV, while helium was used as carrier gas at $1.4kg/cm^2$ pressure. The fraction was dissolved in chloroform (1:20). The injection volume was 1.0µL.

Plant material

The whole plant (2kg) of *Withania coagulans* were purchased from an authentic local herbal store and identified by Prof. Dr. S. I. Ali from Department of Botany, University of Karachi.

Extraction

The extraction of Withania coagulanstwigs (2kg) were done with ethanol repeatedly (x3) at room temperature. The

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combined extract was concentrated under vacuum and partitioned between 90% MeOH and n-hexane. The n-hexane fraction (WTP) was dried over sodium sulphate and concentrated under vacuum (1270g). The WTP small fraction (150mg) was treated with MeOH and HCl for methylation. After work-up the methylated fraction (WTP-M; 120mg) was obtained. The WTP and methylated fractionWTP-M was subjected to GC-FID and GC-EIMS.

Antibacterial Activity

Antibacterial activity of these extracts were tested against *M. luteus*, *B. subtilis*, and gram negative pathogens, *S. typhi* and *S. dysentrae by* agar well diffusion method described by Con and Gokalp (2000).

RESULTS AND DISCUSSION

The analysis of non-polar fraction of ethanol extract of twigs of *Withania coagulans* and its methylated fraction were performed on GC-FID and followed by GC-EIMS. The mass spectral survey (NIST Mass Spectral Search Program, ver. 2.0g, GC-Library NIST-11) with Automated Mass Spectral Deconvolution and Identification System (AMDIS) were used for the characterization of phytochemicals which further supported by Kovat's Retention Indices reported in literature and NIST Library. For calculation of retention indices available pure n-alkanes C6, C8, C10, C12 and C22 were used as primary reference and cover the range from C8 to C25 by

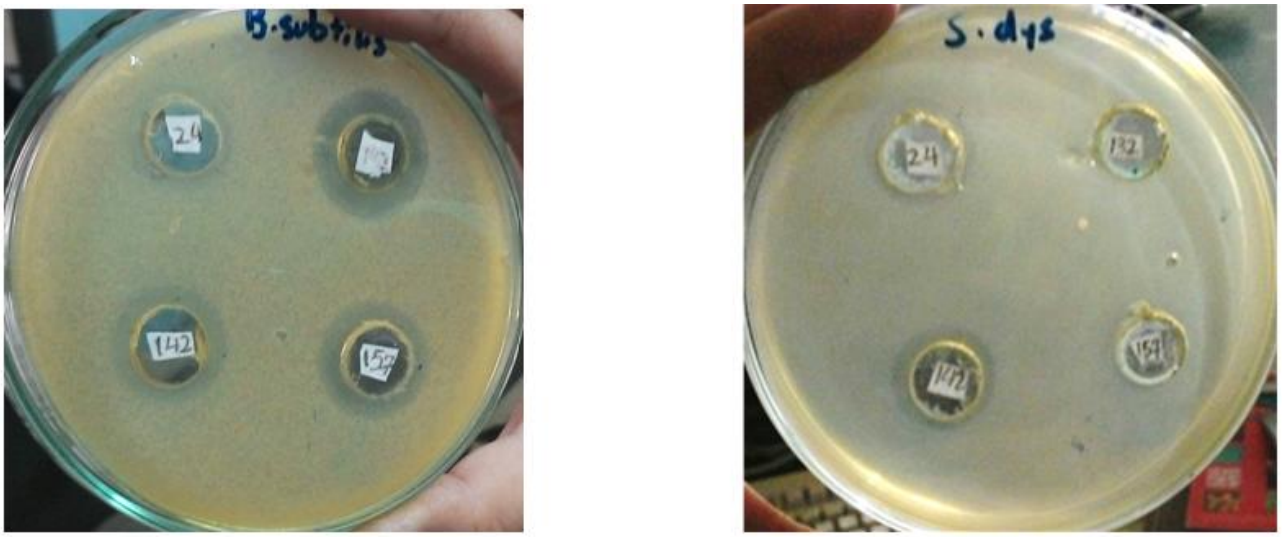

Fig.1. Antibacterial activity of WTP extracts against *B. subtitles* and *S. dysentery* by Agar well method

