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Development of Novel Cost Efficient Process for Extraction of Sterols and Tocopherols from Wild Plant Seed

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The present work focuses on the compositional characterization of tamarind and sea buckthorn seed oils, which have a wide range of uses in the cosmetic, pharmaceutical and food sectors. The characterization includes the analysis of tocopherols, sterols and fatty acid profile in both oils. The tamarind and sea buckthorn oils were converted to fatty acid methyl ester and the fatty acid composition was analyzed by using gas chromatography technique. The quantitative and qualitative analysis of tocopherols performed using high performance liquid chromatography. This study reveals that the oil samples consist of a variety of saturated and unsaturated fatty acids, such as myristic acid (0 and 0.4%), palmitic acid (11 and 30.5%), palmitoleic (0 and 39.6%) stearic acid (0 and 0.5%), oleic acid (18.3 and 26.1%), linoleic acid (45.8 and 2.1%), linolenic acid (4.1% and 0.5%), arachidic acid (1.8% and 0%) behenic acid (9.6 and 0%) and lignoceric acid (9.4 and 0%). Additionally, the analysis detects various tocopherol components, including α-tocopherol (21.4 and 32.6%), β-tocopherol (14.49 and 7.24%), γ-tocopherol (53.72 and 45.66%), δ-tocopherol (9.64 and 9.11%) and α-tocotrienol (0 and 5.13%) in tamarind and sea buckthorn oil, respectively.

Keywords: Oils, Antioxidants, Distillation, Solvent extraction, High performance liquid chromatography.

INTRODUCTION

Tamarind plant can be found in over 50 countries throughout the world. Tamarind (*Tamarindus indica*) flourished abundantly throughout India. Globally, agricultural sectors are concerned about reducing or preventing the environmental impact of waste disposal [1]. Reduced raw material usage led to waste production, which caused financial losses as well as the loss of potential industrial and nutritional production [2,3]. As one of the leading global producers of tropical fruits, India generates tons of garbage per day during raw material processing [4,5]. These wastes were removed from the production process because they contained undesired materials. However, a sizeable portion of this garbage may be recycled and used as a source of beneficial chemicals for creation of new products.

The Leguminosae (Fabaceae) family includes the tamarind plant. Fruit pulp, flowers and leaves can all be eaten and offer a variety of beneficial elements. In Asian gastronomy, tamarind is the principal natural source of acid [6]. Tamarind fruit pulp contains around 16 volatile components and is highly recognized for its flavouring characteristics [7]. Soft beverages, jams, hot sauces and sweet sauces all use tamarind pulp to add flavour [6]. In many sectors, the seed is regarded as a byproduct of the fruit pulp extraction process. The endosperm of tamarind seed has antioxidant and colouring capabilities [8]. Its endosperm is rich in carbohydrates and high-quality proteins; the carbohydrates are utilized as gums and thickeners and contain interesting lipid fractions [9]. Tamarind seed powder has recently emerged with a variety of applications. Tamarind seeds as glossy, flattened, hard, orbicular to rhomboid in shape and purple-brown to red in colour. Tamarind pulp and seed both are low cost and easily available raw materials for industrial use [9]. The brown coat of the tamarind kernel accounts for approximately 20-30% of its weight, whereas the kernel itself accounts for 70-75% [10]. The textile industry uses tamarind kernel powder (TKP) as a warp-sizing agent [11]. Because of its polysaccharide composition, TKP is also used in the food industry for jelly formation [12].

Several authors have described various yields for tamarind seed oil extracted from various seed samples. Tamarind seed

