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Extraction & Characterization Of Oil From Seeds Of Medicinal Plant Withania Coagulans

Neena Srivastava[1,*], Priti Singh[2], Ashutosh Singh[2], Sangeeta Verma[3] and Abhishek Singh[4]

[1]Department of Chemistry, Mahila Vidyalaya Degree College, Lucknow-226018, India.

[2]Department of Chemistry, Shri Jai Narain Misra Post Graduate College, Lucknow- 226001, India.

[3]Department of Chemistry, K.S. Saket P.G. College, Ayodhya-224001, India.

[4]Department of Chemistry, U.P. College, Varanasi-221002, India.

Abstract

In the present study oil was extracted from seeds of Withania coagulans which was subsequently subjected to FTIR and GC-MS analysis to identify phytochemical components of extracted oil. FTIR analysis revealed presence of diverse group of compounds including alcohols, alkane, alkene, aldehyde, ketone and halo compounds. GC-MS analysis depicted presence of 49 phytocompounds in the oil extracted from seeds of Withania coagulans. Among which Hexanoic acid, n-hexadecnoic acid, Vitamin E, gamma-Tocopherol, squalene, Feucosterol, 2-Pyrrolodinone, 1-methyl, Octadecnoic acid represent major phytocompounds identified. Several compounds identified to be present in extracted oil have been reported to possess one or more biological\pharmacological activity. Hence, the study suggests validation of plant oil to be utilized as ingredient of different pharmacological, cosmetic and other food products.

Keywords: Withania coagulans, seeds, oil, phytocompounds, FTIR, GC-MS.

Introduction

The genus Withania belongs to Family Solanaceae is a well-recognized genus comprising of several medicinal plants (Jain et al.[26]). Among 23 reported species of Withania, Withania somnifera and Withania coagulans, are economically important (Panwar et al [11]). W. coagulans Dunal, is usually referred to as 'Indian cheese maker' or 'vegetable rennet' due to its milk coagulating properties (Ali et al.[20]). W. coagulans has been reported to possess several medicinal properties including cancer, ulcers, asthma, dyspepsia, cardiovascular, constipation, and nervous system. It is very much in use in recent

years due to the presence of a large number of steroidal alkaloids and lactones known as withanolides (Hemalahta et al.[27]).

Extraction of essential oil from numerous plant species has been successfully accomplished for medicinal as well as traditional purposes. Medicinal and commercial significance of plant oil is attributed to presence of aromatic compounds, secondary metabolites with biological activities. Published literature reports antifungal, anti-bacterial, anti-viral, anti-diabetic, anti-cancer, anti-inflammatory, anti-oxidant and repellent activities to be major proportion of essential oil extracted from plant species. (Adorjan et al [3]., Upadhyay et al [5]., Teixeira da Silva et al. [31]). Hydro distillation, steam distillation and Soxhlet extraction represents most commonly practical methods for extraction of oil from different parts of plant species. Commercial application of plant essential oil includes their respective utilization in food industry, cosmetics, pharmaceuticals and health care, sanitary products (Salehi et al. [13]). Due to wide spread application of essential oil from numerous plant species have been extracted and utilized for various purposes. Scientific studies have been conducted to analyze and assess medicinal potential of oil extracted from medicinal plants, aromatic plants and other plant species. Still there are species for which only few studies have been conducted pertaining to extraction of oil along with its phytochemical characterization. W. coagulans is one such plant, study conducted by (Ali et al.[2]) is the only prominent published literature pertaining to extraction and characterization of oil from the plant. Further studies are required to optimize the protocol for extract of oil (along with its biochemical characterization). In the present study protocol for extraction of oil from seeds of W. coagulans was optimized along with its phytochemical characterization.

2. Material and Methodology

2.1 Plant material

Commercially available seeds of W. coagulans were utilized as study sample for the present work. The sample was authenticated by Dr. Manjul Dhiman, Head, Department of Botany KLDAV (PG) College Roorkee.

