

Characterisation of Seed Lipids from *Bixa orellana* and *Trachyspermum copticum*

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Abstract *Bixa orellana* L. seeds possess a resinous lipid (6.3 %), which has a pungent and spicy odour. The seed is known for its medicinal properties such as anti-inflammatory, antipyretic activity and as a cure for tonsillitis. *Trachyspermum copticum* L. seed is a well known digestive aid and relief from colic pain. *T. copticum* possesses essential oil rich in thymol (>50 %) and lipid (15.6 %). The present study was aimed to quantify lipid classes of these two species by silicic acid chromatography and analyze their fatty acid composition by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). It was observed that the seed lipids are rich in neutral lipids with 98.1 and 95.2 % and lower quantities of glycolipids of 1.5 and 3.8 % and phospholipids of 0.36 and 1.0 % in *B. orellana* and *T. copticum*, respectively. The fatty acid composition of *B. orellana* seed lipid showed major quantities of palmitic (26.9 %), linoleic (26.1 %), oleic (17.5 %), linolenic (15.1 %), stearic acid (10.8 %) and small quantities of eicosanoic acid (3.6 %). In *T. copticum* seed lipids, petroselinic acid (68.3 %) and linoleic acid (25.3 %) together constituted 93 % of the total lipid. The results revealed that the lipids after recovery of the essential components namely, bixin and volatile oil from *B. orellana* and *T. copticum*, respectively can be further explored for industrial applications.

Keywords *Bixa orellana* · *Trachyspermum copticum* · Seed lipids · Lipid classes · Fatty acid composition

Introduction

Oils or fats are present in the seed portion of most plants. In general, triglycerides are the storage lipids in plants [1]. Several seed lipids are reported to possess medicinal value and functional characteristics such as hypercholesterolemic, anti-diabetic and anti-arthritic [2] activities. Several conventional and unconventional seed lipids useful for food and medicinal or cosmetic applications were characterised for their fatty acid composition. Fatty acids such as oleic acid (23–52 %), linolenic acid (7–47.3 %), and linoleic acid (20–65 %) were reported in few seed lipids [3–8]. In general, cocoa butter, coconut oil or hydrogenated palm oil forms the major industrially used oils/fats that are rich in saturated fatty acids.

Bixa orellana L. (*B. orellana*) also known as annatto, is an ancient crop of tropical lowlands of Latin America, where it is grown extensively for bixin. The literature on *B. orellana* is extensively concentrated on the bixin pigment and its extraction methods. Bixin is the principle colouring component in the annatto seeds. Peru is the world's largest producer and exporter of annatto seeds, followed by Brazil and Kenya. Annatto is a naturally occurring tree in the hotter parts of India and is cultivated to some extent in the states of Orissa, Andhra Pradesh and Maharashtra for its seeds (Anonymous, 1988) [9]. More than 80 % of the carotenoids in the annatto seed coat consist of *cis*-bixin [(2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*,16*Z*,18*E*)—20-methoxy-4,8,13,17-tetramethyl-20-oxoicosa-2,4,6,8,10,12,14,16,18 nonaenoic-acid]. Commercial preparations consist of a solution or suspension of the pigment in vegetable oil or

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in water-soluble form in dilute alkaline solution. Three main commercial processes are used to extract the pigment from dried annatto seeds. These are (i) extraction into oil, (ii) extraction with organic solvents and (iii) extraction into aqueous alkali (Preston & Rickard, 1980) [10]. A detailed review of literature on annatto chemistry, processing methods, toxicological aspects and degradation products was made by Satyanarayana, Prabhakara Rao, and Rao (2003) [11].