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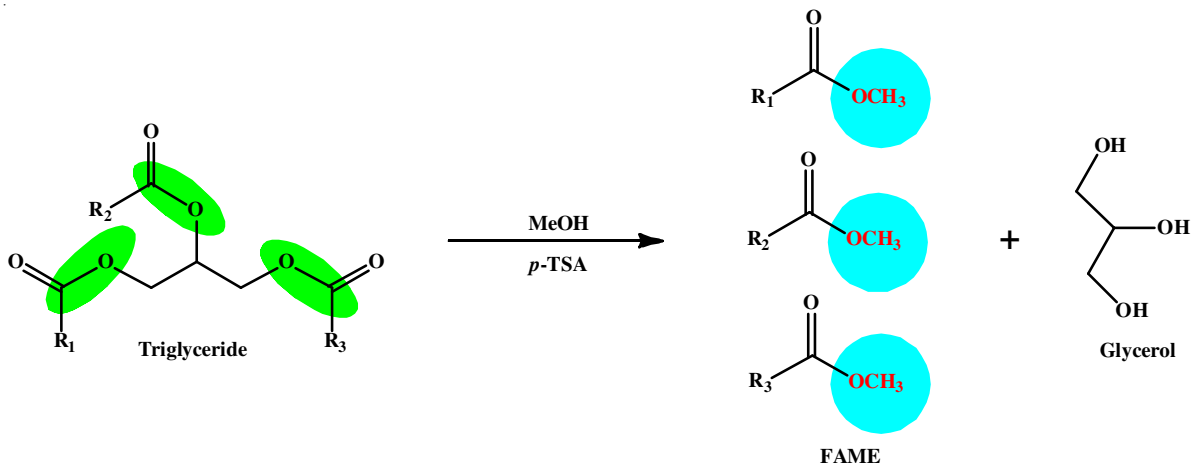
oil's fatty acid content and other properties have been studied. The fatty acid composition of Indian and Egyptian seeds oil differed significantly [13]. Each stated fatty acid composition of tamarind seed oil was discovered to be unique. Morad *et al.* [14] reported a high fatty acid production of approximately 16.25%. The fatty acid and triglyceride composition affects the phase behaviour of fats and oils [15,16]. Pharmaceuticals, flavours, phytochemicals, lipid pigments and scents are among the nutraceuticals and bioactive components found in the tamarind plant. The plant's seeds can be used to extract oil, which is widely used in the food, cosmetic and pharmaceutical industries [17]. Sea buckthorn has been farmed forages throughout Asia and Europe as an ancient crop with modern properties. It was used as fodder in ancient Greece, notably for horses, to improve the gloss of their coats [18]. The sea buckthorn of the hippophae genus has long, narrow leaves and orange-yellow, spherical berries that are 3 to 8 mm in diameter [19]. Due to its cold-resistant nature, sea buckthorn's natural habitat is extensively scattered over China, Mongolia, Russia, Finland, Sweden and Norway [20]. In addition to being used as food for centuries, sea buckthorn has also been used traditionally as a medication to prevent or treat a number of diseases include inflammation, gastric ulcers and skin conditions [21]. Tocopherols, sterols, squalene and phenolic acids are among the bioactive substances found in vegetable oil unsaponifiable content [22,23]. Sea buckthorn leaves, bark and berries are high in bioactive chemicals [24]. Depending on the sea buckthorn's growth environment, geographic area and variety, sea buckthorn oil's chemical composition varies. Due to its high amounts of unsaturated fatty acids and phenolic compounds such phenolic acids, phenolic alcohols and flavonoids, sea buckthorn pulp oil is exceptional among vegetable oils [25]. Because of their distinct fatty acid makeup, fat-soluble vitamins and plant sterols, sea buckthorn seed and pulp oil are considered to be the healthiest components of the berry. The pulp oil contains more saturated fatty acids than the seeds do, especially palmitic and palmitoleic acids, with just trace levels of other polyunsaturated acids.

The current study aims to isolate sterols and tocopherols from tamarind and sea buckthorn oil. Sterols and tocopherols, for example, have several applications in medicine, cosmetics and other industries. Tamarind and sea buckthorn seed oils were analyzed and the results showed that they had different fatty acid compositions. The physico-chemical characteristics of oils are greatly influenced by fatty acids, which are key parts of lipids. Different fatty acids, such as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), might be identified and measured thanks to gas chromatographic analysis. These fatty acid ratios are important factors to consider for industrial applications since they can affect the stability, texture and nutritional value of the oils.

EXPERIMENTAL

Tamarind and sea buckthorn seed oil from the local market were used in their raw, unpurified form. Sigma-Aldrich (St. Louis, USA) provided the standards for tocopherols (α-, β-, γ- and δ-tocopherols), sterols and fatty acids. All reagents, solvents and chemicals used in this study, including sulfuric acid, *p*-toluene sulfonic acid (*p*-TSA), acetone, methanol, ethanol, diethyl ether, silica and other chemicals were purchased from Sigma-Aldrich and were of analytical quality.

Preparation of fatty acid methyl esters (esterification): Tamarind and Sea buckthorn seed oil (SBTO) were transesterified utilizing *p*-toluene sulfonic acid (*p*-TSA) as catalyst and methanol as a reagent, respectively. In a round-bottom flask, 500 g of seed oil and 166 g of methanol were mixed to maintain a molar ratio of 1:6 between triglyceride and methanol and a molar ratio of 1:2 between free fatty acid and methanol. The flask was also treated with 1% *p*-TSA. For 1 h, the reaction mixture was refluxed at 70 ºC (**Scheme-I**). At 10 min intervals, acid values were measured by extracting a sample with a dropper and rinsing it with hot distilled water to eliminate the catalyst. The acid value rapidly declined, reaching zero after 40-45 min for TMO and 55-60 min for SBTO, respectively as indicated in Fig. 1. The round-bottom flask had two different layers once the reaction was completed. Fatty acid methyl esters (FAME) and unsaponifiable matter (nutraceuticals) made up the upper layer, while separated glycerol and unreacted methanol made up the lower layer. A separating funnel was used to separate the two layers. The methanol and glycerol containing lower layer was collected in a separate round-bottom flask. The FAME and nutraceutical-containing layers