2.1 Extraction of oil

For the extraction 10 gm seeds of W. coagulans was finely grounded and mixed with 60ml n-hexane and 60ml acetone. After 90 cycles of Soxhlet apparatus and the seeds was filtered through what man filter paper. After evaporation lipid portion was extracted and collected quantity was 12ml. After that the lipid content was poured in separating funnel to which add 12ml di ethyl ether was added for the separation of pure lipids. After the mixing the separating funnel was left undisturbed for 5-6 minutes after which two independent layers were obtained in separating funnel. Upper layer represents ether layer and lower layer is water soluble other containment layer. After the separation of layers ether layer was carefully separated and the ether was gradually evaporated for separation of oil from ether (Fig. 1).

2.2 GC-MS

The extracted oil was subjected to GC-MS analysis. Perkin Elmer Auto system was utilized as GC-MS analyzer. He gasses acted as carrier with as a flowrate (constant) of 1.51ml/min. An injection volume of 2µl was utilized. Mass spectra was analyzed through Turbo mass software. Phytocompounds were identified based upon molecular mass, structure, retention time and mass spectra compared to standard compounds from database NIST98, NIST database.

2.3 FTIR

FTIR Technique has been recognized as an effective bioanalytical tool for identification of different type of compounds along with their functional group and chemical bonds. Specific wavelength of light is absorbed by particular chemical bond which can be analysed in infrared spectrum and the respective chemical bond is subsequently identified. The extracted oil was subjected to FTIR analysis to determine different classes of organic compounds.

