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Withania somnifera Seed Oil: A Potential Source of Unsaturated Fatty Acid from Western Rajasthan

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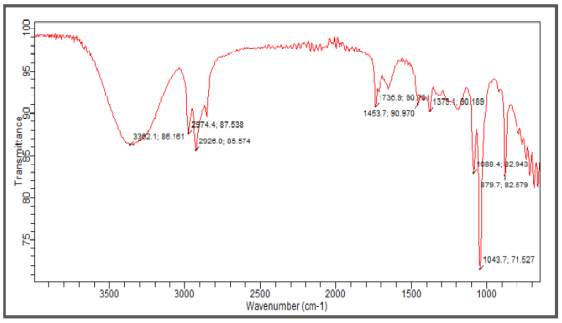
ABSTRACT

Plant seeds are important source of oils of nutritional, industrial and pharmaceutical importance. The suitability of oil for a particular purpose, however, is determined by its characteristics and fatty acid composition. Withania somnifera (commonly known as ashwagandha, winter cherry, poison gooseberry and Indian ginseng) is a short, tender perennial shrub found in the dry and arid regions of western Rajasthan (India). Withania somnifera has medicinal, ecological values in arid and semi-arid ecosystem. All parts of the plant including ripe fruit, leaves, root, stem and flowers may be used for the treatment of stress, anxiety, depression and insomnia and in rheumatic pains. In present work we studied fatty acid composition in seed oil of Withania somnifera from arid region of western Rajasthan. It was observed that Withania somnifera seed oil contain about 14% oil. The fatty acid composition of the oil sample was analyzed by gas chromatography coupled with mass spectrometer. The total amount of saturated, monounsaturated and polyunsaturated fatty acids was found 21.2%, 20.5% and 57.5% respectively. The major fatty acid present was Linoleic acid(56.7%) also named as Omega-6followed by oleic acid (20.5%) and palmitic acid(17.8%), stearic acid(3.1%), linolenic acid(0.8%) and myristic acid (0.3%) were present in very low amounts.

Graphical Abstract



IR Spectra of FAME

Keywords: Withania somnifera, Fatty acids, Omega-6, Linoleic acid, Oleic acid, Palmitic acid.

INTRODUCTION

Withania somnifera, known commonly as ashwagandha [1], Indian ginseng [2], poison gooseberry [3] or winter cherry is a plant in the solanaceae or nightshade family [4]. The solanaceae family is comprised of 84 genera that include about 3,000 species, scattered throughout the world. Members of this family are generally annual shrubs. The genera *Withania* and *Physalis* play an important role in the indigenous medicine e.g. in the Unani and Ayurvedic systems. Several other species in the genus *Withania* are morphologically similar [5].

The twenty-three known Withania species are widely distributed in the drier parts of tropical and subtropical zones, ranging from the Thar desert [6] the Mediterranean region and northern Africa to Southwest Asia [7-10]. In Withania genus there are three species are found in India namely, *W. somnifera*, *W. coagulans* and *W. obtusifolia*. It is distributed in tropical and subtropical region. This grows in the temperate climates. It grows up to a height of 5000 feet in Western India and Himalayan regions. One plant survives for up to 4 to 5 years. It remains green for 12 months near big trees and ponds. This species is a short, tender perennial shrub growing 35-75 cm tall. *Tomentosa* branches extend radially from a central stem. The leaves are bowl shaped, small and without thorns. Its stem contains fiber like texture. The leaves are oval shaped, 2 to 4 inches long and contain fiber. The flowers are blooming at the base of the stems are small, somewhat long with chimney shape and yellowish green in color. The flowers bloom from the base of the leaves and become red when wipe. The ripe fruit is orange-red. The species name *somnifera* means "sleep-inducing" in Latin. The seeds are small, heart shaped, smooth and flat. The roots are rough, white from within, strong, transparent, thick and one to one and half feet long. Crushing the fresh leaves and raw roots gives urine like smell and hence named Ashwagandha, Ashwa means ' horse' and gandha means 'smell'. It is an important medicinal plant that has been used in Ayurvedic and indigenous medicine for over 3000 years [11]. In view of that varied potential therapeutic usage. The plant was traditionally used to promote youthful vigor, endurance, strength, and health, nurturing the time elements of the body and increasing the production of vital fluids, muscle fat, blood, lymph, semen and cells. Ashwagandha is used for arthritis, anxiety, trouble sleeping (insomnia), tumors, tuberculosis, asthma, a skin condition marked by white patchiness (leukoderma), bronchitis, backache, fibromyalgia, menstrual problems, hiccups, and chronic liver disease. Ashwagandha is also used as an adaptogen to help the body cope with daily stress, and as a general tonic.