The seeds and leaves of annatto are used in traditional medicine [9]. The seeds are edible, but slightly purgative and said to be effective against fever, dysentery and kidney diseases. The paste of fruits and seeds is applied against itching; the leaf decoction is taken to stop vomiting, used as a gargle for sore throat and tonsillitis; and as a bath against muscular pain; used in the cosmetic industry in the production of nail gloss, hair oil, lipstick, soap; used in household products like floor wax, furniture polish, shoe polish, brass lacquer and wood stain [9]. Extraction methods, purification, quantification, analysis, stability studies and applications of principle component of *B. orellana* in foods are available in the literature [12–17].

The lipid fraction from *B. orellana* contains mainly tocotrienols of which δ -tocotrienol at 140–147 mg/100 g dry seeds and to an extent of 5.2–5.5 % on a lipid basis [18]. Palmitic, oleic and linoleic acids were found to be major fatty acids, which accounted for 16.4, 33.9, 34.3 %, respectively.

Trachyspermum copticum L. is also known as azwain. In India, the states of Rajasthan and Gujarat are the major producers with Rajasthan alone accounting for 90 % of the total harvest. The monoterpene, thymol is the main component in the essential oil of raw *T. copticum* seeds and its flavour is aromatic similar to thyme. In India, *T. copticum* is used as a digestive relief for abdominal pain, indigestion and as an antiseptic. In southern India, powdered *T. copticum* seeds soaked in milk are fed to babies to provide relief from colic pains. In northern India, people serve it after dinner [19]. Essential oils from *T. copticum* L. obtained by hydro-distillation were studied for antifungal activities with special reference to the inhibition of *Aspergillus parasiticus* growth and aflatoxin production. Aflatoxin production was inhibited at 450 ppm of *T. copticum*. Essential oils were shown to contain thymol (37.2 %), *p*-cymene (32.3 %), γ -terpinene (27.3 %). Preservative effects of essential oils of azwain were confirmed and the natural materials can safely be applied to protect food materials from toxigenic fungal infections [20]. Several therapeutic effects including carminative, diuretic and anti-vomiting effects were reported for *Carum copticum*. GC–MS analysis of essential oil from *C. copticum* fruits showed thymol (54.5 %), γ -terpinene (26.1 %) and *p*-cymene (22.1 %) as the major terpenoid compounds [21]. Thymol, present in

the essential oil was characterised by the chemical shift method using spectral properties, which was confirmed by optical microscopy and gas chromatography-mass spectrometry (GC–MS), respectively [22].

However, the analysis of the total lipid portion of *B. orellana* and *T. copticum* had not been carried out in detail till now and hence, the present study was taken up with the main objective to evaluate residual lipid after recovering the principal components namely, bixin from *B. orellana* seed and the essential oil from *T. copticum*.

Materials and Methods

Materials

B. orellana seeds (10 kg) were procured from M/s. Girijan Cooperative Corporation Ltd., during the three successive seasons of 2009–11, which were collected by tribal population from the Paderu forests of the Visakhapatnam district of Andhra Pradesh, India, where the average temperature ranged between 15 and 22 °C during 2009 and 2011. The plants wildy growing at an altitude of 900–1200 m were harvested for annatto seed during the season of November to December. *T. copticum* seeds (10 kg) were purchased from a grower in the Kurnool district of Andhra Pradesh, India, located at an altitude of 270 m in three consecutive seasons of 2010–2012, where the average temperature was 12–30 °C. The seed was harvested in the months of December and January. Solvents and chemicals used in the study were of laboratory grade obtained from M/s. Loba Chemie, Mumbai, India. Standard fatty acid methyl esters (FAME, C₄–C₂₄) were procured from M/s. Sigma-Aldrich, St Louis, USA.

Sample Preparation

Seeds of *B. orellana* and *T. copticum* were manually cleaned from dust, fibre and stones. The seeds were powdered using a high speed laboratory mixer (Sumeet Food Processor, Nasik, India) and passed through a 30 mesh (500 μ m) sieve to obtain uniform sample.

