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ISOLATION AND PHYSICO-CHEMICAL CHARACTERIZATION OF SEED OIL FROM *RADERMACHERA XYLOCARPA*

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ABSTRACT

Seeds from *Radermachera xylocarpa*, a species from Bignoniaceae were studied for oil content and physico-chemical characteristics for the first time. The seeds were found to contain oil (12.6 %), moisture and volatiles (0.15 %) along with protein (26.5 % on dry basis) and carbohydrate (49.7 % on dry basis). The extracted oil was analyzed for parameters such as acid value (33.35), iodine value (165.8), peroxide value (8.55 ppm), saponification value (193.7), density (0.9046 g/cm^3 at 40 ºC), specific gravity (0.9085 at 40 ºC) and kinematic viscosity (20.0 Cst). The unsaponifiables and the phosphorous content were found to be 3.49 % and 237 ppm respectively. The fatty acid composition of the oil showed that the oil was a good source of alpha linolenic acid (37.8%) and linoleic acid (36.5 %) as major fatty acids followed by palmitic (12.4 %), oleic (7.4 %) and stearic (4.8 %) acids. The oil can be projected as a newer source of alpha linolenic acid which could find potential applications in nutraceutical and other industrial applications.

KEY WORDS: *Radermachera xylocarpa*, Seed oil, Physico-chemical properties, Fatty acids, Alpha linolenic acid.

INTRODUCTION

Tree borne oil seeds have been the topic of interest due to the high imports of vegetable oils in India. It was found that the imports of vegetable oils increased rapidly during the last few decades and during 2013-14, India alone imported more than 11.61 million tonnes of vegetable oils for the edible and industrial applications of the country (http: //www. seaofindia.com / publications). Seeds from lesser known plant varieties can be important sources of oils with potential industrial and nutritional applications. In view of the increasing imports, there have been several studies aiming at the utilization of forest trees and plants as a source of unconventional oil for edible and non-edible applications. In an attempt to screen the unexploited or lesser known tree borne seeds for oil content and fatty acid composition, we have identified *Radermachera xylocarpa* as one of the species which was not studied for seed oil compositional studies.

Radermachera xylocarpa is a middle size deciduous tree which belongs to family Bignoniaceae. It is reported to be mainly distributed in areas of Deccan, Konkan, Khandesh and Western Ghats of India (Shetgiri *et al.*, 2001). The tree is known for its wood which is described as tough and elastic and used for general cabinet work. The oil from the wood is reported to be effective in cutaneous diseases (Chopra *et al.*, 1956). Previous literature on the phytochemical investigation of this species revealed the presence of dinatin-7-glucuronide in the leaves (Subramanian *et al.*, 1972). The roots were reported to contain O-acetyl Oleanolic acid and radermachol which are known to exhibit antitumor and anti-inflammatory activities respectively. (Desai *et al.*, 1977 and Joshi *et al.*, 1984]. It is reported that three spoonful of stem barkdecoction is administered once a day for 10 days for treatment of rheumatism as ethnomedicine in some rural areas of south India. (Ratna Manjula *et al.*, 2013). It is reported that the paste of seeds and root water is administered orally and also applied on the wounds due to snake and insect bites (Misra, 2004). As the paste of seeds is also used for some treatments, it is of interest to know about the seed oil composition. Seed oils are known to be having industrial, nutritional and pharmaceutical importance. However, no physico-chemical data is reported on the seed oil of *Radermachera xylocarpa* and its characterization in the literature so far. Therefore, in the present study, we report the isolation and physico-chemical characterization the oil from seeds of *Radermachera xylocarpa*.

MATERIALS & METHODS

Materials

Seeds of *Radermachera xylocarpa* were supplied by AP Forest Department, Hyderabad, India which were collected during the month of March 2015. All the chemicals and solvents were purchased from M/s. Sd Fine Chemical Co. Ltd. (Mumbai, India) and were of highest grade of purity.