The similarity between these restorative properties and those of ginseng roots has led to Ashwagandha roots being called Indian ginseng [12]. It also helps counteract chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotency, premature ageing, emaciation, debility, and muscle tension. The leaves of the plant are bitter in taste and used as an anthelmintic. The infusion is given in fever. Bruised leaves and fruits are locally applied to tumors and tubercular glands, carbuncles and ulcers [13-14]. The roots are used as a nutrient and health restorative in pregnant women and old people. The decoction of the root boiled with milk and ghee is recommended for curing sterility in women. It is also used for fertility problems in men and women and also to increase sexual desire [15]. The roots are also used in constipation, senile debility, rheumatism, general debility, nervous exhaustion, loss of memory, loss of muscular energy and spermatorrhoea [16]. It has been used owing to its antioxidant property to treat rheumatism and neurodegenerative disorders [17]. Recently, it has been developed as an immunosuppressive agent for the inflammatory diseases [18]. *W. somnifera* may possess anti-inflammatory properties. Cultures of cartilage from patients with osteoarthritis and rheumatoid arthritis have been used to demonstrate *W. somnifera's* protective effects on chondroplasts [19-22]. Ashwagandha exhibited stimulatory effects on the generation of cytotoxic T lymphocytes and demonstrated the potential to reduce tumor growth [23].

MATERIALS AND METHODS

Materials: Seeds were collected at maturity from arid and semiarid region of western Rajasthan (India). The whole seed was used for the analyses, they were freeze-dried and ground to powder using mortar and analyzed immediately.

Oil Extraction: Oil extraction was performed from grounded seeds of *W. somnifera* with light petroleum ether (40-60^{o}C) using Soxhlet extraction technique. The solvent was removed completely under vacuum using rotary evaporator. The analytical values of seed and seed oil were determined according to the standard American Oil Chemist Society (AOCS) methods [**24**]. Methyl esters of oil were prepared using direct analytical TLC test [**25**], 2,4DNP TLC test [**26**], Halphen test [**27**], picric-acid TLC test [**28**], and alkaline picrate test [**29**] were also performed for indication of any unusual fatty acid.

Percentage Yield: The oil which was recovered by complete distilling of most of the solvent on a heating mantle was transferred to measuring cylinder. The measuring cylinder is then placed over water bath for complete evaporation of solvent for about 2-3 h in accordance with the method reported (Pant et al., 2006) and volume of the oil was recorded and expressed as oil content (%) as follow.

$$\text{Oil content} = \frac{\text{Oil weight}}{\text{Sample weight}} \times 100$$

After the oil had been obtained and its percentage of oil content is calculated the same is subjected to physiological test such as acid value test, iodine value test and saponification value test, chemical analysis of seed oil.

Acid Value: 2 g of the pure oil was weighed accurately by transfer method into a (250 mL) conical flask. Neutral ethanol (20 mL) was added by means of a pipette and the flask heated on a steam bath for 3-min. Then the flask was cooled and the contents titrated with 0.1N alcoholic potassium hydroxide solution using phenolphthalein as an indicator. A blank titration was also conducted side by side.

Iodine Value: Oil (0.2 g) was weighed accurately by transfer method into a 250 mL iodine flask and dissolved in chloroform (20 mL). Wij's reagent (20 mL) was added by means of a pipette. The flask was stoppered and kept in darkness for 1h. with intermittent shaking. Then 15% of potassium iodide solution (10 mL) and 50 mL of distilled water were added to the flask and mixture was shaken well. The liberated iodine was titrated with 0.1 N sodium thiosulphate solution using fresh starch solution as indicator. A blank titration was also conducted side by side [**30**].

Saponification Value: 2 g of oil was weighed accurately by transfer method into a 250 mL round bottom flask. Freshly prepared 0.5 N alcoholic potassium hydroxide solution (25mL) was added to the sample by means of pipette and the mixture gently refluxed on a water bath using an air-condenser for one hour. Then the flask was cooled, the condenser tip washed with little distilled water and the contents were titrated with 0.5 N hydrochloric acid solution using phenolphthalein as indicator.