Scheme-I: Reaction scheme conversion of fatty acid methyl ester (FAME) from oil

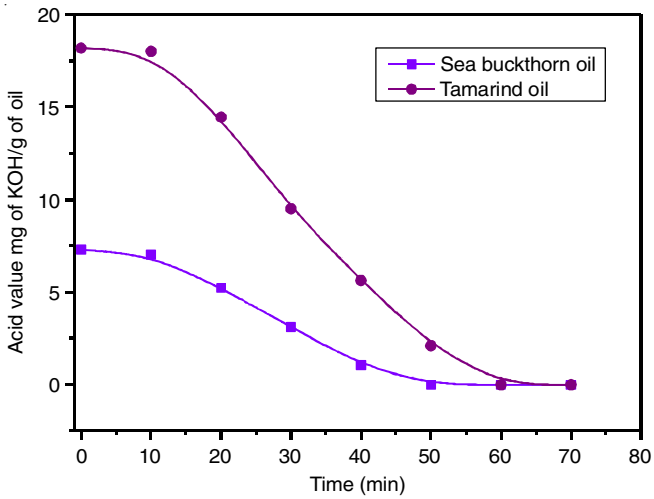


Fig. 1. Reaction growth monitoring by using acid values at different time interval

were washed 9-10 times with hot distilled water to thoroughly remove the methanol, glycerol and catalyst. Any leftover water residues were removed by centrifuging the mixture and passing it through anhydrous sodium sulphate.

Distillation of FAME: The resultant combination of nutraceuticals and FAME was transferred to a round-bottom flask for distillation, with vacuum distillation used to separate the FAME. The residue was high in sterols, tocopherols and other unsaponifiable materials. At room temperature, this unsaponifiable residue was dissolved in acetone and treated to column chromatography to remove contaminants and compounds other than sterols and tocopherols. Complete extraction of sterols and tocopherol was checked by Salkowski's test and vitamin E detection test. Other compounds and waxes were removed in column chromatography. The mixture of sterols and tocopherols collect in round bottom flask and did rotary evaporator for removal of more solvent after solvent removing sterols and tocopherols solid present in round bottom flask.

Effect of solvents on sterols and tocopherols: A 40 g of acetone was added per gram of sterols and tocopherols mixture. The sterols were subsequently separated by chilling the mixture for 5 h at -10 ºC and performing cold centrifugation at -10 ºC. Acetone and tocopherols were extracted from the residual solution by evaporating the acetone on a watch glass. High-performance liquid chromatography (HPLC) with methanol and acetonitrile solvent systems was used to characterize the acquired sterols and tocopherols. Tamarind oil (TMO) and sea buckthorn oil (SBTO) were both subjected to the identical method.

Physico-chemical characterization: The oil samples moisture content was determined using a hot air oven technique. The oil samples were specifically placed in a hot air oven maintained at a temperature of 100 ºC for 30 min. Following that the samples were chilled in vacuum desiccators to avoid moisture absorption and the moisture content was calculated. The density of the oil samples was evaluated using a hydrometer, a device built particularly to detect liquid density. The hydrometer was immersed in the oil samples and the density values obtained were recorded.

Calculation of acid value: The acid value of the oil samples was estimated using eqn. 1. The oil sample (2 g) were combined with 50 mL of neutral alcohol and then titrated using 0.1 N NaOH solution, with the endpoint of the titration visualized using a phenolphthalein indicator. The acid value was calculated using the amount of NaOH solution necessary to neutralize the free acids in the oil sample.

$$\text{Acid value} = \frac{56.1 \times V \times N}{W} \quad (1)$$

where N = normality of NaOH, V = volume of NaOH (mL), W = weight of oil sample (g).

Saponifiable matter calculation: Eqn. 2 was used to calculate the saponifiable matter content of the oil samples. Oil (2 g) were combined with 25 mL of 0.5 N alcoholic KOH and refluxed for 1 h. Using a 1 mL phenolphthalein indicator, the resultant liquid was titrated with 0.5 N HCl. This titration allowed the saponifiable matter content of the oil samples to be determined, which refers to the fraction of oil that may be hydrolyzed and transformed into soap or fatty acids.

$$\text{Saponifiable matter} = \frac{56.1 \times (B - S) \times N}{W} \quad (2)$$

where B = volume of HCl required during blank titration, S = volume of HCl required during sample titration.

As it shows the fraction of oil that may undergo saponification processes, the saponifiable matter content is an essential measure that gives insights into the composition and prospective applications of the oil.

Calculation of ester value: The ester value of the oil samples was calculated by subtracting the acid value from the saponification value.

Calculation of unsaponifiable matter: Eqn. 3 was used to compute the unsaponifiable matter in the oil samples. The resulting mixture was transferred to a separating funnel and petroleum ether was added after the saponifiable ingredient had been refluxed. The insoluble material was extracted and subsequently dissolved in petroleum ether. A portion of the unsaponifiable material was taken out and dissolved in petroleum ether. To ensure maximum extraction of unsaponifiable compounds, this extraction process was repeated three to four times, or until the petroleum ether phase was transparent. To ensure maximum extraction of unsaponifiable compounds, this extraction process was repeated three to four times or until the petroleum ether phase was transparent.