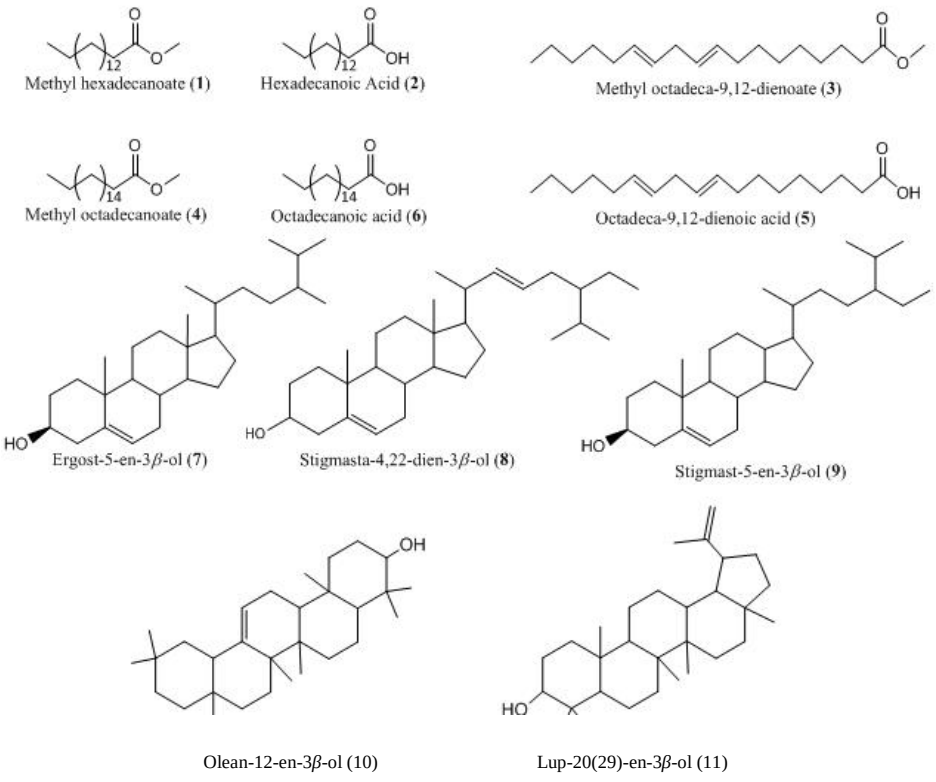

Table 1. Qualitative analysis of WTP

S.#	Retention Time (min)	Constituent Name	CAS#	Chemical Formula	Molecular Weight	Calc. R.I.[a]
1	28.83	Methyl hexadecanoate (1)	112-39-0	$C_{17}H_{34}O_2$	270	1924
2	29.78	n-Hexadecanoic acid (2)	57-10-3	$C_{16}H_{32}O_2$	256	1968
3	31.25	Methyl octadeca-9,12-dionate (3)	2462-85-3	$C_{19}H_{34}O_2$	294	2015
4	31.62	Methyl octadecanoate (4)	112-61-8	$C_{19}H_{38}O_2$	298	2028
5	31.70	Octadeca-9,12-dionic acid (5)	60-33-3	$C_{18}H_{32}O_2$	280	2145
6	32.03	n-Ocadecanoic acid (6)	57-11-4	$C_{18}H_{36}O_2$	284	2157
7	47.21	Ergost-5-en-3 -ol (7)[b]	4651-51-8	$C_{28}H_{48}O$	400	-
8	48.18	Stigmasta-4,22-dien-3β-ol (8)[b]	57815-94-8	$C_{29}H_{48}O$	412	-
9	50.45	Stigmast-5-en-3β -ol (9)[b]	83-47-6	$C_{29}H_{50}O$	414	-
10	51.46	Olean-12-en-3β -ol(10)[b]	559-70-6	$C_{30}H_{50}O$	426	-
11	53.70	Lup-20(29)-en-3β-ol (11)[b]	545-47-1	$C_{30}H_{50}O$	426	-

spiking pure n-alkanes in kerosene oil and diesel, used as secondary standard. All these were analyzed on GC-FID followed GC-EIMS under the identical conditions as for the fraction and further confirmed by mass spectral survey (NIST Mass Spectral Search Program, ver. 2.0g, GC-Library NIST-11) with Automated Mass Spectral Deconvolution and Identification System (AMDIS). The non-polar fraction of ethanol extract of twigs of *W. coagulans*was obtained as a dark brown semi solid (123g). Eleven compounds were identified in this fraction which includessterols, fatty acid and their methyl esters. For further confirmation and analysis for presence of any carboxylic acid, fraction WTP was subjected to methylation. The methylated fraction (WTP-M) was obtained as a dark brown semi solid (100mg).

