

Isolation and physico-chemical characterization of *Butea parviflora* seed oil

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SUMMARY: The seeds of *Butea parviflora* were investigated for oil extraction and the oil was studied for complete physico-chemical properties. The fatty acid profile of the seed oil showed oleic acid (18:1) at 27.5%, linoleic acid (18:2) at 26.4%, palmitic acid (16:0) at 16.1% and behenic acid (22:0) at 14.1% as the major fatty acids. The physico-chemical characteristics of the seed oil were studied for parameters such as free fatty acids (0.71%), iodine value (76.2 g/100g), peroxide value (5.95 ppm), saponification value (177.32 mg KOH/g), unsaponifiable matter (0.82%), phosphorous content (197 ppm), triglyceride analysis, tocopherols, specific gravity and refractive index following standard procedures.

KEYWORDS: *Butea parviflora*; Fatty acids; Seed oil; Sterols; Tocopherols; Triterpenoid

RESUMEN: *Aislamiento y caracterización físico-química de aceites de semillas de Butea parviflora.* Se ha estudiado la extracción de aceite de semillas de *Butea parviflora* así como las características físico-químicas completas del aceite. El perfil de ácidos grasos está compuesto de un 27,5% de ácido oleico (18:1), 26,4% de ácido linoleico (18:2), 16,1% de ácido palmítico (16:0) y 14,1% de behénico (22:0) como principales ácidos grasos. Se han estudiado las características físico-químicas del aceite de las semilla tales como los ácidos grasos libres (0,71%), índice de yodo (76,2 g/100 g), índice de peróxido (5,95 ppm), índice de saponificación (177,32 mg KOH/g), materia insaponificable (0,82%), contenido en fósforo (197 ppm), análisis de triglicéridos, gravedad específica e índice de refracción, siguiendo los protocolos recogidos en procedimientos estandarizados.

PALABRAS CLAVE: *Aceite de semilla; Ácidos grasos; Butea parviflora; Esteroles; Tocoferoles; Triterpenoides*

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1. INTRODUCTION

The genus *Butea* of the Fabaceae family includes *Butea monosperma*, *Butea parviflora*, *Butea minor* and *Butea superba* and is widely distributed throughout India (Wealth of India, 1998). Among these species *Butea monosperma*, popularly known as Palash, is widely studied for its medicinal and economic value. There are several reports on the medicinal and pharmacological uses of *Butea monosperma* in Ayurveda, Unani and Homeopathic medicine for various conditions (Madhavi, 2013). Seed oils from forest origin are gaining importance as focus is now shifted on the underutilized seeds as they are renewable raw materials for various edible and non-edible applications. A literature review revealed that previous research on the chemical examination of the seed oil from *Butea parviflora* showed that it contained palmitic, stearic, lignoceric, oleic, and linoleic acids and the unsaponifiable matter of the oil was found to contain only β -sitosterol (Garg, 1971). However, there were no reports on the complete physico-chemical characterization of the seed oil from *Butea parviflora* focusing on the unsaponifiable fraction, which is a potential source for minor bioactive components. As the import of vegetable oils is high in India, there is an increased interest in investigation on tree borne seed oils for utilization in edible and industrial applications, hence seeds from lesser known plant varieties can be important sources of oil (Amit, 2012). Information on the fatty acid composition, minor constituents and other physico-chemical characteristics of tree borne oil seeds is important to judge the potential usefulness of such oils for edible and non-edible applications. The oils from seeds are known to be natural sources for fatty acids, carotenoids, phytosterols, squalene and other micro-nutrients with a wide range of biological activities for applications in the cosmetic and pharmaceutical sectors (Górnas *et al.*, 2013). The increasing awareness of the nutritional effects of fatty acids in the diet along with the demand for vegetable oils has prompted many research groups to study and characterize newer or lesser known vegetable oils for fatty acid profile and other components (Paz *et al.*, 2014; Anwar *et al.*, 2006; Kaki *et al.*, 2015). In view of these observations, and as a part of a screening program on unknown and lesser known tree borne seeds for oil content and composition, we have identified *Butea parviflora* seed and its oil for complete physico-chemical studies.

2. MATERIALS AND METHODS

2.1. Plant material

The *Butea parviflora* seeds were supplied by the Regional Center for Development Cooperation,

Bhubaneswar, Odisha State, India. Standards of tocopherols and tocotrienols and HPLC grade solvents were purchased from Merck (Darmstadt, Germany). All the chemicals and solvents were purchased from M/s. Sd Fine Chemical Co. Ltd. (Mumbai, India) and were of the highest grade of purity.