Methods

Physical and proximate analysis of seeds Physical and proximate analysis of the seed was carried out following reported protocol (Montes *et al.*, 2011).

Extraction of oil

The dried total seed of Radermachera xylocarpa were ground to powder and in an electrical grinder and oil was extracted in a Soxhlet apparatus using hexane as solvent. The oil content was determined as a percentage of the

extracted oil to the sample weight (w/w). The extracted oil was stored at 4 °C in a glass bottle under nitrogen blanket for further analysis.

Physico-chemical analysis of oil

Physico-chemical analysis of the oil such as acid value (AOCS, 1994), iodine value (AOCS, 1994), saponification value (AOCS, 1994), peroxide value (AOCS, 1994), Moisture and volatiles (AOCS, 1994) unsaponifiable matter (AOCS, 1994), RI at 40°C (AOCS, 1994), p-anisidine (AOCS, 1994), density (ASTM method D 4052), Tocopherol (AOCS, 1994) and colour (AOCS 1994). Colour was determined using Lovibond Tintometre (Lovibond model PFX 995) and density was determined using Anton Paar density meter (Type DMA4500M, Austria) at 40 °C. The samples were analyzed in triplicate and the average of the three measurements is reported. Phosphorous content was estimated following IUPAC method (Pacquot and Hautfenne, 1987). The Kinematic viscosity (Cst) was measured following ASTM standard method D-445 [Cannon instrument Co., state college, PA].

Fatty Acid Composition by Gas Chromatography

The fatty acid composition of the extracted oil was determined by gas chromatography (GC). The oil was converted to fatty acid methyl esters using methanol-sulphuric acid (2 % v/v) reagent. GC analysis of the fatty acid methyl esters (FAME) was performed using a Agilent 6890 gas chromatograph coupled to a flame ionization detector (FID) equipped with a DB 225 capillary column (30 m x 0.25 mm x 0.25 µm, (J & W Scientific, USA). The column temperature programme was 2 min at 160 °C, 5 °C/min to 230 °C and 20 min at 230 °C. The injector temperature was 230°C with a split ratio of 10:1. The carrier gas was N_2 at a flow rate of 1 mL/min. The detector temperature was 270°C with air and hydrogen flow rates of 300 mL/min and 30 mL/min, respectively. The fatty acids were identified by comparing the retention times with mixture of standard FAMEs, C4-C24 (Supelco, USA). Each FAME sample was analyzed in duplicate and average values are reported.

Analysis of Unsaponifiable Matter by Gas Chromatography

GC analysis of unsap matter was performed using a Agilent 6850 gas chromatograph coupled to a flame ionization detector (FID) equipped with a HP-1 capillary column (30 m x 0.25 mm x 0.25 µm, 100% dimethyl polysiloxane stationary phase material; company, J & W Scientific, USA). The column temperature programme was 2 min at 150 °C, 10 °C/min to 300 °C and 20 min at 300 °C. The injector temperature was 280°C with a split ratio of 50:1. The carrier gas was N_2 at a flow rate of 1 mL/min. The detector temperature was 300°C with air and hydrogen flow rates of 300 mL/min and 30 mL/min, respectively. The unsaponifiable matter was also analyzed by GC-MS on Agilent 6890N Gas Chromatograph connected to Agilent 5973 mass selective detector at 70 ev (m/z 50-550; source at 230°C and quadruple at 150°C) in the EI mode with a HP-5 ms capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The oven temperature was programmed at 160°C for 2 min, increased to 230°C at 5°C/minute and hold for 10 minutes at 230°C further continued up to 300°C. The carrier gas, helium was used at a flow rate of 1.0 ml/min. The inlet temp was maintained at 300°C and split ratio was 50:1. Structural assignments were based on interpretation of mass spectrometric fragmentation and confirmed by comparison of retention times as well as fragmentation pattern of authentic compounds. The unsaponifiables were identified by comparing the retention times with those of mixture of standard compounds (Vitapherol, INDIA). Each sample was analyzed in duplicate.