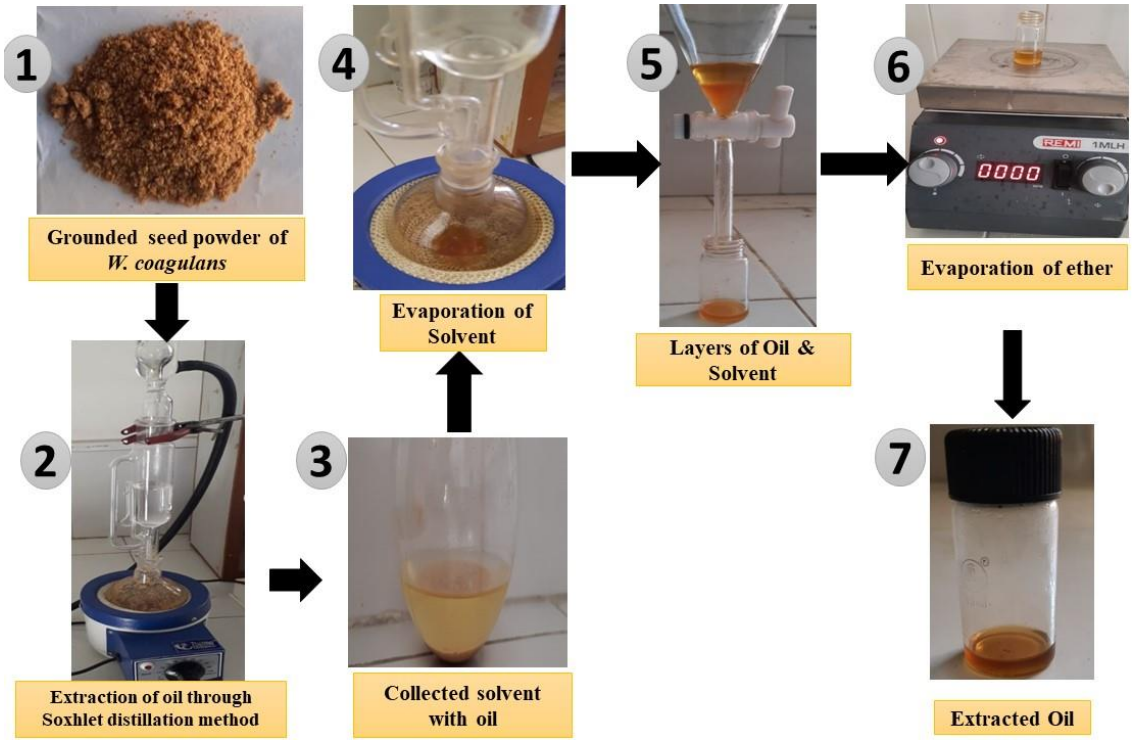

Fig.1: Methodology adopted for extraction of oil from seeds of W. coagulans

Result and Discussion

The protocol followed for extraction of oil from seeds of W. coagulans was found to be effective for extraction of oil free from impurities. About 6.8 ml oil was extracted from 100 gm powder of seeds. The oil was highly viscous and when extracted was yellowish brown in colour.

GC-MS Analysis

GC-MS analysis of oil extracted from seeds of W. coagulans revealed presence of 49 phytocompounds in the oil. Fig. 2 represents the gas chromatogram of the oil and Table 1 represents phytocompounds identified to be present in the extracted oil with their retention time and concentration (%). Along with retention time and structure (molecular mass) mass spectra of compounds have been collaboratively utilized to identify the respective phytocompounds. Fig. 3 represents the mass spectrum of major\ biologically active phytocompounds present in extracted oil. Major compounds found to be present in oil include hexanoic acid, n-hexadecnoic acid, 9,12-octadecadienoic acid (Z, Z)-, Squalene, gamma-Tocopherol, Vitamin E, Vanillin and Phorone. In an earlier study conducted by (Ali et al. [2]) oil was extracted from fruits of W. coagulans and the same was subjected to GC-MS analysis. A total of 29 phytocompounds including unsaturated (52.36%), saturated (22.15%) fatty acids, alkenes (5.65%), phytosterols (4.39%), fatty alcohols (4.145) were reported to be major constituent of oil extracted from W. coagulans fruits. W. coagulans has been recognized as an important genus of Withania species with medicinal potential as well as commercial value. Available literature reports several medicinal properties of the plant which are mainly confined to extracts of fruits and seeds of W. coagulans. (Peerzade et al. [22]) reported antibacterial activity of methanolic fruit extract of Withania coagulans against various bacteria (Salmonella parathyphi, Klebsiella pneumoniae, E. coli., Bacillus subtillus, Straphylococcus aureus & Micrococcus leuteus with highest activity reported against E. coli. In a phytochemical analysis conducted by (Pramanick et al**.** [6]) fruit and seeds of W. coagulans were found to be rich in alkaloids, steroids, esterase, phenolic compounds, tannins, & organic acids. (Azhar et al.[17]) reported presence of phytochemical components with biological activity in W. coagulans and W. somnifera which can be utilized as components of several pharmacological formulations. Comparatively higher antioxidative potential was reported in W. coagulans as compared to W. somnifera. In a specific study conducted by (Kitukale et al. [16]) antidiabetic potential of methanolic and aqueous extract of W. coagulans flower was studied. The study reported decrease in blood glucose of STZ induced diabetic rats compared to control rats after 28 days. As evident from the reported studies most of the medicinal and pharmacological properties reported of W. coagulans have utilized extracts prepared from either seeds / fruits /flower for their respective study. Findings of the present study reveal presence of several compounds in the oil extracted from seeds which have been earlier reported to possess medicinal value. Hexanoic acid, octadecnoic acid possess anti-oxidant activity & anti-inflammatory activity (Ramya et al.[4] Abdelhamid et al.[19].) Feucosterol, 2-Pyrrolodinone, 1-methyl possess anti-cancer activity (Abdul et al.[24], Hosseinzadeh et al.[32]). Stigmasta-5,22-dien-3-ol and 2-Methoxy-4-vinyphenol, vanillin, octadecanoic acid comprise phytocompounds present in oil extracted from seeds of W. coagulans with reported antimicrobial activity (Jebastella et al.[10], Rubab et al.[12]) (Table 2).