2.2. Extraction of oil

The seeds of *Butea parviflora* were oven dried in a Cintex precision hot air oven (Mumbai, India) at 50–55 °C for 5 hours. The kernels from dried seeds were ground to powder in an electrical grinder and the oil was extracted in a Soxhlet apparatus for 6 hours using hexane as solvent according to the AOCS method (Firestone, 2013). 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 a nitrogen blanket for further analysis.

2.3. Physico-chemical analysis of oil

Free fatty acids, iodine value, saponification value, peroxide value, unsaponifiable matter, tocols, density, refractive index and color were measured following official methods of AOCS (Firestone, 2013). Phosphorous content was estimated following the IUPAC method. The Kinematic viscosity (Cst) was measured following the ASTM standard method (www.astm.org).

2.3.1 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 as described in the literature (Christie, 1982). GC analysis of the fatty acid methyl esters (FAME) was performed using an 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 μ m, (J&W Scientific, USA). The column temperature program 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 N₂ 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 a mixture of standard FAMES, C4-C24 (Supelco, USA). Each FAME sample was analyzed in duplicate and average values are reported.

2.3.2 Regio-specific fatty acid analysis of *Butea parviflora* oil

The fatty acid distribution of triacylglycerols in the extracted oil was determined by regio-specific hydrolysis using pancreatic lipase following a reported method (Christie, 1982). Briefly, oil (about 20 mg) was taken in a mixture of tris buffer (1M; pH 8.0; 4 mL), calcium chloride solution (2.2%; 0.4 mL) and 0.05% bile salt solution and the mixture was shaken for 1 min at 40 °C. Pancreatic lipase (porcine pancreatic lipase, crude type II, 10 mg) was added to the contents and stirred for 3 min at 40 °C. Ethanol (1 mL) followed by HCl (1.5 mL of 6N solution) were added and the products were extracted with diethyl ether (2 x 10 mL), washed until neutral and dried over anhydrous sodium sulphate and concentrated. The hydrolysis product mixture was purified using thin-layer chromatography (TLC) in a mobile phase of hexane/ethyl acetate/acetic acid (70/30/1, v/v/v) to separate the 2-monoglyceride band which was converted to methyl esters and analyzed by GC for the *sn*-2 position. The mean composition of each fatty acid in positions 1 and 3 is calculated from the intact triacylglycerol using the equation.

$$\text{Positions 1 and 3} = \frac{3 \times [\text{TAG}] - [\text{sn-2}]}{2}$$

All analyses were conducted in duplicate and average values were calculated.

2.3.3 Determination of sterols and terpenoids via GC

The unsaponifiable matter obtained after complete saponification followed by extraction was analyzed by gas chromatography. A gas chromatographic analysis was performed using an 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 N₂ 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 unsaponifiables were identified by comparing the retention times with those of mixture of standard compounds (Vitapherol, India).

2.3.4 Determination of triglycerides via RP-HPLC/ELSD

The triglyceride profile was determined using the reversed phase HPLC which was performed on Waters HPLC equipped with an evaporative

light scattering detector, Waters 2424 (ELSD) with a quaternary pump. The samples (about 10 µL of 1 mg/mL concentration) were injected into two reversed phase columns (XBridge column from Waters; C18-RP; 4 x 250 mm; 5µm particle size) connected in series for better separation. The molecular species of TAGs were eluted in 15 min using a mobile phase of acetone (100%) at a flow rate of 1 mL/min. The operating conditions for ELSD were: drift tube temperature 55 °C, flow of nitrogen 50 psi with a gain of 100. The triglyceride molecular species of the extracted oil were identified by their equivalent carbon numbers (ECN) and the elution order was tentatively predicted according to fatty acid composition (Reena *et al.*, 2009). The samples were analyzed in duplicate and the average value is reported.

2.3.5 Analysis of tocopherols and tocotrienols (Tocols)

Tocols in the oil were analyzed by High performance liquid chromatography (HPLC) with fluorescent detection according to the AOCS Official Method Ce 8-89 (Firestone, 2013). The HPLC analysis was performed on an Agilent 1100 series instrument equipped with a fluorescent detector. The sample (20 µL of 4 mg/mL) 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). 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 fluorescent detector was set at an excitation wavelength of 292 nm and emission wavelength at 330 nm. The total tocopherol and tocotrienol content was expressed as micrograms per gram (µg/g).