The petroleum ether phase containing the unsaponifiable materials was separated and weighed together with the dry unsaponifiable residue using a rotary evaporator. Using a phenolphthalein indicator, the residue was dissolved in warm neutral alcohol and titrated with 0.02 N NaOH solution. The unsaponifiable matter content of the oil samples was determined using this titration.

$$\text{Unsaponifiable matter} = \frac{100 \times (A - B)}{W} \quad (3)$$

where the weight of free fatty acid extract B = 0.282 V N; N = normality of NaOH; V = volume of NaOH; A = weight of residue.

Determination of iodine value: The iodine value of the sample was assessed using the standard techniques established by the American Oil Chemists' Society (AOCS). The iodine value, given as the number of gram of iodine absorbed per 100 g of sample, indicates the amount of unsaturation in the sample. The samples were treated in a combination of 15 mL CCl_4, 10 mL of 5% fresh KI solution and 25 mL of Wij's solution reagent to calculate the iodine value. The solutions were then left in the dark for 30 min to allow the iodine to react with the unsaturated compounds in the sample. Iodine was freed from the sample during this procedure. The freed iodine was then titrated using a 0.1 N standard $Na_2S_2O_3$ solution, with the end-point detected using a starch indicator. The amount of sodium thiosulphate solution needed for the titration was measured and recorded.

The iodine value was then estimated using eqn. 4, which connects the amount of $Na_2S_2O_3$ solution consumed during the titration to the sample's iodine value.

$$\text{Iodine value} = \frac{12.69 \times (B - S) \times N}{W} \quad (4)$$

where N = Normality of $Na_2S_2O_3$, B = volume of $Na_2S_2O_3$ required for blank titration, S = volume of $Na_2S_2O_3$ required for the sample.

Gas chromatography: Fatty acid methyl esters were analyzed using a gas chromatograph (Chemito GC1000 (MPC), Thermo Fisher Scientific) equipped with a data processor (Iris 32 Lite) and provided with an FID detector. The column was used for characterization having BPX-5 of 30 m long, 0.25 mm id and 0.25 µm film. The equipment was operated under the following conditions: nitrogen 40 mL/min, hydrogen 40 mL/min, injector temperature 250 ºC, detector temperature 250 ºC, oven initially at 150 ºC, held for 2 min and heated to 250 ºC (5 ºC min^{-1}, then held for 5 min). The flow rate of nitrogen as carrier gas was 1 mL min^{-1}; manual injection; the injection volume was 0.2 µL with a split ratio of 1:50. The identification of fatty acids was carried out using standard samples of fatty acid methyl esters.

High-performance liquid chromatography: Qualitative and quantitative analysis of tocopherols were measured by Thermo-Scientific HPLC comprising of C18 column (5 µm, 120 Å) 4.6 × 250 mm, autosampler injection valve injected 10 volumes of sample and diode array detector. The Dionex ultimate pump (3000) with high pressure and 85:13:1.5 (% v/v). The tocopherol separation was carried out on Kromasil, KR60-5-SIL-4.6 × 250 column with 5 µm particle size, using as mobile phase 99:1 hexane/ethanol at 0.500 mL/min and 30 ºC temperature.

RESULTS AND DISCUSSION

Table-1 displays the physico-chemical characterization of tamarind oil (TMO) and sea buckthorn oil (SBTO). TMO and SBTO both had densities that were lower than water, 915 kg/L for TMO and 920 kg/L for SBTO. TMO had a greater viscosity (86 cP) than SBTO (80 cP) due to a larger quantity of free fatty acids in SBTO, which are more freed from glycerol. The oils' colours also vary with TMO having an Amber and SBTO having a light yellow. TMO had a greater moisture absorption capacity (4.01%) than SBTO (2.02%). SBTO had a higher acid value than TMO, indicating a larger concentration of acidic components. When compared to SBTO, TMO had more saponifiable materials, whereas TMO's ester value was likewise greater than SBTO's. TMO also had a higher amount of unsaponifiable materials, indicating the inclusion of nutraceuticals. TMO had a higher iodine value than SBTO, indicating that it was more unsaturated. TMO has superior qualities over SBTO based on the chemical characterization summarized in Table-1. Based on the literature available, the *p*-TSA catalyst shows the best results in esterification and transesterification reactions. As a result, the emphasis moved to minimizing the oil/methanol ratio and reaction time while determining the appropriate concentration of *p*-TSA. Fig. 2 depicts the weight percentages of several components in the sample; including triglycerides, free fatty acids, unsaponifiable matter and moisture content. Triglycerides are the primary components of oils, consisting of three fatty acid molecules connected to a glycerol molecule. Free fatty acids are unbound fatty acids found in the sample, whereas unsaponifiable matter refers to components that cannot be saponified into soap or salts. Moisture content is the quantity of absorbed water and other volatiles in the sample.