FTIR analysis

FTIR has been recognized as an effective analytical technique to identify different types of chemical bonds as well as functional groups present in organic compounds (Devi et al.[7]). The functional groups of different phytocompounds were identified according to the peak values in region of infra-red

radiation. The analysis (Fig.4) revealed extracted oil to possess organic compounds belonging to different classes including alcohol at 3473cm^{-1}, alkenes at 3008.9 cm^{-1} and 2924.64 cm^{-1}, aldehydes at 2854.12 cm^{-1}, 2672.75 cm^{-1} and 1377.49 cm^{-1}, alkanes at 1465.08 cm^{-1}, ketones at 1654.08 cm^{-1}, cyclopentanone at 1744.13 cm^{-1} and sulfone at 1164.17 cm^{-} (Saleem et al.[1], Janakiraman et al.[21], Subrahmanian et al.[9]). Presence of diverse nature of organic compounds indicates the extracted oil to be highly rich in containing different metabolites with characteristic properties and function. FTIR is a commonly utilized technique to identify different classes of organic compounds present in plant species. (Chaudhary et al. [30]) analyzed FTIR profile of Tamra bhasma. The study reported FTIR spectra of T. bhasma to possess hydrogen stretching region (3700-2700Cm^{-1}), triple bond region (2700-1950 Cm^{-1}), a double bond region (1950-1550 Cm $^{-1}$) & fingerprint region (1500-700Cm^{-1}) which indicated presence of large number of functional groups. In another study conducted by (Satapathy et al.) FTIR analysis of water, methanol, ethyl acetate and acetone extract of Pderia foetide was accomplished for identification of organic secondary metabolites. The study revealed that leaf extract of P. foetide possesses phytochemicals of different functional groups such as alkenes, aromatic compounds, aldehydes, saturated fatty acids, alcohols, carboxylic acids, esters and alkyl halides. (Thenmozhi et al.[14]) analyzed FTIR of ethanolic and hexane extract of leaf & fruit of Ziziphus oenopila Mill. Presence of alkaloids, amino acids, carbohydrates, phenols, phytosterols, gums & mucilage was reported. Higher proportion of alkaloids was reported in hexane extract compared to ethanolic extract of leaves and fruit of Z. oenopila.

