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RESEARCH ARTICLE

Extraction and characterization of oil from seeds of the medicinal plant *Withania coagulans* (Stocks) Dunal (Doda paneer)

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Abstract

Withania coagulans (Stocks) Dunal commonly known as Doda panner/ Indian Rennet is a well-known medicinal plant but remains underutilized mainly due to scarce distribution in nature and extremely poor germination rate. 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 phytoconstituents in the oil extracted from seeds of *W. coagulans*. among which hexanoic acid, n-hexadecenoic acid, Vitamin E, gamma-Tocopherol, squalene, fucosterol, 2-Pyrrolidinone, 1-methyl, octadecanoic acid represent major phytoconstituents identified. Several compounds identified to be present in extracted oil have been reported to possess one or more pharmacological activity. Hence, the study suggests validation of plant oil to be utilized as ingredient of different pharmacological, cosmetic and other food products.

Keywords

FTIR, GC-MS; oil, phytoconstituents, seeds, *Withania coagulans*

Introduction

The genus *Withania* belonging to the family Solanaceae is a well-recognized genus comprising of several medicinal plants (1). Among 23 reported species of *Withania, Withania somnifera* and *Withania coagulans,* are among economically important (2). *W. coagulans* Dunal, is usually referred to as 'Indian cheese maker' or 'vegetable rennet' due to its milk coagulating properties (3, 4). *W. coagulans* has been reported to possess several medicinal properties such as anti-cancer, anti-diabetic, anti-oxidant, anti-fungal, anti-asthmatic, anti- bacterial, and antianxiety (5). Withanolides (such as steroidal alkaloids and lactones) being characteristic phytoconstituents of *W. coagulans* besides which flavonoids, tannis (6).

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 the 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 prominent biological activities of essential oil extracted from plant species (7-9). Hydro distillation, steam distillation and Soxhlet extraction

represents most commonly practiced 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 etc. (10, 11). 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 which is not yet explored to its medicinal potential and hence the plant is not commercially utilized in pharmaceutical and other industries whereas related species *Withania somnifera* is a component of several consumable healthcare and cosmetic products due to several validated *in vitro* and clinical Studies. With reference to research conducted on oil of *W. coagulans*, a study reported (12) is among the prominent published literature pertaining to extraction and characterization of oil from the plant. Further studies are required to optimize the protocol for extraction 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.

Extraction of oil

For the extraction 10 g seeds of *W. coagulans* were finely powdered and mixed with 60 ml n-hexane and 60 ml acetone. After 90 cycles of Soxhlet apparatus and the extract was filtered through Whatman filter paper. After evaporation, lipid portion was extracted and collected following which lipid content was poured in separating funnel to which 12 ml diethyl ether was added for separation of lipids. The separating funnel was left undisturbed for 15 min. after which 2 independent layers were obtained in separating funnel. Upper layer represents ether layer and lower layer is water soluble layer. Once the two layers (ether and aqueous) got separated, ether layer was carefully removed and the ether was subsequently evaporated to obtain oil (Fig. 1).