High performance liquid chromatography (HPLC) analysis for TAG composition

The reversed phase HPLC analysis was performed on waters semi prep HPLC equipped with an evaporative light scattering detector, waters 2424 (ELSD) with a quaterny pump. The samples (about 10 µl of 1 mg/ml concentration) were injected in to the X bridge RP column (C18-RP). The molecular species of TAGs were eluted within 10 min using mobile phase of acetone (100%) at a flow rate of 1 ml/min. The operating conditions for ELSD were: drift tube temperature 50ºC, flow of nitrogen 50 psi with gain 100. The molecular species of oils were identified by their equivalent carbon numbers (ECN) and the elution order was tentatively predicted according to reported method (Reena et al., 2009).

Analysis of Tocopherols and tocotrienols (tocols)

Tocols were analysed by High performance liquid chromatography (HPLC) with fluorescence detection according to AOCS Official method Ce 8-89 [AOAC, 1994]. The HPLC analysis was performed on an Agilent 1100 series instrument equipped with fluorescence detector. 20 µL of the sample (4 mg/mL concentration) was injected into a normal phase silica column (LiChrospher Si-60 (250 x 4.0 mm having a mean particle size of 5 µm from Merck) and the fluorescence detector was set at an excitation wavelength of 292 nm and emission wavelength at 330 nm. The isocratic mobile phase consisting of hexane and isopropyl alcohol (99.5:0.5, vol/vol) was used at a flow rate of 1.0 ml/min. The total tocopherol and tocotrienol content was expressed as micrograms per gram (µg/g).

RESULTS & DISCUSSION

The physical characteristics and proximate analysis of the seed of *Radermachera xylocarpa* are shown in Table 1.

TABLE 1. Physical analysis of seed

Properties	*R. xylocarpa* seed
100 seed weight (g)	0.6311 ± 0.1
Length (cm)	1.78 ± 0.21
Width (cm)	0.5 ± 0.01
Relative density (g/cm3)	0.012 ± 0.1
Apparent density (g/cm3)	0.07 ± 0.1
Protein (%)	26.53
Ash (%)	2.6
Moisture (%)	8.5
Carbohydrate (%)	49.75

Values are mean ± standard deviation of triplicate determinations

The physico-chemical characteristics of the *Radermachera xylocarpa* seed oil revealed that it contains about 12.65% oil. The seeds were small in size with very light weight. The carbohydrate content was obtained by subtracting the values of other parameters. The other chemical characteristics of the oil like acid value, moisture content, iodine value, sap value, peroxide value, p-anisidine value, unsap matter and phosphorous content along with physical characteristics such as colour, density, refractive index were determined according to standard protocols and are listed in Table 2.

TABLE 2. Physico-chemical characteristics of *R.xylocarpa* seed oil

Characteristics	*R. xylocarpa* seed oil
Oil content (Wt %)	12.65 (±) 0.21
Moisture content (Wt %)	0.15 (±) 0
Acid value	33.35 (±) 0.07
Iodine value	165.8 (±) 0.14
Saponification value	193.75 (±) 0.07
Peroxide value (ppm)	8.55 (±) 0.07
p- Anisidine value	10.05 (±) 0.07
Unsaponifiable matter (Wt %)	3.49 (±) 0.01
Phosphorous content (ppm)	237.45 (±) 0.7
Colour in 10 mm cell	36.5 (3.5 R, 19Y) (±) 0.01
Density at 40˚C	0.9046 (±) 0
Specific gravity at 40˚C	0.9085 (±) 0
Viscosity at 40˚C	20.0 CST (±) 0
Refractive Index at 40˚C	1.4664 (±) 0
Tocols (ppm)	78 (±) 0

Values are mean ± standard deviation of triplicate determinations

The fatty acid composition was determined by gas chromatography and is shown in Table 3. The fatty acid composition of the seed oil suggests that the oil is a good source of alpha linolenic acid which was the major fatty acid present (37.8%). ALA was followed by linoleic acid (36.4%) and palmitic acid (12.4%) as the next major fatty acids. *R. xylocarpa* seed oil can be projected as a source of ALA which is reported to have many nutraceutical applications. Among the vegetable oil sources, flax seed oil is reported to possess highest amount of linolenic acid which is being used as a n-3 supplement in food products (Ankit Goyal *et al.,* 2014).