TABLE-1
PHYSICO-CHEMICAL CHARACTERIZATION OF TMO AND SBTO

Parameters	TMO (SD)	SBTO (SD)
Density (Kg/L)	915	920
Viscosity (cP)	86	80
Colour	Amber	Pale yellow
Moisture content (%)	4.01	2.02
Acid value (mg KOH/g)	7.3 ± 0.18	18.17 ± 0.09
Saponifiable matter (mg KOH/g)	202.75 ± 0.05	179.00 ± 0.16
Ester value (mg KOH/g of esters)	195.45 ± 0.12	160.83 ± 0.12
Unsaponifiable matter (%)	3.07 ± 0.25	2.74 ± 0.06
Iodine value (mg I_2/g)	107.32 ± 0.07	60.22 ± 0.13

*SD standard deviation.

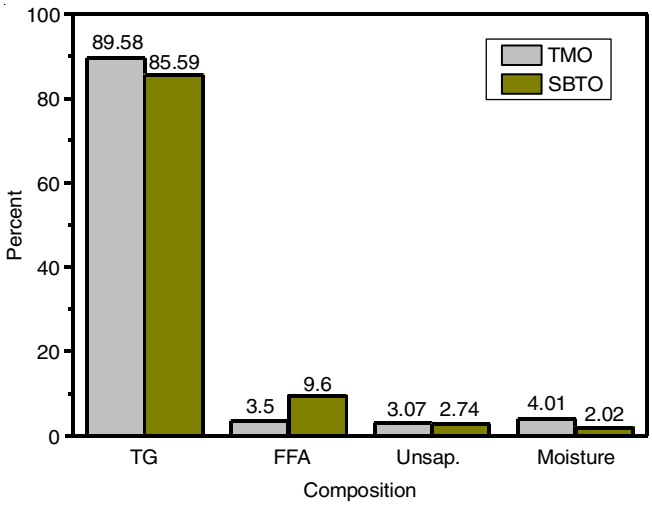


Fig. 2. Oil content and other components TMO & SBTO

Tamarind oil has a greater proportion of triglyceride content (89.58%) than SBTO (85.5903%). Furthermore, tamarind oil

has higher total oil content. TMO has just 3.5% free fatty acids (FFA), whereas SBTO has a larger dissociation of free fats from glycerol, resulting in lesser cold stability. TMO (3.07%) has a larger percentage of nutraceutical-rich residue than SBTO (2.74%). Furthermore, TMO absorbs more moisture than SBTO, with a moisture content of 4.01% against 2.02%. These findings underline the unique compositional variations between TMO and SBTO, emphasizing the potential implications for their utilization in various applications. The tamarind and sea buckthorn oil reaction mixture's acid value gradually decreased, along with a rise in the formation of fatty acid methyl esters. At regular intervals, systematic acid value measurements were made to track the reaction's development. A difference in the length of time needed for the reaction to complete between TMO and SBTO (60 min and 50 min, respectively) were found. The empirical finding that the esterification of free fatty acids required a longer time than the esterification of triglycerides gave rise to this disparity. Similarly, a stepwise temperature elevation method was used to calculate the ideal reaction temperature, starting at room temperature. The optimal reaction temperature was determined to be 70 ºC by gradually raising the temperature by 10 ºC.

Studies were also conducted to examine the impact of various solvents on the separation of sterols and tocopherols. It was found that acetone outperformed the other three tested solvents in terms of isolating sterols and tocopherols. In particular, the acetone extraction method produced 1.0438 g of tocopherols and 0.460 g of sterols from the unsaponifiable matter of TMO and 0.685 g of tocopherols and 0.274 g of sterols from the unsaponifiable matter of sea buckthorn oil. In contrast, methanol was more effective than ethanol and diethyl ether at extracting sterols and tocopherols. Notably, as seen in Figs. 3 and 4, it was shown that diethyl ether extracted much less sterols and tocopherols. The ratio of solvent to unsaponifiable matter was found to be crucial after choosing the right solvent for the isolation process. The yield of sterols and tocopherols decreased when a solvent quantity less than the required amount was used for the isolation. Similarly, increased solvent amounts also led to lower yields. Through testing, it was shown that 40 g

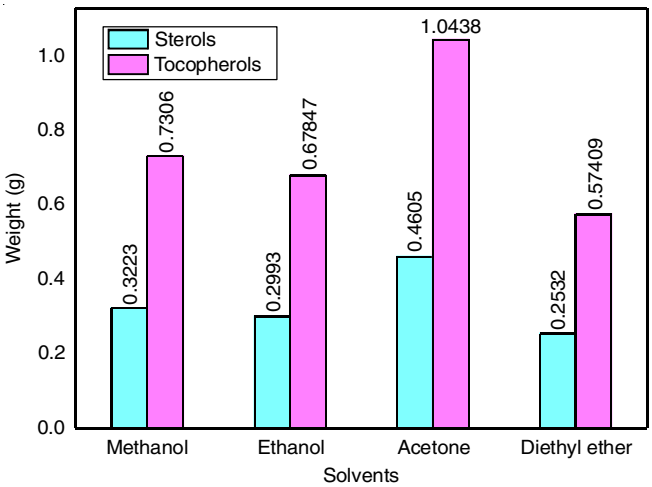


Fig. 3. Effect of different solvent on quantitative isolation of sterols and tocopherols from tamarind oil