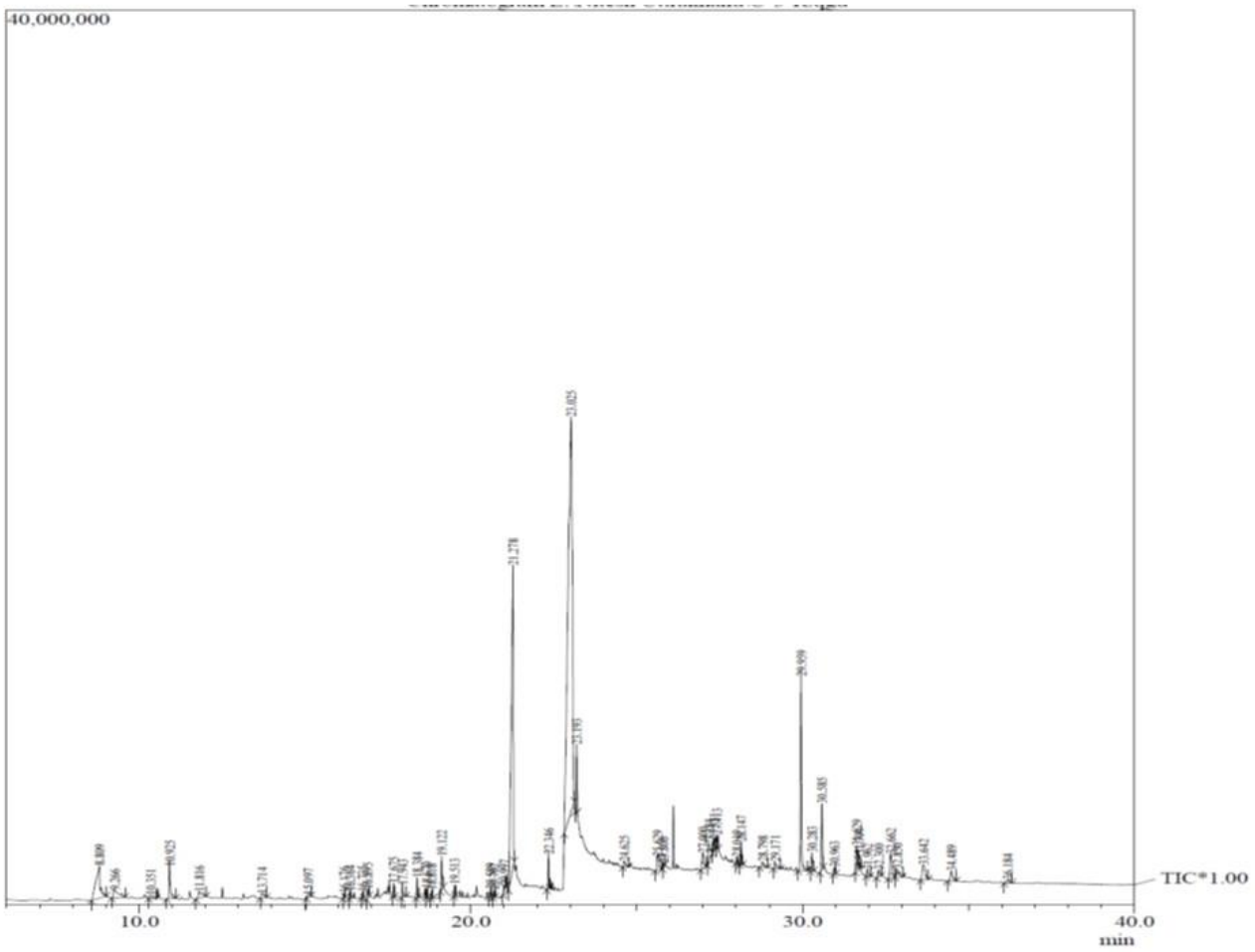

Fig.2: GC-MS chromatogram of oil extracted from seeds of W. coagualns

Table 1: Phytocompound identified to be present in oil of seeds of Withania coagulans through GC-MS analysis

Peak	R. Time	Area%	Name of the compound
1	8.809	3.98	Hexanoic acid
2	9.266	1.09	2-Pyrrolidinone, 1-Methyl-
3	10.351	0.23	Phorone
4	10.925	1.29	2,6-Dimethyl-6-nitro-2-hepten-4-one
5	11.816	0.66	2-Pentanol, 2,3-Dimethyl-
6	13.714	0.37	2-Methoxy-4-vinylphenol
7	15.097	0.21	Vanillin
8	16.176	0.12	Phenol, 3,5-bis(1,1-dimethylethyl)-
9	16.364	0.13	Benzene, (1-butylhexyl)-
10	16.735	0.13	Benzene, (1-Ethyloctyl)-
11	16.895	0.29	Dodecanoic Acid