3. RESULTS AND DISCUSSION

It was found that the *B. parviflora* seed is a good source of protein based on the nutritional composition. The results of the proximate analysis of the *B. parviflora* are given in Table 1 where it can be observed that protein, carbohydrate and oil are

TABLE 1. Proximate analysis of *Butea parviflora* seed.

Component	%
Moisture (%)	7.45 ± 0.07
Protein (%)	42.65 ± 0.49
Oil (%)	18.15 ± 0.07
Ash (%)	5.30 ± 0.14
Fiber (%)	4.50 ± 0.21
^a Carbohydrate (%)	22.40 ± 0.14

^aCarbohydrate: obtained by difference

TABLE 2. Physico-Chemical Properties of *Butea parviflora* seed oil.

Characteristic	<i>B. parviflora</i>
FFA (%)	0.71 ± 0.01
Iodine value (g/100g)	76.2 ± 0.28
Density at 40 °C (g/cm ³)	0.89883 ± 0
Specific gravity at 40 °C	0.90588 ± 0
Saponification value (mg KOH/g)	177.32 ± 0.7
Unsap matter (%)	0.82 ± 0.03
Peroxide value (ppm)	5.95 ± 0.07
Phosphorous content (ppm)	197 ± 2.8
Color (Y+5R in 1 "cell)	14.25 ± 0.35
Kinematic Viscosity at 40 °C (Cst)	42.49 ± 0
Refractive index at 40 °C	1.4648 ± 0

TABLE 3. Fatty acid composition (wt%) of the *Butea parviflora* seed oil

Fatty acid	TAG	<i>Sn-2</i>	<i>Sn-1,3</i>
16:0	16.15 ± 0.07	5.23 ± 0.07	19.4 ± 0.56
16:1	0.10 ± 0	0.10 ± 0	0
18:0	7.75 ± 0.07	3.61 ± 0.28	11.55 ± 0.21
18:1	27.50 ± 0.14	32.82 ± 0.14	22.35 ± 0.14
18:2	26.45 ± 0.07	48.12 ± 0.21	14.65 ± 0.35
18:3	0.10 ± 0	0.10 ± 0	0
20:0	2.0 ± 0	1.05 ± 0.07	3.15 ± 0.07
20:1	1.75 ± 0.07	3.34 ± 0.21	0.45 ± 0.07
22:0	14.05 ± 0.07	4.63 ± 0.21	20.85 ± 0.14
22:1	0.60 ± 0.14	1.0 ± 0	0.55 ± 0.07
24:0	3.60 ± 0	0	7.05 ± 0.21
SFA	43.55	14.52	62
UFA	56.45	85.48	38

TAG: triacylglycerols; SFA: saturated fatty acids; UFA: unsaturated fatty acids

the major constituents with lower amounts of ash and fiber.

The oil content in *B. parviflora* seeds was found to be 18.1% and the physico-chemical properties of the extracted oil are presented in Table 2.

It was observed that the oil content was similar to pear seed oil which is being projected as a new source of oil in China (Rui *et al.*, 2009). The oil yield was found to be just above goose berry seed oil which is reported to contain 15.6% oil in a recent report in which nine varieties of seeds with oil yield in the range of 11.8 to 28.5% were studied for their potential use in biodiesel and other biomedical applications (Górnas *et al.*, 2016). The free fatty acids and peroxide values of the extracted *B. parviflora* oil were found to be 0.7% and 5.9 ppm respectively which was observed to be in the range

of soybean oil (Hammond *et al.*, 2005). The oil was characterized for the fatty acid composition along with the positional distribution of fatty acids and the data is presented in Table 3.