Determination of Physical Parameters: The physical properties of the liquids depend on their chemical composition, pressure and temperature. An unknown oil physical parameter can be measured and compared with the literature and standard values, the oil can then be identified.

Specific Gravity: Density bottle was used in determining the specific gravity of the oil. A clean and dry stoppered bottle of 25 mL capacity was weighed (W0) and then filled with the oil stoppered and reweighed to give (W1). The oil was substituted with distilled water after washing and drying the bottle and weighed to give (W2). The expression for specific gravity (Sp.gr) is:

$$Sp.gr = \frac{W1 - W2}{W2 - W0}$$

Where, W0 = weight of dry empty density bottle; W1 = weight of density bottle + oil; W2 = weight of density bottle + distilled water [31-32].

Viscosity: A clean, dried Ostwald viscometer with a flow time above 200 sec for the fluid to be tested was elected. The sample was filtered through a sintered glass (fine mesh screen) to eliminate dust and other solid materials in the liquid sample. The viscosity meter was charged with the sample by inverting the tube's thinner arm into the liquid sample and suction force drawn up to the upper timing mark of the viscometer, after which the instrument was turned to its normal vertical position. The viscometer was placed into a holder and inserted to a constant temperature bath set at 29°C. The oil was kept for approximately 10 min for it to come to the bath temperature of 29°C. The suction force was then applied to the thinner arm to draw the sample slightly above the upper timing mark. The afflux time was recorded by timing the flow of the sample as it flowed freely from the upper timing mark to the lower timing mark [33]. Three recordings were taken and using water as a standard, a viscosity for each sample was recorded.

Refractive Index: Abbe's refractometer was used in the determination of refractive index. This instrument measures the index of refraction by measuring the critical angle of total reflection. In this case, a few drops of the sample were transferred into the glass slide of the refractometer. Water at 30°C was circulated round the glass slide to keep its temperature uniform. Through the eyepiece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index. The refractometer was calibrated using distilled water where the refractive index of water at that temperature was obtained. The procedure was repeated by using castor oil from the six regions and their refractive indices were obtained at 30°C. The mean value for each region was noted and recorded as the refractive index.

pH: In a dry clean 25 mL beaker, 2 g of the sample was placed followed by 13 mL of hot distilled water and the mixture was stirred slowly. The mixture was then cooled in a cold-water bath to 25°C. The pH electrode was standardized with buffer solutions (pH 4 and 7) and the electrode immersed into the sample where an average pH of three recordings per sample were recorded.

Preparation of Mixed Fatty Acid: The fatty acid mixture was obtained by hydrolysis of oil and fats. Firstly took 2 g of oil sample is an oven dried round bottom flask then saponified with 2.5 mL of 1N standard alcoholic NaOH solution and 10 ml alcohol as solvent and refluxed over water bath(70-80 ^{o} C) for 2 h monitored with TLC. The final mixture contained both saponified and unsaponified matter. Mixture was further diluted with 30 ml double distilled water. The saponified matter was removed by repeated washing with diethyl ether in a separating funnel. The upper organic (ether) layer contained unsaponified matter which was taken in another beaker. After rotatory evaporation diethyl ether is recollected. The lower aqueous solution, contained salt fatty acids was acidified with dilute hydrochloric acid (6N HCl). Fatty acid was extracted by repeated washing of diethyl ether from this mixture of the lower aqueous layer discarded and the upper combined ether extract which contained mixture of fatty acid was collected in oven dried flask. After evaporate excess ether, these mixed fatty acid (MFA'S) were further washed with double distilled water and dried over anhydrous Na_2SO_4 clean and pure MFA'S were collected. The whole procedure was monitored with help of TLC. TLC plates were prepared by coating approx.0.05 mm of layer silica gel on glass plate. The mobile phase used in a consisting of mixture of petroleum ether; diethyl ether; acetic acid (70:29:1) the spot was made visible in iodine chamber.

The mixture of fatty acids was further derivatized into their methyl esters and further quantized by HPLC and GC-MS (gas chromatography-mass spectrometer). For the preparation of fatty acid methyl ester (FAME) mixed fatty acid (MFA'S) were refluxed in a round bottom flask with excess methanol (1:6) on a water bath (100^{o}C) for the approx. 1-2 h using 1% H_2SO_4 as catalyst. After completion of

esterification (monitored by TLC plate) assembly was removed and flask was cooled at room temperature evaporate excess solvent and cooled over ice-bath and 30 mL double distilled water was added in it. Stirred well and fatty acid methyl esters (FAME) were extracted with diethyl ether. The lower aqueous layer was discarded and upper combined ether extracted was collected in another flask. After evaporation of solvent it was dried over anhydrous Na_2SO_4.Thus, obtained FAME were collected and stored at low temperature for further analysis.