12	17.675	0.27	Benzene, (1-propyloctyl)-
13	17.943	0.37	Benzene, (1-Ethylnonyl)-
14	18.384	0.42	Benzene, (1-Methyldecyl)-
15	18.670	0.18	Benzene, (1-butyloctyl)-
16	18.818	0.17	Benzene, (1-Propylnonyl)-
17	19.122	0.84	Tetradecanoic acid
18	19.513	0.23	Benzene, (1-methylundecyl)-
19	20.589	0.17	Benzene, (1-Methyldodecyl)-
20	20.702	0.17	Hexadecanoic Acid, Methyl Ester
21	20.997	0.24	9-Hexadecenoic Acid
22	21.278	18.05	n-Hexadecanoic acid
23	22.346	0.80	9,12-Octadecadienoic acid (Z, Z)-, methyl ester
24	23.025	47.82	9,12-Octadecadienoic acid (Z, Z)-
25	23.193	1.50	Octadecanoic acid
26	24.625	0.33	Cyclohexane, 1,1'-Hexylidenebis-
27	25.629	0.94	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z, Z)-
28	25.806	0.11	3-Heptadecene, (Z)-
29	27.000	0.87	Cyclopropane, 1,1-dichloro-2,2,3,3-tetramethyl-
30	27.184	1.16	(R)-(-)-14-Methyl-8-hexadecyn-1-OL
31	27.331	0.47	7-(3,4-Methylenedioxy)-tetrahydrobenzofuranone
32	27.413	0.35	9,12-Octadecadienoic acid (Z, Z)-, 2-hydroxy-1- (hydroxym
33	28.019	0.19	9-Octadecenamide
34	28.147	0.46	Squalene
35	28.798	0.32	Androst-5-en-3-ol, 4,4-dimethyl-, (3. beta.)-
36	29.171	0.28	delta. -Tocopherol
37	29.959	6.17	gamma. -Tocopherol
38	30.283	0.54	beta. -Tocopherol
39	30.585	1.91	Vitamin E
40	30.963	0.21	Lanostan-7-One
41	31.629	0.68	Stigmasta-5,24(28)-DIEN-3-OL, (3. beta.)-
42	31.704	0.31	Ergost-5-en-3-ol, (3. beta.)-
43	31.962	0.31	Stigmasta-5,22-DIEN-3-OL
44	32.300	0.34	. Delta. 24-24-Methylcholester
45	32.662	1.43	. gamma. -Sitosterol
46	32.850	0.68	Fucosterol
47	33.642	1.29	Lanost-8-en-3-ol, 24-methylene-, (3. beta.)-
48	34.489	0.69	9,19-Cyclolanostan-3-ol, 24-methylene-, (3. beta.)-
49	36.184	0.21	Octadecanoic Acid, 2,3-Bis [(1-Oxotetradecy