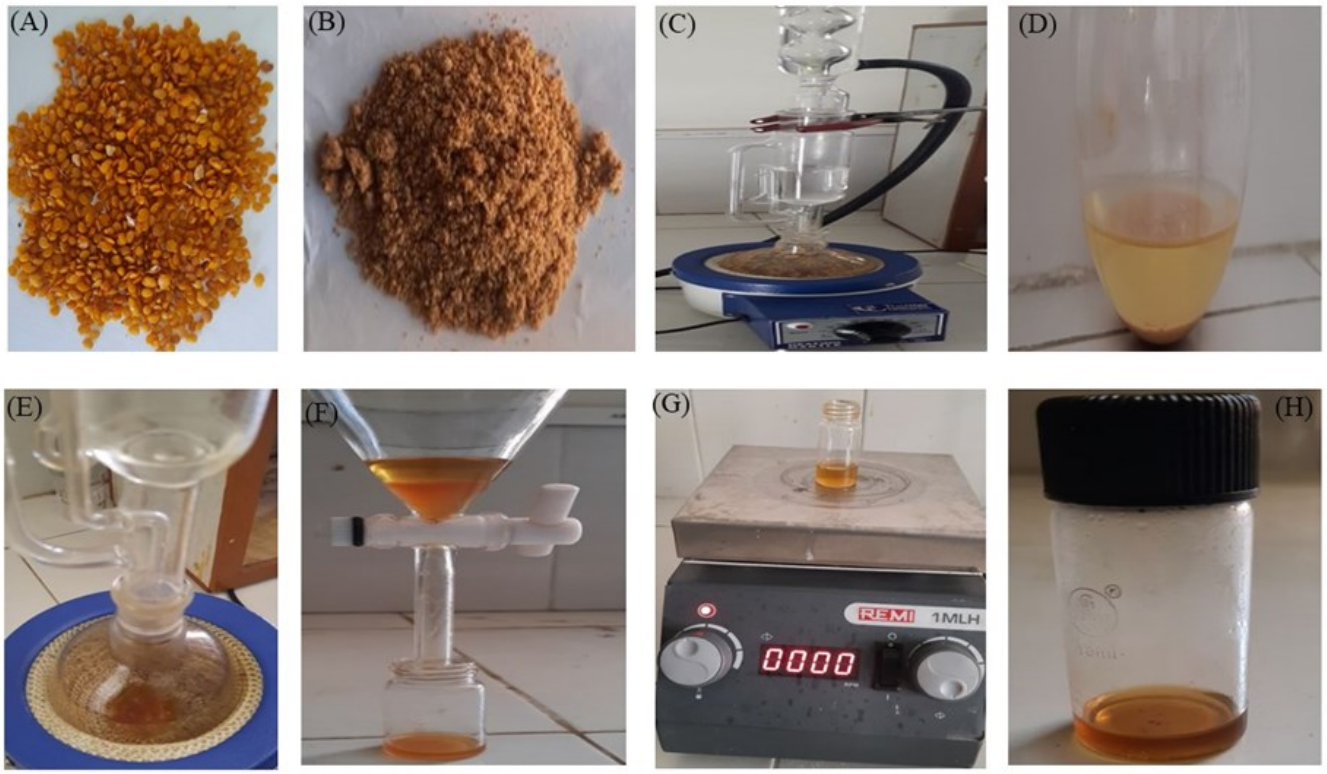

Fig. 1. Methodology adopted for extraction of oil from seeds of *W. coagulans* (**A**) Seeds of *W. coagulans*, (**B**) Grounded seed powder of *W. coagulans* **C**) Extraction of oil through Soxhlet distillation method (**D**) Collected Solvent with Oil (**E**) Evaporation of Solvent (**F**) Layers of Ether and Solvent (**G**) Evaporation of Ether (**H**) Extracted oil.

Materials and Methods

Plant material

Commercially available seeds of *W. coagulans* (Stocks) Dunal 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.

Gas chromatography – Mass spectrophotometry (GC-MS)

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

Fourier Transmission Infrared Spectroscopy (FTIR)

FTIR Technique has been recognized as an effective bioanalytical tool for identification of different type of compounds or identification of 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 (400-4400 nm) to determine different classes of organic compounds.

Results 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 2.8 ml oil was extracted from 10 g 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 the presence of 49 phytoconstituents in the oil. Fig. 2 represents the gas chromatogram of the oil and Table 1 reports different phytoconstituents 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 phytoconstituents. Fig. 3 represents the mass spectrum of major biologically active phytoconstituents present in extracted oil. Major compounds present in oil include hexanoic acid, n-Hexadecenoic acid, 9,12-octadecadienoic acid (Z, Z)-, Squalene, gamma-Tocopherol, Vitamin E, Vanillin and Phorone. Reports are on the presence of twenty-nine phytoconstituents to be present in oil extracted from fruits of *W. coagulans* including unsaturated and saturated fatty acids, alkenes, phytosterols and fatty alcohols (12). *W. coagulans* has been recognized as an important species of *Withania* genus 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*. It was reported that antibacterial activity of methanolic fruit extract of *Withania coagulans* against various bacteria including *Salmonella paratyphi, Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Micrococcus leuteus* with highest activity reported against *E. coli* (13). In a phytochemical analysis conducted in fruit and seeds of *W. coagulans* it was found to be rich in alkaloids, steroids, esterase, phenolic compounds, tannins and organic acids (14). Reports are on the presence of phytochemical components with biological activity in *W. coagulans* and *W. somnifera* which depict their potential application in pharmacological formulations (15). Comparatively higher antioxidative potential was reported in *W. coagulans* as compared to *W. somnifera*. Reports are on the extraction of oil from fruits of *W. coagulans* through steam distillation technique (16). GC-MS analysis of oil revealed presence of 20