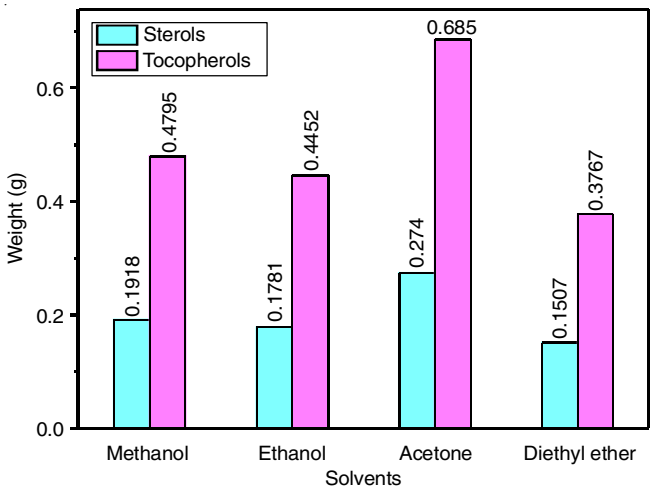


Fig. 4. Effect of different solvent on quantitative isolation of sterols and tocopherols from sea buckthorn oil

of acetone was the ideal solvent ratio for isolating sterols and tocopherols from 1 g of unsaponifiable material in both TMO and SBTO. The accompanying Figs. 5 and 6 gives a visual representation of this ideal ratio.