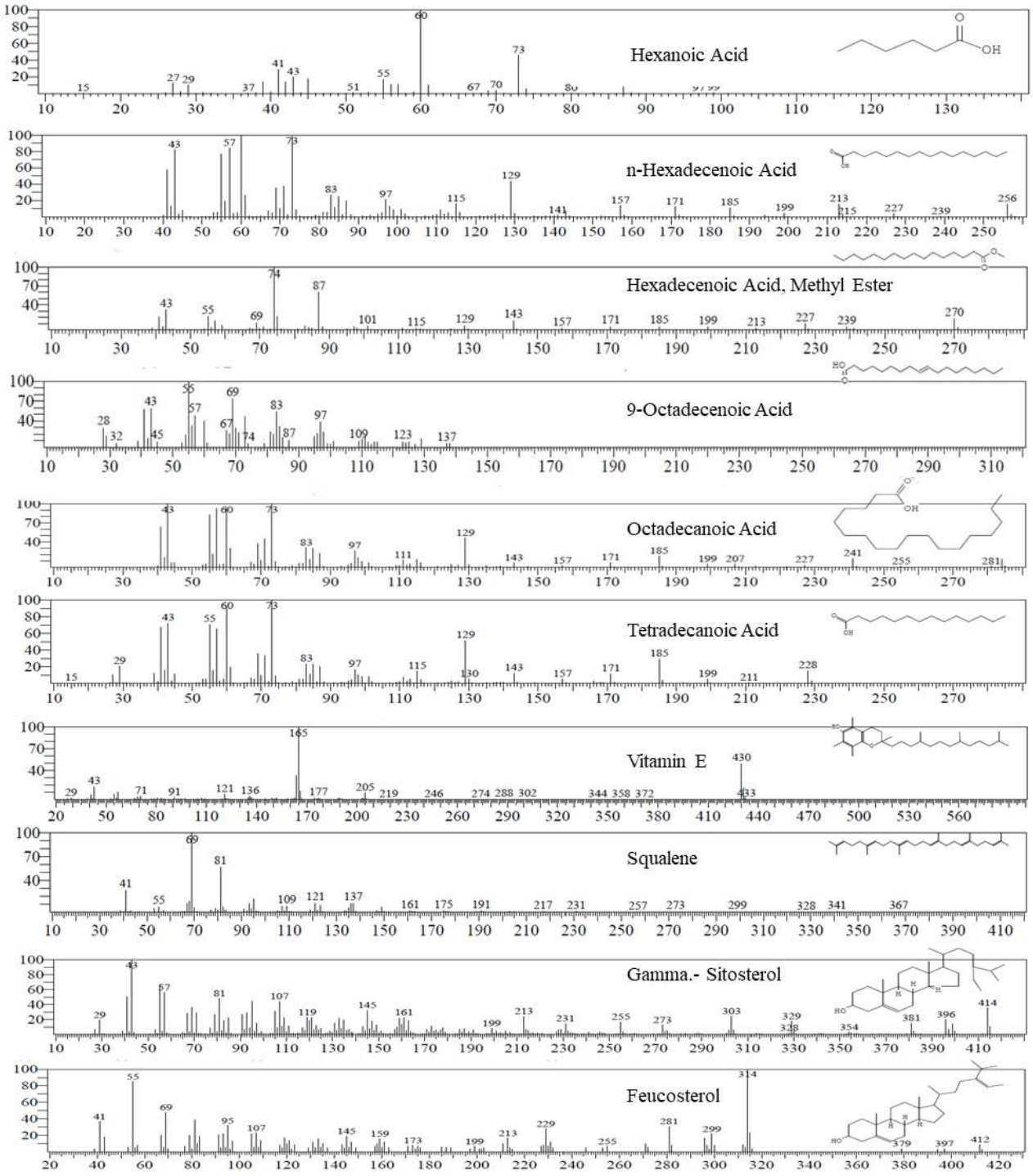

Fig.3: Mass spectrum of major compounds identified to be present in oil extracted from seeds of W. coagualns

Table 2: Biological activities of major phytocompounds found to be present in oil extracted from seeds of W. coagulans

Sr. nm.	Compound Name	Biological Activities	References
1.	Hexanoic acid	Flavoring agents Antidiabetic activity, anticancer activity.	Ramya B, et al.[4]
2.	Octadecanoic acid	Anti HIV, Antiamoebic, Antianaemic, Antianxiety, Antiasthmatic, Antibacterial, Antibiotic, Anticancer, Anticarcinogenic, Anticoagulant, Antidiabetic, Antidiarrheic, Antifatigue, Antigastric, Antihemorrhagic, Anti-inflammatory, Antimalarial, Antiobesity, Antioxidant,	Abdelhamid MS. et al. [19]
3.	9-octadecenoic acid	Antipreventive, Flavour, Fungicide, pesticide, perfumery Anti -inflammatory, hypocholesterolemic, Cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, anticoronary.	Sayeda F.A et al. [8]
4.	n-Hexadecanoic acid	Anti-inflammatory, Antioxidant, hypocholesterolemic nematicide, pesticide, anti -androgenic flavor, hemolytic, 5-Alpha reductase inhibitor, Anti-alopecic, Haemolytic, Lubricant.	Achi NK et al. [23]
5.	Tetradecanoic acid	Antioxidant, cancer preventive, nematocide, hypercholesterolemic, lubricant. Hypercholesterolemic, antiarthritic, nematocide,	Chandrasekaran M. et al. [15]
6.	Hexadecanoic acid methyl ester	Antioxidant, Hypercholesterolemic, Lubricant, Nematicide, Pesticide, Hemolytic 5-Alpha reductase inhibitor, Flavour, Antiandrogenic	Hema R. et al. [25]
7.	Feucosterol	Anti -cancer, anti-diabetic, anti-oxidant, anti-fungal, anti-asthematic, anti-hyperlipidemic, cholinergic, adipogenic, photodamaging.	Abdul QA.et al. [24]
8.	2-Pyrrolodinone, 1-Methyl-	Anti-bacterial, anti-fungal, anti-cancer,	Hosseinzadeh Z. et al. [32]
9.	Vanillin	Neuroprotection, Anti-carcinogenic, Anti-microbial, wound healing, Anti-cancerous, Anti-oxidant, Cardioprotective.	Arya SS. et al. [29]