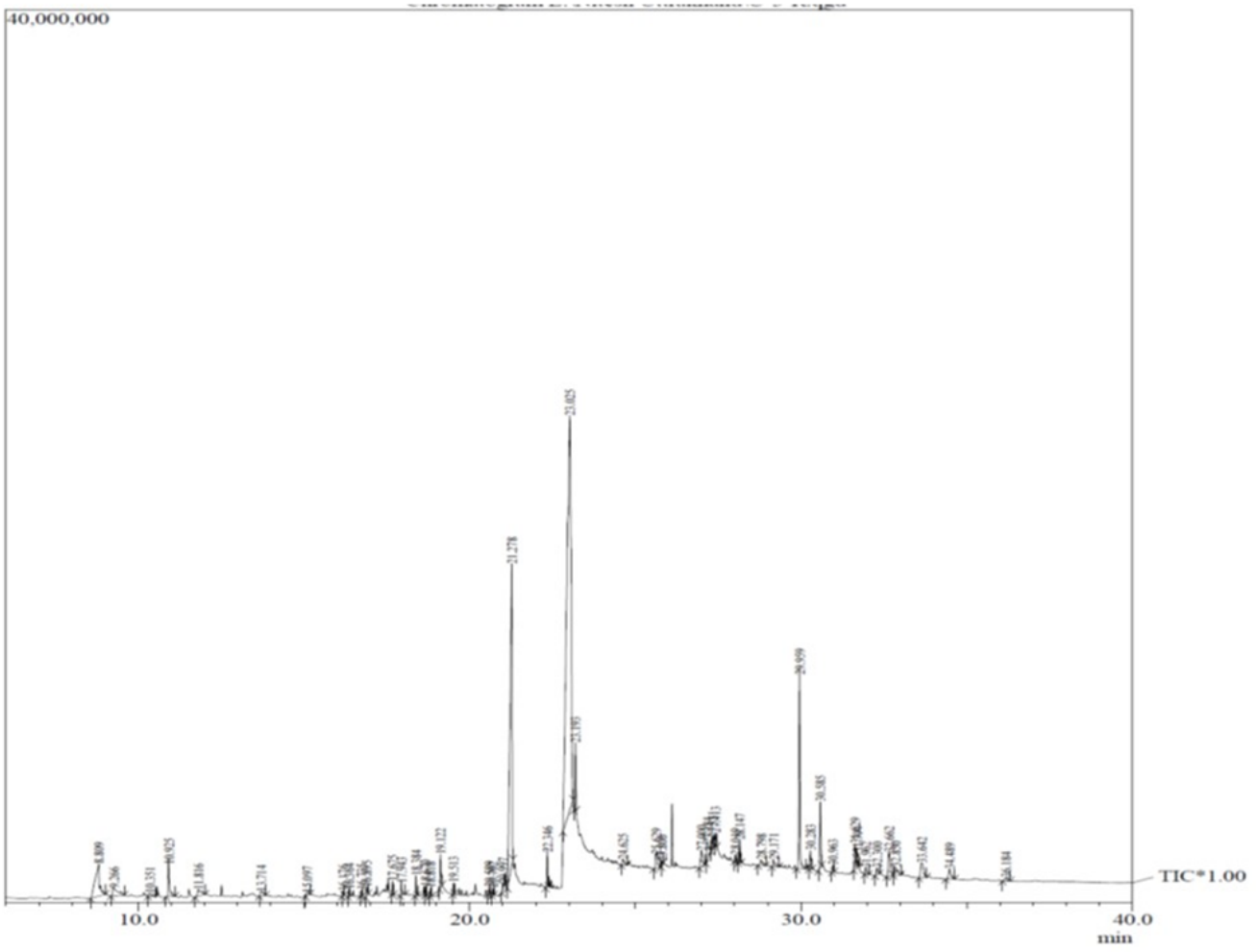

Fig. 2. GC-MS chromatogram of oil extracted from seeds of *W. coagulans.*

Table 1. Phytoconstituents 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

phytoconstituents among which sesquiterpenes and esters to represent major phytoconstituents while acids, alkanes and aldehydes comprised minor proportion of oil. In a specific study conducted antidiabetic potential of methanolic and aqueous extract of *W. coagulans* flower was studied (17). 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 the presence of several compounds in the oil extracted from seeds which have been reported to possess medicinal value. Hexanoic acid and octadecanoic acid possess anti-oxidant activity and anti-inflammatory activity (18, 19) Fucosterol, 2-Pyrrolidinone, 1-methyl possess anti-cancer activity (20, 21). Stigmasta-5, 22-dien-3-ol and 2-Methoxy-4-vinyphenol, vanillin, octadecanoic acid comprise phytoconstituents present in oil extracted from seeds of *W. coagulans* with reported antimicrobial activity (22, 20-30). Similar to the findings of present earlier studies conducted have also effectively utilized the technique of GC-MS to identify phytoconstituents with reported medicinal properties and biological activities (31, 32).