Activity

Antimicrobial activity of non-polar fraction WTP was determined against four different bacterial strains which include gram positive parhogens, *M. luteus, B. subtilis, and gram negative pathogens, S. typhi* and *S. dysentrae by* agar well diffusion method described by Con and Gokalp (2000) has been observed that n-hexane faction (WTP) was effective against tested bacteria. The inhibitory effected was recorded as positive after observing zone of inhibition around the well containing the extract. The experiments were performed in triplicates, however, further investigations are recommended to compare the antimicrobial activity of *W. coagulans* extracts with commercially available antibiotics.

		Indicators			
		Gram Positive		Gram Negative	
		Zone of inhibition in mm		Zone of inhibition in mm	
S.No	Chemical Extracts	*Bacillus subtilis*	*Micrococcus luteus*	*Shigella dysenteriae*	*Salmonella typhi*
1	WPS 0.0157	17 mm	Negative	14 mm	Negative
2	W.C.S(M) 0.0024	18 mm	Negative	18 mm	Negative

Spectral data

Characterization of constituents

Methyl hexadecanoate (1): GC-EIMS *m/z* (rel. int.); $C_{17}H_{34}O_2$, 270 (M^+, 3),227 (11), 143 (20), 74 (100), 88(70), 57 (19), 55 (31), 41 (42)

n-Hexadecanoic acid (2): GC-EIMS *m/z* (rel. int.); $C_{16}H_{32}O_2$, 256 (M^+, 10), 213 (8), 129 (25), 73 (90), 60 (84), 57 (63), 55 (61), 43 (100)

Methyl octadeca-9,12-dienoate (3): GC-EIMS *m/z* (rel. int.); $C_{19}H_{34}O_2$, 294 (M^+,32) , 109 (23), 95 (50), 81 (78), 67 (100), 55 (75), 41 (90)

Methyl Octadecanoate (4): GC-EIMS *m/z* (rel. int.); $C_{19}H_{38}O_2$, 298 (M^+,15), 255 (15), 199 (13), 143 (24), 87 (74), 74 (100), 55 (20); 43 (35)

Octadeca-9,12-dienoic acid (5): GC-EIMS *m/z* (rel. int.); $C_{18}H_{32}O_2$, 280 (M^+, 50) , 110 (45), 95 (55), 81 (87), 67 (100), 55 (60), 41 (55)

Octadecanoic acid (6): GC-EIMS *m/z* (rel. int.); $C_{18}H_{36}O_2$, 284 (M^+, 47), 241 (20), 185 (23), 129 (50), 73 (95), 57 (85); 43 (100)

Ergost-5-en-3β-ol (7): GC-EIMS *m/z* (rel. int.); $C_{28}H_{48}O$, 400 (M^+,40), 315 (28), 289 (30), 213 (25), 145 (35), 105 (40), 95 (47), 81 (52), 55 (72), 43 (100)

Stigmasta-4,22-dien-3 -ol (8): GC-EIMS *m/z* (rel. int.); $C_{29}H_{48}O$, 412 (M^+, 100), 255 (66), 271 (42), 255 (67), 107 (40), 95 (54), 83 (77), 69 (60)

Stigmast-5-en-3β-ol (9): GC-EIMS *m/z* (rel. int.); $C_{29}H_{50}O$, 414 (M^+, 45), 396 (15), 329 (10), 213 (13), 145 (32), 107 (45), 81 (45), 55 (67), 43 (100)

Olean-12-en-3β-ol (10): GC-EIMS *m/z* (rel. int.); $C_{30}H_{50}O$, 426 (M^+, 5), 218 (100), 203 (55), 189 (13), 107 (10), 95 (15), 55 (13)

Lup-20(29)-en-3β-ol (11): GC-EIMS *m/z* (rel. int.); $C_{30}H_{50}O$, 426 (M^+, 30), 207 (53), 147 (40), 107 (62), 95 (75), 81 (77), 68 (98), 55 (87), 43 (100)

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