Chemical Composition of Seed Samples

Estimation of Moisture

Moisture was determined using a hot air oven method [23]. The sample weighed in a glass dish was dried in a hot air oven maintained at 103 ± 2 °C and the drying was continued for 16–18 h. The dish was cooled in a desiccator, and weighed. The sample was reheated and checked for weight until two consecutive weights show no significant variation. Loss on drying is expressed as moisture present in sample.

Estimation of Total Ash

The ash content of a food material determines the inorganic metals after combustion of organic matter. High ash or low alkalinity of ash, may suggest the presence of adulterants. The acid-insoluble ash is a measure of sand or silica. About 5 g of the sample was weighed into each silica dish and the contents were ignited on a Bunsen burner. Further, the material was exposed to 525 °C for 4–6 h in a muffle furnace (Adair Dutt and Company, Chennai, India) [23]. The crucibles were cooled and weighed and the difference in weight gave the total ash content, which was expressed as a percentage.

Estimation of Protein

Nitrogen content was estimated by the Kjeldahl method, which is based on determination of the amount of reduced nitrogen (NH₂ and NH) present in the sample. Various nitrogenous compounds are converted into ammonium sulphate, when digested with concentrated sulphuric acid in the presence of a digestion mixture consisting of copper sulphate, potassium sulphate and selenium dioxide. Ammonia was liberated by boiling with an alkali (NaOH), which was absorbed in neutral boric acid solution and was titrated with a standard acid. Percentage protein was calculated using a general conversion factor $N \times 6.25$. *B. orellana* and *T. copticum* seed powders from three consecutive seasons were analysed for moisture, total ash, and protein contents following standard methods (Ranganna 1986) [23].

Extraction of Total Lipids

Extraction of total lipids from *B. orellana* and *T. copticum* powder was conducted using the solvent mixture chloroform:methanol (2:1, v/v) at room temperature (RT, 27 ± 2 °C) under magnetic stirring [24]. Extraction was repeated 3 times for maximum recovery of lipids. Recovery of solvent was conducted at <50 °C in a rotary film evaporator.

Estimation of Volatile Oil in *T. copticum*

The volatile oil content of *T. copticum* seed was estimated by hydro-steam distillation using a Clevenger apparatus. The powdered sample (25 g) was dispersed in water in a round-bottomed flask, which was connected to a Clevenger apparatus and a water cooled condenser. The contents of the flask were heated to reflux for collecting the volatile oil in the graduated side arm. The percent volatile oil (v/w) in the sample was calculated by measuring the volume of volatile oil.

Determination of Bixin Content in the Total Lipid of *B. orellana*

Bixin content in the total lipid of *B. orellana* was analysed using a spectrophotometric method [25] by dissolving in chloroform and suitably diluting aliquots for measuring optical density at 470 nm. The optical measurements were conducted in a Shimadzu (Kyoto, Japan), UV-160 A UV-Vis spectrophotometer. The percentage of bixin was calculated using the following expression:

$$\text{Bixin content, \%} = \frac{\text{OD of the diluted aliquot} \times \text{Volume} \times \text{Dilution factor}}{3230 \times \text{weight of lipid}}$$

where $E_{1\text{ cm}}^{1\%}$ at the 470 nm value of bixin is 3230

Lipid Quality Analysis

The total lipids of *B. orellana* and *T. copticum* were analysed for chemical parameters by standard AOCS methods such as free fatty acids (FFA) Cd 3d-63 [26], saponification value (SV) Cd 3–25 [27], peroxide value (PV) Cd 8–53 [28] and iodine value Cd 1–25 [29].