TABLE 3. Positional distribution of fatty acids (wt %) of *R.xylocarpa* seed oil

Fatty Acid	Composition (wt %)		
	Total	*Sn*-1,3	*Sn*-2
16:0	12.4	16:2	4.8
16:1	0.2	0.3	-
18:0	4.8	5.9	2.6
18:1	7.4	3.5	15.2
18:2	36.4	32.4	44.4
18:3	37.8	40.6	32.2
20:0	0.8	0.9	0.7
20:1	0.2	0.3	-

Oleic and stearic acid were the minor fatty acids present in 7.4 and 4.8 % respectively along with traces of other fatty acids. Saturated fatty acids were present in about 18% and unsaturated fatty acids were found to be about 82% which suggests that the oil has to be stored properly. Due to the higher content of linolenic and linoleic acid, the oil can be

projected as a renewable source for applications in paints, varnishes, etc compared to petroleum based oils (Przybylski Roman, 2005). In addition, ALA is reported to have beneficial effects on cardiovascular diseases and it was found that ALA has cardioprotective effects (Pierce, 2014). There have been reports on seed oil studies where the oil content was low, but the presence of ALA was about 30% as in the case of raspberry seeds where the oil content was found to be 10.7% (Oomah *et al.,* 2000). Further the oil was studied for its positional distribution of fatty acids on the triglyceride by pancreatic lipase hydrolysis with 1, 3-specific lipase to yield free fatty acids and 2-monoacylglycerols. The fatty acid distribution over the triglyceride is shown in Table 3.

It was interesting to observe that the seed oil of *R xylocarpa* showed the presence of linoleic acid in higher amounts compared to linolenic acid at *sn*-2 position. Normally, the unsaturated fatty acids are known to be distributed to a greater extent at *sn*-2 compared to *sn*-1 position. This was true in case of oleic and linoleic acids where as not in the case of linolenic acid as per present study which shows that *sn*-1 and 3 positions contained higher amounts of linolenic acid compared to *sn*-2 position. The oil was also studied for its triglyceride molecular species composition by RP-HPLC and the results are given in Table 4.

TABLE 4. Composition (% area) of TAG Molecular Species of *Radermachera xylocarpa* Seed Oil

ECN	Expected Molecular Species	%
C36	LnLnLn	8.4
C38	LnLLn	23.2
C40	LLLn	26.1
C42	LLL/LnLP/LnLO	22.4
C44	LLP/LLO	16.9
C46	PLP/POL/SLL	2.9

The major molecular species of the oil were found to be C38, C40 and C42 dominated by unsaturated fatty acids which constituted to about 71.7%. As the unsaponifiable matter was found to be about 3%, it was further analyzed by GC for quantitative analysis and the components were confirmed by GC-MS analysis and the results are shown in Table 5.

TABLE 5. GC Composition (wt %) of Unsaponifiable Matter

Component	%
Hydrocarbon	46.0
Squalene	0.6
Stigmasterol	1.2
Beta sitosterol	51.2
Beta Amyrin	1.0

It was found that major components were sterols and hydrocarbons along with traces of squalene in the unsaponifiable matter. Sterols constituted up to 52% in which beta sitosterol was about 51%. In hydrocarbons, it was observed that the chain length ranged from C12 to C30 with both saturated and unsaturated straight hydrocarbons.

CONCLUSION

In conclusion, the present study revealed seed oil of *Radermachera xylocarpa* as a newer source for oil. The fatty acid composition revealed that the oil was rich in alpha linolenic acid and linoleic acid followed by other common fatty acids in small amounts. Complete analysis of the seed oil was carried out and all the physico-chemical properties were similar to other vegetable oils.

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