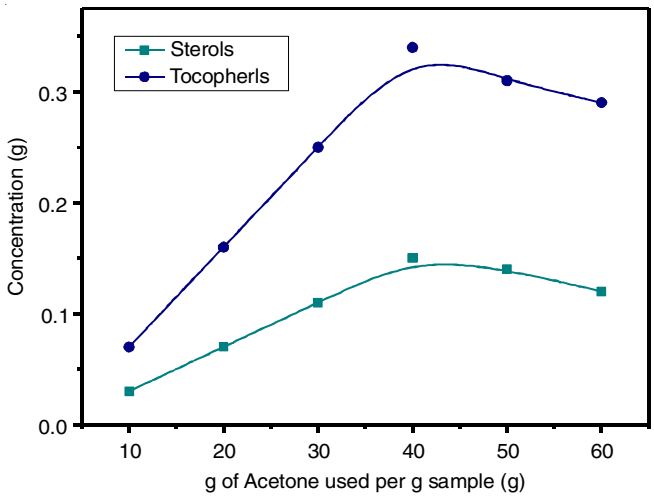


Fig. 5. Effect of acetone ratio to sterols and tocopherols of tamarind oil

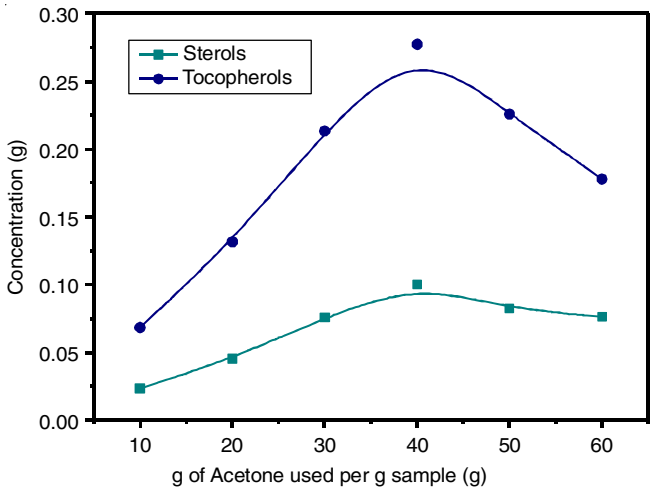


Fig. 6. Effect of acetone ratio to sterols and tocopherols of sea buckthorn oil

Fatty acid composition: TMO and SBTO have different fatty acid compositions. Arachidic acid (1.8%), palmitic acid (11%), oleic acid (18.3%), linolenic acid (4.1%), behenic acid (9.6%) and lignoceric acid (9.4%) are present in TMO. Myristic acid (0.4%), palmitic acid (30.5%), palmitoleic acid (39.6%), stearic acid (0.5%), oleic acid (26.1%), linoleic acid (2.1%) and linolenic acid (0.5%) are the main acids found in SBTO. Linoleic acid, which contained in TMO in a higher percentage than other fatty acids [9], whereas arachidic acid was found to be the least. On the other hand, myristic acid is only present in very small amounts in SBTO, where palmitoleic acid is the most prevalent fatty acid. The wider range of fatty acids present in TMO is shown by the fact that it has a greater variety of fatty acids than SBTO. As far as variations in fatty acids are concerned, the fatty acid profiles of TMO and SBTO exhibit significant differences. Certain fatty acids found in SBTO, such as myristic acid, palmitoleic acid and stearic acid, are absent from TMO. In contrast, SBTO lacks arachidic, behenic and lignoceric acids, which are all present in TMO. Common fatty acids including palmitic acid, oleic acid, linoleic acid and linolenic acid are present in both oils, though in varied amounts. In terms of unsaturated fatty acids, SBTO has a larger concentration of monounsaturated fatty acids (MUFA) than TMO, which shows a higher presence of polyunsaturated fatty acids (PUFA). Saturated fatty acid (SFA) levels in the two oils are comparatively similar (Table-2). These variations in fatty acid compositions draw attention to the unique nutritional qualities and potential health advantages of each oil.

TABLE-2
FATTY ACID CONTENT OF TMO & SBTO

Common name	No. of carbon	Std. RT (min)	TMO FAME % area	SBTO FAME % area
Lauric acid	C 12:0	8.802	–	–
Myristic acid	C 14:0	10.630	–	0.4
Palmitic acid	C 16:0	11.583	11.0	30.5
Palmitoleic acid	C 16:1	–	–	39.6
Stearic acid	C 18:0	12.877	–	0.5
Oleic acid	C 18:1	13.013	18.3	26.1
Linoleic acid	C 18:2	13.695	45.8	2.1
Linolenic acid	C 18:3	14.110	4.1	0.5
Arachidic acid	C 20:0	14.390	1.8	–
Behenic acid	C 22:0	15.340	9.6	–
Lignoceric acid	C24:0	16.857	9.4	–
SFA	–	–	31.8	31.4
MUFA	–	–	49.9	65.7
PUFA	–	–	18.3	2.6

Sterols and tocopherols are components of unsaponifiable matter. Fig. 7a-b depicts the content of sterols and tocopherols in the unsaponifiable matter of TMO and SBTO, respectively. When the amounts of sterols and tocopherols are compared, it is clear that the concentration of tocopherols is higher in both oils. A 3.07 g of unsaponifiable matter from TMO comprises 0.77 g of tocopherols and 0.32 g of sterols, in addition to other components such as nutraceuticals and contaminants. Similarly, 1.1904 g of tocopherols and 0.5101 g of sterols are present in 2.74 g of unsaponifiable matter (nutraceutical-rich residue)

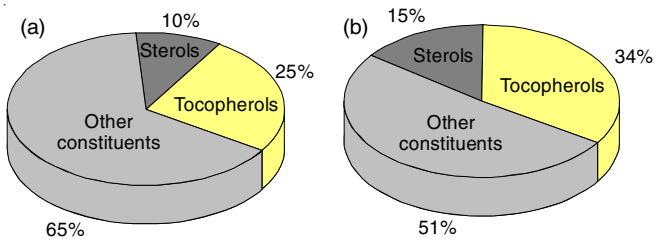


Fig. 7. Concentration of sterols & tocopherols in total unsaponifiable matter (a) unsaponifiable matter of SBTO, (b) unsaponifiable matter of TMO

in SBTO. These data indicated the presence of sterols and tocopherols in unsaponifiable matter, with tocopherol concentrations being greater in TMO and SBTO. Fig. 8 depicts a complete overview of the exact amounts of sterols and tocopherols, providing for a thorough comprehension of the nutraceutical content of these oils.

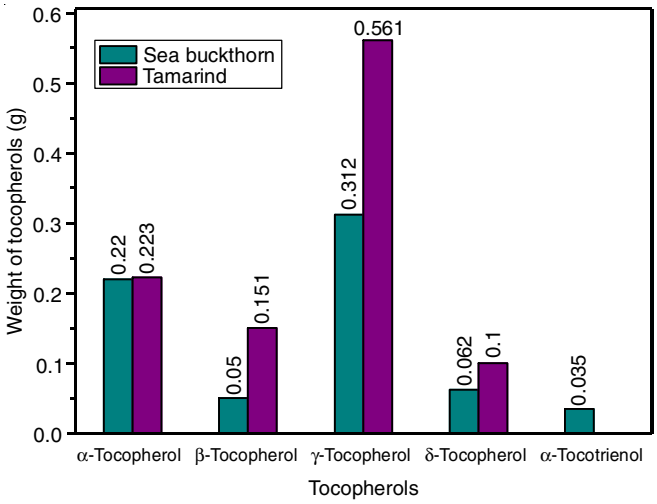


Fig. 8. Concentration of tocopherols and tocotrienols in 100 g of oil (TMO & SBTO)