Acid value (AV) and Free Fatty Acid Content (FFA)

The fat sample (2 g) was put into a 250-ml Erlenmeyer flask and 50 ml petroleum ether was added. The contents were thoroughly swirled until the sample dissolved. To the contents, 50 ml neutral ethyl alcohol was added and mixed. Phenolphthalein indicator (1 % in 95 % ethyl alcohol) was added and titrated with 0.1 N KOH solution till the pink color appeared. The acid value was calculated as:

$$\text{Acid value (mg/g sample)} = \frac{\text{Titre value} \times N \times 56.1}{\text{Weight of sample}}$$

where, N = normality of KOH; (% FFA as oleic acid = Acid value/2)

Iodine Value

Wijs Procedure: the iodine value is the number of grams of iodine absorbed per 100 g of the oil or fat, when determined using Wijs solution. The material is treated in carbon tetrachloride medium with a known excess of iodine monochloride in glacial acetic acid. The excess of iodine monochloride is treated with potassium iodide, and the liberated iodine is estimated by titrating with standard sodium thiosulphate (Na₂S₂O₃) solution using starch indicator. A blank determination was carried out along side without the addition of fat.

$$\text{Iodine value (g / 100 g of fat)} = \frac{(\text{Blank titre value} - \text{Sample titre value}) \times N \text{ of Na}_2\text{S}_2\text{O}_3 \times 12.69}{\text{Weight of sample}}$$

Peroxide Value

The peroxide value of an oil or fat is the amount of peroxides present and expressed as milli-equivalents of peroxide per 1000 g of sample. The sample was dissolved in solvent (2 volumes of glacial acetic acid and one volume of chloroform), treated with potassium iodide, and the iodine liberated by the peroxides present in rancid fat or oil was titrated with standard sodium thiosulphate solution.

(hexane:ethyl acetate, v/v). After completion of the reaction, the reaction mixture was concentrated by distillation under a vacuum and the FAME were extracted into ethyl acetate and the excess acid was removed by repeatedly washing with water [30]. The extracts were concentrated by removing the solvent for conducting GC and GC–MS analysis.

The FAME were analysed by GC and GC–MS for fatty acid composition by applying the following conditions.

$$\text{Peroxide value (mequiv. /1000 g of fat)} = \frac{(\text{Titre value} - \text{Blank titre value}) \times N \text{ of Na}_2\text{S}_2\text{O}_3 \times 1000}{\text{Weight of fat taken}}$$

Saponification Value

When a fat is boiled with an excess of alcoholic KOH, the triglycerides become hydrolysed resulting in glycerol and soap. The alkali consumed for this hydrolysis is a measure of the saponification value (SV) and is defined as the number of milligrams of KOH required to saponify one gram of oil or fat completely. A blank determination was carried out along side without the addition of fat.

- The GC-FID analysis was performed with an Agilent 6850 (Agilent Technologies, Palo Alto, CA, USA) series gas chromatograph equipped with an FID detector, DB-225 capillary column (30 m × 0.25 mm i.d. and 0.25 μm film) with temperature programming maintaining 160 °C for 2 min, increasing to 230 °C at 6 °C/min, and finally maintained for 10 min at 230 °C [31]. The carrier gas was nitrogen at a flow rate of 1.5 ml/min.

$$\text{Saponification value, mg KOH/g} = \frac{\text{Blank titre value} - \text{Sample titre value} \times N \text{ of KOH} \times 56.01}{\text{Weight of sample (g)}}$$

Chromatographic Separation of Lipid Classes

The neutral, glyco- and phospholipids of the total lipids were quantitatively separated by successively eluting with chloroform, acetone and methanol using silicic acid column chromatography [30]. The separation was carefully monitored by thin layer chromatography for ascertaining the complete elution of each class. Neural lipids, glycolipids and phospholipids were monitored by TLC using solvent systems namely, 90:10 (hexane:ethyl acetate, v/v), 170:25:24:4 (chloroform:methanol:acetic acid:water, v/v/v/v) and 65:25:4 (chloroform:methanol:water, v/v/v), respectively [30].