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. The functional groups of different phytoconstituents were identified according to the peak values in region of infrared radiation. The analysis (Fig. 4) revealed extracted oil to possess organic compounds belonging to different classes including alcohol at 3473 cm^{-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^{-1} (33-35). 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 or functional groups present in plant species (36, 37). In an earlier study conducted, FTIR analysis of extract of fruits of *W. coagulans* revealed the presence of steroidal lactones (13). FTIR technique (along with NMR and UVvis) was utilized (4) for identification of withanolides in *W. coagulans.* Most of the reported literature pertaining to FTIR of *W. coagulans* have utilized plant extract and comparatively few studies have been conducted related to extraction as well as analytical analysis of oil of *W. coagulans.*

Conclusion

Withania coagulans (Stocks) Dunal is a well-known medicinal plant which is not yet fully explored to its potential for utilization in pharmaceutical, cosmetics and food industries. The commercial utilization is challenged by slow propagation rate (due to extremely poor

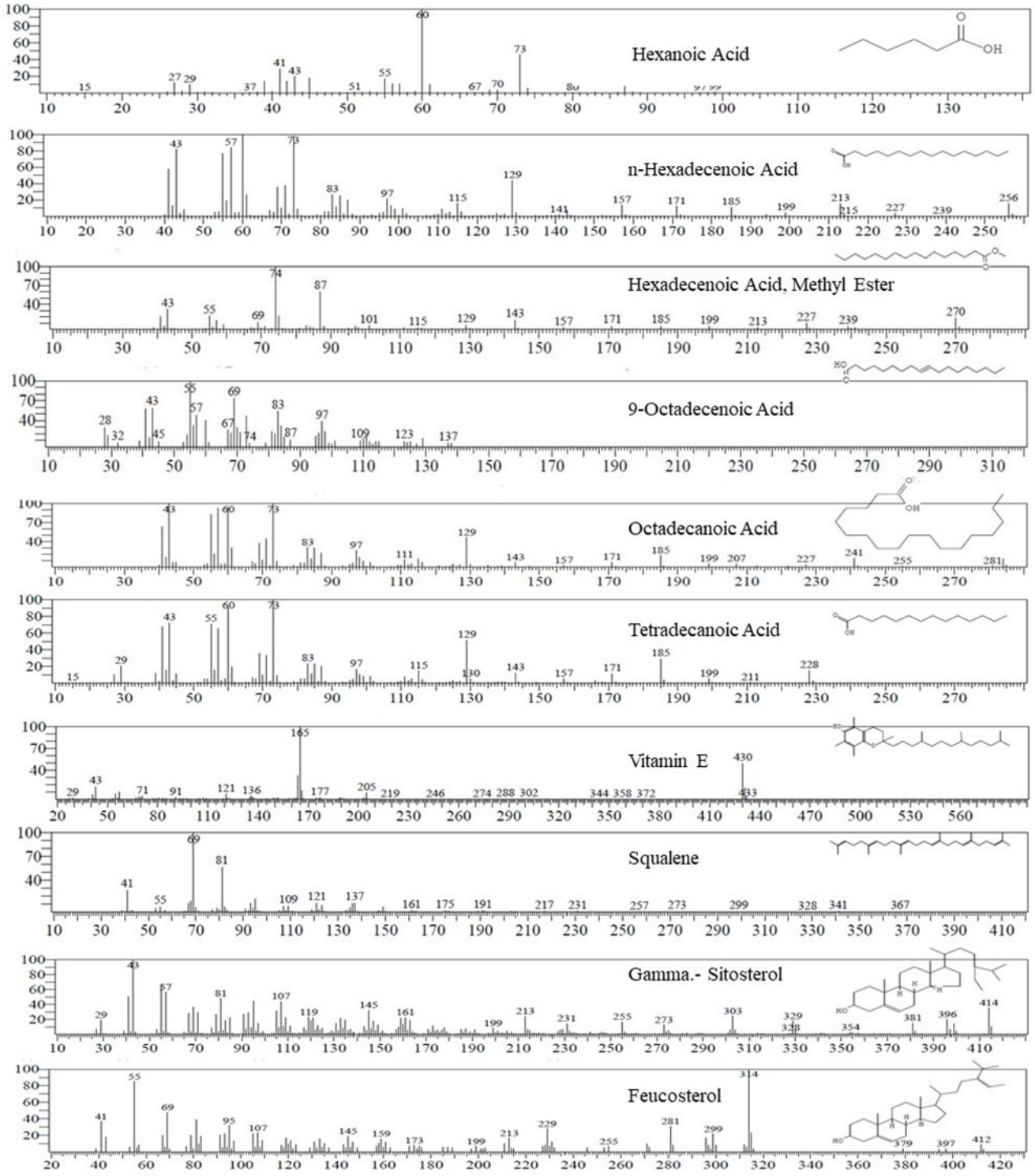

Fig. 3. Mass spectrum of major compounds identified to be present in oil extracted from seeds of *W. coagulans.*

germination rate) and present endangered status 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. Along with commercial cultivation, micropropagation can also prove extremely beneficial for mass propagation as well as conservation. 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.

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Authors contributions

NS and PR identified the research problem and designed the experimental study. PR and PC conducted the experimental study supervised by NS and RS. AS contributed in analysis of GC-MS and FTIR. Initial draft of manuscript was written by RS and AS. Manuscript was revised by PR and NS.

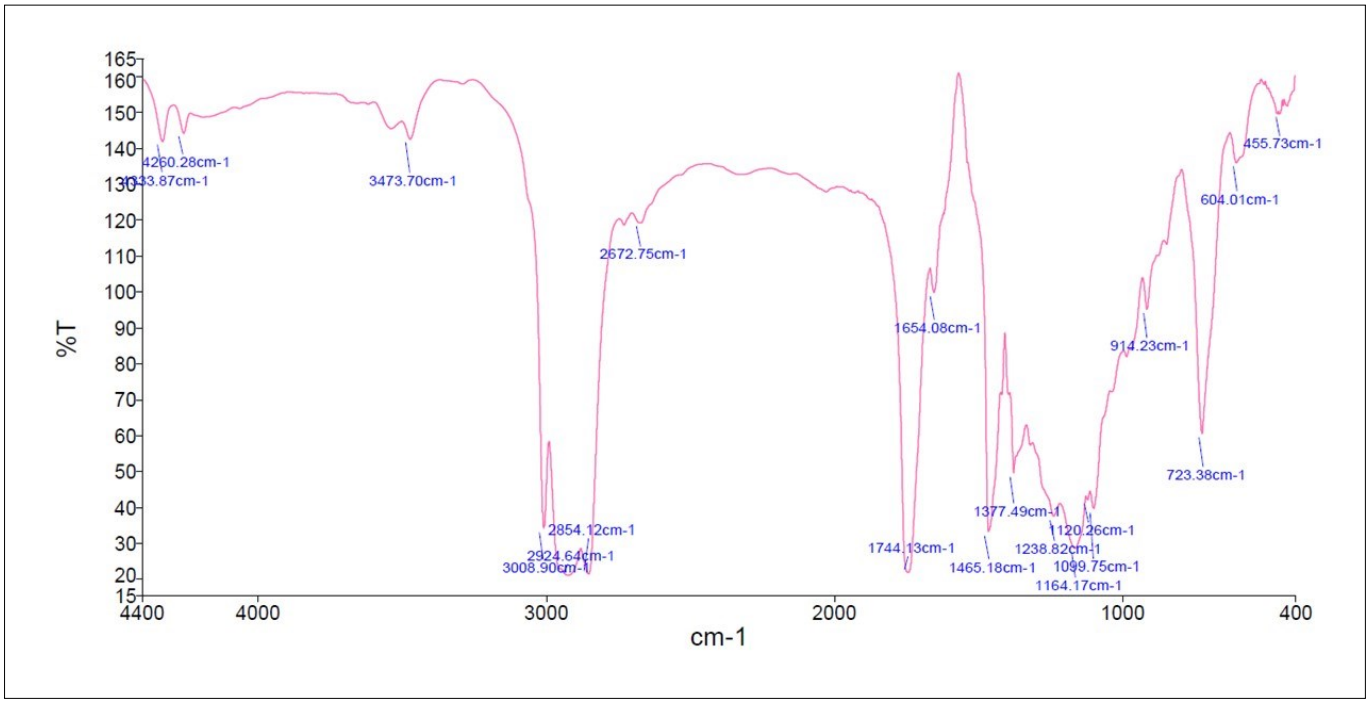

Fig. 4. FTIR analysis of oil extracted from seeds of *W. coagulans.*

Compliance with ethical standards

Conflict of interest: Authors declare that there exists no conflict of interest.

Ethical issues: None.

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