10.	Vitamin E	Anti-oxidant, prevent cancer, heart disease, Anti-inflammatory.	Rizvi S. et al. [28]
11.	gamma.-Sitosterol	hypolipidemic property	Jebastella J et al. [10]
12.	Stigmasta-5,22-dien-3-ol,	antibacterial activity, antinflammatory, antiarthritic antiasthma, diuretic	
13.	Squalene	Anti-oxidant, Anti-tumor	Huong ZR. et al. [33]
14.	2-Methoxy-4-vinyphenol	Antimicrobial, antioxidant, anti-inflammatory, analgesic, anti-germination	Rubab M. et al. [12]
15.	9,12-octadecadienoic acid (Z,Z)-, methyl ester	Anti-cancer	Abdelhamid MS. et al. [19]

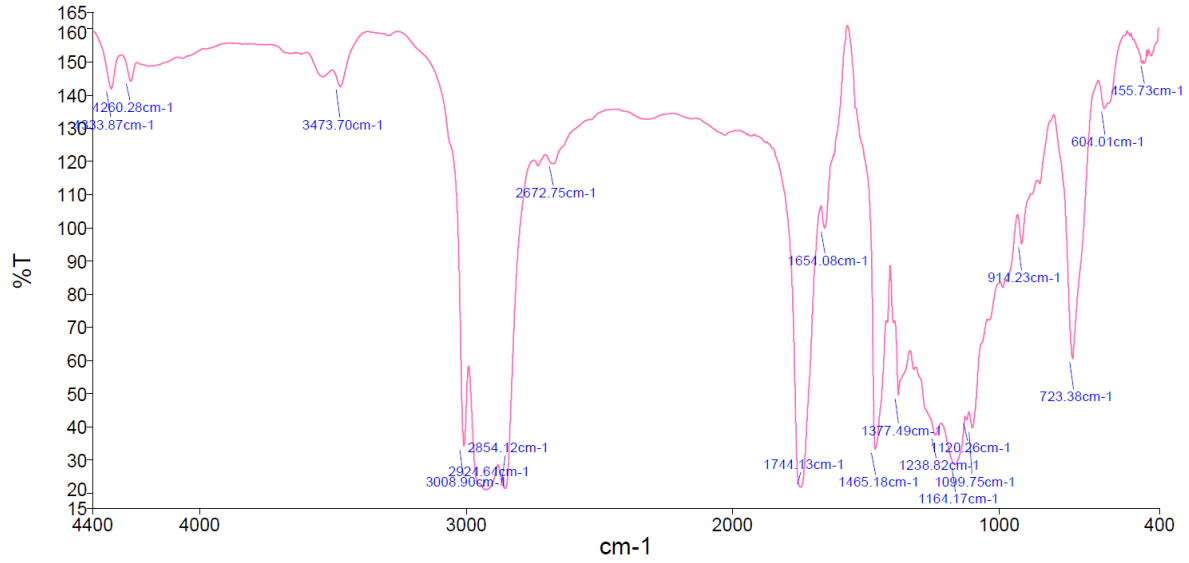

Fig. 4: FTIR analysis of oil extracted from seeds of W. coagulans

Conclusion

Withania coagulans is a well-known medicinal plant which is not yet utilized to its potential in pharmaceutical, cosmetics, food industry. The commercial utilization is challenged by slow propagation rate (due to extremely poor germination rate) and present endangered status of the plant. Also, at present them exist a research gap to validate the utilization plant extract, oil for medicinal purposes and as component of other cosmetic and food products. Studies are required for optimization of process and protocols to fully utilize medicinal potential of the plant. Efforts are also required to accomplish commercial cultivation of the plant to produce sufficient raw material which can serve as industrial feedstock.

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