Determination of the Fatty Acid Composition of Lipid Classes

The total lipids and the lipid classes were converted into their respective fatty acid methyl esters (FAME) by refluxing with methanol containing 2 % sulphuric acid for 6 h ensuring the complete conversion [30] of the triacylglycerol by testing with TLC using a solvent system of 90:10

- The injector and detector temperatures were maintained at 230 and 250 °C, respectively with a split ratio of 50:1.
- The GC–MS analysis of FAME was conducted with an Agilent (Palo Alto, USA) 6890 N gas chromatograph equipped with a HP-5 MS capillary column (30 m × 0.25 mm i.d. 0.25 μm film) connected to an Agilent 5973 mass spectrometer operating in the EI mode (70 eV; *m/z* 50–550; source temperature 230 °C and a quadrupole temperature 150 °C).
- The column temperature was programmed with an initial temperature of 200 °C for 2 min, increasing to 300 °C at a rate of 4 °C/min, and maintained for 20 min at 300 °C.
- Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The inlet temperature was maintained at 300 °C and a split ratio of 50:1 [31].
- Structural assignments were based on comparison of retention times as well as the fragmentation pattern of authentic compounds and confirmed by the spectral data obtained from the Wiley and NIST libraries.

Fig. 1 Photographs of *Bixa orellana* fruiting branch and seeds



Fig. 2 Photographs of *Trachyspermum copticum* flowering branch and seeds



Results and Discussion

The photographs of the fruiting branch depicting the pods and seed of *B. orellana* and *T. copticum* are presented in Figs. 1 and 2 respectively.

Chemical Characterisation of the Total Lipids

Results indicated that not much variation in chemical composition with change of season was observed. The results of chemical analyses of seed powders are presented in Table 1. The ash and protein contents in *B. orellana* were

Table 1 Physico-chemical analysis of *Bixa orellana* and *Trachyspermum copticum* seed

Parameter	<i>B. orellana</i>	<i>T. copticum</i>
Moisture (%)	9.8 ± 0.16	8.2 ± 0.04
Total ash (%)	5.3 ± 0.21	7.0 ± 0.10
Total lipid (%)	6.3 ± 0.10	15.6 ± 0.46
Protein content (%)	11.2 ± 0.37	16.3 ± 0.61
Bixin content	17.0 ± 0.47	–
Volatile oil content	–	10.03 ± 0.24

Values are the means of triplicate analyses ± SD

5.3 and 11.2 % respectively. The yield of total lipids in *B. orellana* was 6.3 %, which possessed a bixin content of 17 %. The lipids with bixin can be applied to fatty foods such as butter and cheese to yield an orange yellow colour in the products. Higher peroxide (10.3 mequiv O₂/kg) and iodine values (146 g I₂/100 g fat) were noted in *B. orellana* lipids. The quantity of FFA present in the seed oils was reported to have a significant effect on the formation of soap or biodiesel manufacture wherein a free fatty acid content of <1 % is recommended [32, 33]. Therefore, in the present study, *B. orellana* with a lower FFA content of 0.2 % is in good agreement with the reported literature and hence can be suggested for examining its compatibility in industrial applications.

The ash and protein contents in *T. copticum* were 7 and 16.3 %, respectively. The yield of total lipid from *T. copticum* was 15.6 % and the essential oil was found to an extent of 10 %. A higher free fatty acid content of 1.5 % and a saponification value of 188 mg KOH/g was observed in *T. copticum* total lipid (Table 2). The essential oil can be recovered using a Clevenger apparatus and may find application as a flavourant in sweets and fried foods.

Lipid Classes and Fatty Acid Composition of Total Lipids

Silicic acid column chromatography revealed that both the plant seeds were rich in neutral lipids (Table 2). Neutral, glyco- and phospholipids were 98.12, 1.52 and 0.36 %, respectively in *B. orellana*. Neutral, glyco- and phospholipids were found to be 95.17, 3.76 and 1.07 % in *T. copticum*. The presence of low PL content indicates that the lipid of *T. copticum* can be used as a frying oil. Similarly, the higher glycolipid content suggests its use in the preparation of bio-surfactants. Glycolipid based bio-surfactants are the most promising ones due to versatile biochemical properties in