RESULTS AND DISCUSSION

The oil yield is 17.2% and different chemical properties like as acid value, saponification value, iodine value, protein content give structural stability in table 1. The oil contents and physiochemical properties of oil *Withania somnifera* seed from arid zone are presented in table 2. Fatty acids are the primary component of oil and fats. The saturated fatty acids were identified as palmitic acid (17.8%), stearic acid (3.1%) and myristic acid (0.3). The major Poly-unsaturated fatty acid was linoleic acid (56.7%), linolenic acid (.08%) present. The Mono-unsaturated fatty acid was oleic acid (20.5%). The fatty acid composition indicates the presence of higher amount of unsaturated fatty acids (77.5%) compared to saturated fatty acids (21.2%).

Table 1. Physico-chemical properties of the seeds and oil of *Withania somnifera*

Characteristic	Value
Oil content (%)	17.2
Protein content (%)	12.8
Moisture (%)	3.8
Specific gravity	0.871
Iodine value (wij's)	158
Acid value	7.02
Saponification value	132
Unsaponifiable matter	3.6

Table 2. Fatty acid composition of the oil from seed of *Withania somnifera*

Fatty acid		Common / IUPAC Names	Percentage Composition
Linoleic acid	C 18:2	Omega-6	56.7
Linolenic acid	C 18:3	Omega-3	0.8
Oleic acid	C 18:1	Omega-9	20.5
Palmitic acid	C 16:0	Hexadecanoic acid	17.8
Stearic acid	C 18:0	Octadecanoic aid	3.1
Myristic acid	C 14:0	Tetradecanoic acid	0.3

The composition of fatty acid obtained from GLC and GC-MS were in good agreement and confirm the position of double bonds. The IR spectra of FAME exhibited peak at 1737 cm^{-1} for carbonyl ester besides the usual peaks for hydrocarbon end, confirmed the absence of any other functional group in figure 1. The IR and UV-Vis spectra of FAME exhibited no absorption band for the presence of any trans unsaturation and conjugation. The significance of fatty acid analysis has gained much attention because of the nutritional and health implications. The most common procedure for the analysis is the conversion of fatty acid components to methyl esters in order to improve their volatility. Methyl esters of fatty acids are separated and analyzed by high performance liquid chromatography.

The acid value (7.02 mg g^{-1}) of sample obtained in the present study is in the less range with the value obtained by S. N. Mishra *et al.* (1994); (13.16 mg g^{-1}). The oil shows quite a high saponification value (327.4 mg g^{-1}), which was less obtained by S. N. Mishra (1994), (185.3 mg g^{-1}). The difference

in the saponification value may be due to the quality of the oil and environmental conditions. The Iodine value (158) is obtained with the almost same value (144.33) [**34**].

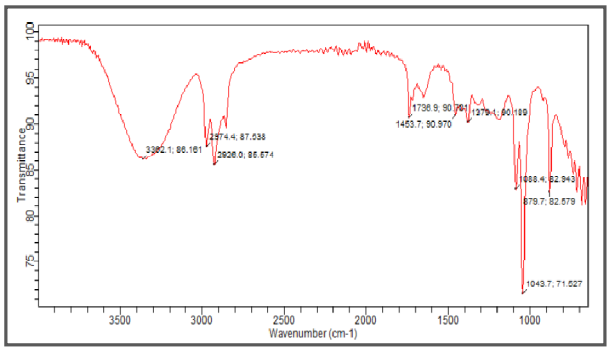


Figure 1. IR Spectra of FAME

APPLICATION

The *Withania Somnifera* seed oil is good source of essential Omega-6 fatty acid which is good for human health and useful for industrial uses, byproducts of oilseeds and plant parts could be used as animal feedstock and as biomass.

CONCLUSION

The seed oil is good source of essential Omega-6(linoleic acid C18:2) fatty acid. Linoleic acid is the most significant PUFA in human diet, as it prevents heart and vascular diseases. Therefore, the seed oil is good for human health. Linoleic acid contains high PUFAs (>55%) content, could be proved as alternate source of paints-varnishes. Apart from human consumption and industrial uses, byproducts of oilseeds and plant parts could be used as animal feedstock and as biomass in various applications.

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