The tocopherols in TMO and SBTO have different compositions, according to a comparison of the two oils. α-, β-, γ- and δ-Tocopherols are all present in TMO in amounts of 0.223 g, 0.151 g, 0.561 g and 0.1 g per 100 g of oil, respectively. The amounts of α, β, γ and δ-tocopherols in SBTO were 0.22 g, 0.05 g, 0.312 g and 0.062 g per 100 g of oil, respectively. Tocotrienols are not present in TMO, which is an exceptional finding. The amount of α-tocotrienol present in SBTO was 0.035 g/100 g of SBTO, nevertheless. This mismatch emphasizes the different tocopherol profiles of the two oils, with SBTO containing both tocopherols and tocotrienols whereas TMO mostly containing tocopherols. It is clear from a careful examination of the tocopherol compositions in TMO and SBTO that different tocopherol variants are present in different concentrations. γ-Tocopherol is present in higher quantity in both oils than other tocopherols [15]. In contrast, SBTO has a smaller content of β-tocopherol than TMO and *vice versa* for δ-tocopherol. It is interesting to observe that the two oils appear to have a similar amount of α-tocopherols per 100 g of oil, as seen in the accompanying figure. TMO and SBTO both contain various amounts of α-, β-, γ- and δ-tocopherols when their tocopherol composi-

tions are compared. SBTO has a relatively lower concentration of these tocopherols than TMO, which exhibits a relatively larger concentration. In particular, TMO has higher concentrations of α-, β-, γ- and δ-tocopherols per 100 g of oil, compared to SBTO, which has substantially lower concentrations of these tocopherols in the same unit of measurement.

There are distinguishable peaks on the HPLC chromatogram that correspond to tocopherols. α-Tocopherol (21.04%), β-tocopherol (14.49%), γ-tocopherol (53.77%) and δ-tocopherol (9.64%) were the tocopherols with the highest quantities in the TMO (Fig. 9), according to the analysis. Similar quantities of α-tocopherol (32.06%), β-tocopherol (7.24%), γ-tocopherol (45.66%), δ-tocopherol (9.11%) and α-tocotrienol (5.13%) were found in SBTO (Fig. 10).

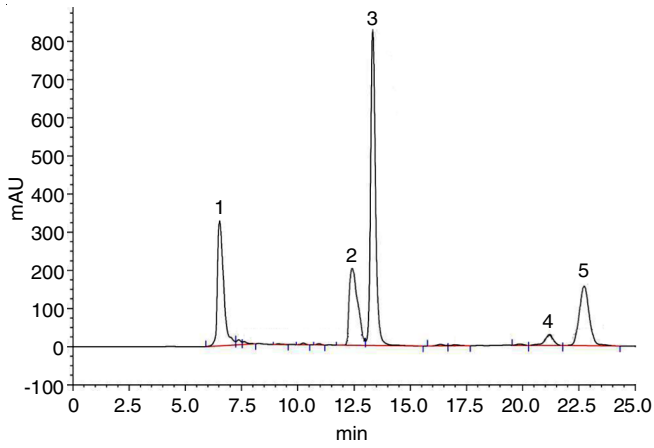


Fig. 9. Chromatogram of tocopherols of tamarind seed oil (TMO)

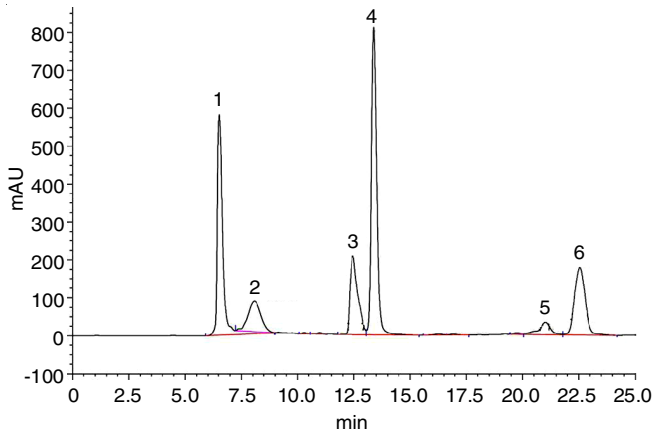


Fig. 10. Chromatogram of tocopherols of sea buckthorn seed oil (SBTO)

Conclusion

By employing the solvent extraction method, the successful extraction of sterols and tocopherols from tamarind seed oil (TMO) and sea buckthorn seed oil (SBTO) was achieved. Cold centrifugation was used to obtain sterols, while solvent evaporation was used to isolate tocopherols. Furthermore, a comprehensive analysis of the fatty acid composition of seed oils was also conducted. The study found that TMO has the greatest content of linoleic acid, underlining its importance in the oil's composition. According to this study, among all the tocopherols studied, γ-tocopherol had the greatest concentration and δ-tocopherol the lowest. However, α-tocotrienol was only found in SBTO, emphasising its importance as a differentiating trait. Furthermore, TMO contained a greater quantity of the nutraceutical rich residue than SBTO. As a result, tamarind oil is an excellent alternative for extracting nutraceuticals.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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