Table 2 Chemical characteristics of total lipids from *B. orellana* and *T. copticum*

Parameter	<i>B. orellana</i>	<i>T. copticum</i>
Free fatty acid content (mg/g)	2.0 ± 0.2	15.0 ± 0.4
Peroxide value (mequiv O ₂ /kg)	10.3 ± 0.14	2.8 ± 0.39
Saponification number (mg KOH/g)	44.8 ± 1.00	188 ± 1.53
Iodine value (g I ₂ /100 g fat)	146 ± 2.52	103 ± 1.02
Lipid composition % by weight		
Neutral lipids	98.12 ± 0.44	95.17 ± 1.35
Glycolipids	1.52 ± 0.14	3.76 ± 0.44
Phospholipids	0.36 ± 0.05	1.07 ± 0.09

Analyses were carried out in triplicate and mean values with ± SD are presented

the food, cosmetic, and pharmaceutical industries and in environmental protection [34].

Based on GC and GC–MS data, the fatty acid composition of the seed lipids and their lipid classes are tabulated (Tables 3 and 4). The total lipid from *B. orellana* was characterised by substantial amounts of palmitic (26.9 %), linolenic (26.1 %), oleic (17.5 %), linolenic (15.1 %) and stearic acids (10.8 %). In the case of phospholipids, palmitic and linoleic acids were 25.6 and 43.8 %, respectively, making up to 69.4 % of total lipids.

The lipids from *Bixa orellana* were reportedly used in cosmetic application [11]. However, the polyunsaturated fatty acid (PUFA) content of the total lipid of *B. orellana* (41.2 %) was comparable to the PUFA contents of sesame seed oil (38 %) and *Sterculia urens* cotyledons (36.7 %) [35, 36]. Palm oil with higher saturated fatty acids (~50 %) was reported as being used for food applications and the spent fried oil for bio-diesel production [37]. Saturated fatty acid content was noted as being higher in glycolipids (42.9 %) compared to neutral or phospholipid fractions of *B. orellana*. Higher PUFA of 39.1, 35.7 and 51.5 % were found in neutral, glyco- and phospholipids, respectively in *B. orellana*.

The fatty acid composition of *T. copticum* was characterised by lower amounts of palmitic (4.5 %), stearic (0.9 %), linolenic (1.0 %) acids, and substantial amounts of linoleic (25.3 %) and petroselinic acids (68.3 %). The total lipid content of *T. copticum* was highly aromatic

Table 3 Fatty acid composition (wt%) of lipid classes of *Bixa orellana*

Fatty acid	Total lipid	Neutral lipids	Glycolipids	Phospholipids
16:0	26.9 ± 0.56	20.3 ± 0.20	27.2 ± 0.78	25.6 ± 0.36
18:0	10.8 ± 0.17	13.9 ± 0.46	17.2 ± 0.70	9.0 ± 0.17
20:0	3.6 ± 0.13	2.0 ± 0.09	–	–
18:1	17.5 ± 0.26	20.7 ± 0.33	26.4 ± 0.62	13.9 ± 0.18
18:2	26.1 ± 0.30	35.4 ± 1.47	18.6 ± 0.26	43.8 ± 0.36
18:3	15.1 ± 0.26	7.7 ± 0.26	10.6 ± 0.70	7.7 ± 0.30

Values are the means of triplicate analyses ± SD

Table 4 Fatty acid composition (wt%) of lipid classes of *Trachyspermum copticum*

Fatty acid	Total lipid	Neutral lipids	Glycolipids	Phospholipids
16:0	4.5 ± 0.05	5.1 ± 0.10	17.5 ± 0.56	24.6 ± 0.40
18:0	0.9 ± 0.08	2.0 ± 0.17	8.3 ± 0.19	7.3 ± 0.04
18:1 Δ 6	68.3 ± 2.46	71.1 ± 0.95	46.9 ± 0.79	32.4 ± 0.44
18:2	25.3 ± 0.70	21.1 ± 0.66	27.3 ± 0.35	35.7 ± 0.61
18:3	1.0 ± 0.06	0.7 ± 0.03	–	–