The fatty acid composition showed that the oil is composed of 43.55% of saturated fatty acids and 56.45% of unsaturated fatty acids. The major fatty acids were oleic (27.5%) and linoleic (26.4%) acids in almost similar amounts followed by palmitic and behenic acids in 16.1 and 14% respectively. The content of oleic acid was observed to be similar to one of the cultivar of *Pyrus communis* seed oil where the percent of oleic acid for different cultivars ranged from 27.3 to 38.1% (Górnas *et al.*, 2016). Considerable amounts of very long chain saturated fatty acids such as behenic and lignoceric acids were found at 14.05 and 3.6%, respectively, in the present study. Previous studies on seed oil of *Butea parviflora* showed higher contents of oleic (40.62%), palmitic (20.89%), stearic (15.89%) and lignoceric acids (5.5%) with a lower content of linoleic acid (17.05%) without the presence of behenic acid (Garg, 1971). Literature reports also show that behenic and lignoceric acids were present in the seed oils of the *Albizia* genus which also belongs to the Fabaceae family. A recent report on the fatty acid composition of *Albizia lebbeck* and *Albizia saman* revealed that the seed oil of *Albizia saman* was found to contain 13.6 and 2.3% of behenic and lignoceric acid, respectively; whereas *Albizia lebbeck* showed lower contents of these fatty acids (Knothe *et al.*, 2015). The presence of very long chain fatty acids in seed oils in higher amounts is advantageous as it provides an opportunity to employ these oils in low calorie fat applications (Haumann, 1997). The studies involving structured triacylglycerols with long chain saturated fatty acids showed that in addition to reducing the calories, the structured lipids also exhibited hypocholesterolemic effects on animal models (Kanjilal *et al.*, 2013). Hence, oils containing long chain saturated fatty acids can be projected as potential substrates for preparing structured lipids of nutraceutical applications after a complete toxicological evaluation of such oils. Further, the extracted oil was studied for fatty acid distribution on triglyceride by regio-specific hydrolysis using pancreatic lipase. It was found that unsaturated fatty acids were selectively located at the *sn-2* position and the *sn-1* position was rich in saturated fatty acids according to the present study.

The seed oil of *B. parviflora* was also analyzed for triglyceride molecular species composition by HPLC analysis and the data is presented in Table 4.

The triglyceride molecular species revealed that the oil was composed of molecular species ranging from C42 to C60. Among all the molecular species, it was found that C48 was the major triglyceride molecular species followed by C46

TABLE 4 HPLC analysis of *Butea parviflora* seed oil

ECN	EMS	%
C42	LLL	9.54
C44	LLO/LLP	5.63
C46	LOO/LOP/PLP	21.95
C48	OOO/POO/POP/LOS/PLS	31.5
C50	POS/OOS/LLB/SLS	3.25
C52	LOB/PLB	10.84
C54	OOB/POB/PPB/SLB	8.88
C56	BOS/BPS	5.69
C58	BLB	2.17
C60	BOB/BPB/BBS	0.54

ECN: effective carbon number; EMS: expected molecular species; L: linoleic; O: oleic; P: palmitic; S: stearic; B: behenic acids.

molecular species. The molecular species with C52 and C54 were present in the range of 8.8 to 10.8% and other molecular species were present in lower amounts.

The unsaponifiable matter which represented about 0.82% of the oil was analyzed by GC and GC-MS and was found to be composed of sterols, triterpenoids and tocopherols. Sterols constituted up to 84.7% of the unsaponifiables with β -sitosterol as the major phytosterol followed by stigmasterol and campesterol as minor phytosterols whereas the remaining component was found to be β -amyrin at 15.3%. The composition of tocopherols in the oil was analyzed by HPLC and was found that 581.7 $\mu\text{g/g}$ of tocopherols were present in the seed oil. Among the three tocopherols identified, gamma tocopherol was present in major amounts (560 $\mu\text{g/g}$) followed by alpha tocopherol (12.2 $\mu\text{g/g}$) and delta tocopherol (9.5 $\mu\text{g/g}$) in smaller amounts. The gamma tocopherol concentrations were close to the contents reported for canary melon and gooseberry seed oils where these seed oils were recovered from industrial fruit by-products. However, the tocopherol contents in these two seeds were slightly higher and were reported to contain about 630.8 and 603.5 $\mu\text{g/g}$ (Górnas *et al.*, 2015). Thus the *Butea parviflora* seed oil is a good source of gamma tocopherol which is known as the most potent free radical scavenger among the other tocopherol isomers and is also reported to be exhibiting an inhibitory effect on carcinogenesis (Ju *et al.*, 2010).

4. CONCLUSIONS

The *Butea parviflora* seed was identified as a potential source for oil from the Fabaceae family. The seed was found to contain about 18% oil and the fatty acid composition showed the presence of considerable amounts of behenic and lignoceric acids. The seed oil may find applications for use in low calorie fat related studies due to the presence

of very long chain fatty acids and natural lipophilic bioactive compounds. Further studies on the toxicological evaluation of the seed oil can be of interest for potential edible applications.

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