Values are the means of triplicate analyses ± SD

with the flavour of thymol. The total lipid and other lipid classes were typically composed of high concentrations of oleic and linoleic acids, which together constituted 93.6 % of the total lipids. Such a high percentage of unsaturation is similarly observed in the non-edible oil, jatropha seed oil, oleic (44.7 %) and linoleic acid (32.8 %), which is one of the important sources for bio-diesel production [38]. Hence *T. copticum* can also be exploited for biodiesel preparation. In case of glyco- and phospholipids of *T. copticum*, palmitic and stearic acid contents were higher and a corresponding decrease in oleic and linoleic acids was observed. Seasonal variation was not observed to be significant in the lipid or fatty acid composition of the seed samples. Higher monounsaturated (68.3 %) fatty acids were observed in *T. copticum* lipids. The glycolipid fraction possessed higher saturated fatty acids (11.8 %) compared to phosphor- or neutral lipids. In addition, the *T. copticum* lipid classes were found to be rich in monounsaturated fatty acids (Table 2).

The ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) and the ratio of polyunsaturated to monounsaturated fatty acids (PUFA/MUFA) were found to be 1.0 and 2.35 in *Bixa orellana* total lipids, respectively. The ratio of PUFA/SFA is generally used to evaluate the nutritional value of fat. Chang and Huang [39] studied the effects of monounsaturated fatty acids (MUFA) and the ratio of the sum of polyunsaturated and monounsaturated fatty acids to saturated fatty acids (PUFA + MUFA/SFA) on plasma and liver lipid concentrations in rats. They suggested that a low MUFA/SFA ratio, high PUFA/MUFA ratio and PUFA + MUFA/SFA ratio not to exceed 2 are the prerequisites for keeping low plasma and liver lipid concentration. In the present study, low MUFA/SFA (0.42) ratio and high PUFA/MUFA (2.35) ratio, and PUFA + MUFA/SFA (1.42) ratio of total lipid of *B. orellana* were observed. The findings of Chang and Huang [39] are in agreement with the fatty acid profile of *B. orellana* seed lipid. Similarly highly nutritious foxtail millet bran oils were found to be rich in polyunsaturated fatty acids (83.47 %) with very low saturated fatty acids [40].

In the present study *T. copticum* lipid was found to be rich in monounsaturated fatty acids (68.3 %) and PUFA (26.3 %) and low quantities of saturated fatty acids (5.4 %). The ratio of PUFA to SFA was found to be 4.87 and the ratio of PUFA to MUFA was 0.45 in *T. copticum* lipid. In the total lipid, higher MUFA/SFA ratio (12.65) and lower PUFA/MUFA ratio (0.38) and very high PUFA + MUFA/SFA ratio (17.52) was observed. In this context, *B. orellana* seed lipid can be exploited for nutritional applications. Jojoba seeds with major monounsaturated fatty acid (20:1) (66.3 %) [41], was employed in biodiesel preparation. Hence, *T. copticum* seed lipid with high yields of lipid and rich in monounsaturated fatty acids can be employed

in industrial applications especially for the production of biodiesel.

Conclusions

The seed samples collected during three consecutive seasons did not show considerable variation in physico-chemical, lipid and fatty acid composition. This study showed that the by-product of resin after extraction of the principle natural dye from *B. orellana*, could be a good source of lipids for the food, pharmaceutical, cosmetic and varnish industries. Similarly, *T. copticum* oil a rich source of monounsaturated fatty acids could be nutritionally good for minimising the risks of cardiovascular diseases. The results revealed that the lipids after recovery of the essential components of bixin and volatile oils rich in thymol, respectively from *B. orellana* and *T. copticum* can be further explored for industrial applications.

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