J Food Sci Technol
DOI 10.1007/s13197-014-1674-2

ORIGINAL ARTICLE

Evaluation and Characterization of Malabar Tamarind [*Garcinia cambogia* (Gaertn.) Desr.] Seed Oil

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Revised: 27 November 2014 / Accepted: 1 December 2014

Abstract The objective of this study is to evaluate the chemical compounds present in the Malabar tamarind seed oil. The oil was extracted from the seeds of Malabar tamarind fruits collected from NBPGR Regional station, Thrissur. The seeds yielded 46.5 % of oil. Parameters such as the peroxide value, iodine value, saponification value, and acid value of the extracted Malabar tamarind seed oil were determined. These values were used to predict the quality of fatty acid methyl esters present in the oil. UV absorption spectroscopy of the oil showed hypsochromic shift, and the maximum absorbance was at 269 nm. The Fourier Transform Infrared Spectrum revealed the presence of olefin hydrogen and carbonyl group of ester compounds in the oil sample. The evaluation of the chemical compounds in the oil using gas chromatography coupled with mass spectrometry (GC-MS) revealed that, a total of five fatty acid methyl esters were present in the oil sample. Among the five fatty acid esters present in the Malabar tamarind seed oil, Methyl 16-methyl heptadecanoate (54.57 %) was found to be the predominant compound. This study also supports the presence of olefins in the long chain fatty acids from Nuclear Magnetic Resonance (NMR) data. There is a significant correlation between the properties and the characteristic profile of the oil sample. This study is the first report that shows Malabar tamarind as a promising source of oil seeds.

Keywords Malabar tamarind · Gas chromatography · ?Seed oil · NMR spectroscopy · *Garcinia cambogia*

Research highlights 1. This study is a first report for the Malabar tamarind seed oil profiling.
2. GC-MS of seed oil revealed the presence of saturated and unsaturated fatty acid methyl esters
3. Methyl 16-methyl heptadecanoate (ester of margaric acid) was the predominant compound.
4. Oleic acid is the second most abundant fatty acid (39 %) next to margaric acid.
4. NMR studies revealed the presence of olefins in the long chain fatty acids.
5. Malabar tamarind seed is one of the viable sources for oil production.

Electronic supplementary material The online version of this article (doi:10.1007/s13197-014-1674-2) contains supplementary material, which is available to authorized users.

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Introduction

Garcinia cambogia (Gaertn.) Desr. famously known as Malabar tamarind or Kodampuli is a member of the family Clusiaceae (Guttiferae) and is distributed throughout the world. Africa and Malaysia appear to be the main regions with large number of endemic species of the genus *Garcinia* (Hemshekhar et al. 2011). It is a multipurpose tree naturally found in the evergreen and semi-ever green wild forests of Western Ghats, but is also grown in the home gardens of Kerala for the acidic fruit rind (Abraham et al. 2006). It is a rich source of (−) hydroxycitric acid and is a potent regulator of blood lipids such as cholesterol and triglycerides (Kim et al. 2011). The fruits of *Garcinia cambogia* are yellowish, large and globular with deep vertical grooves enclosing six to eight multi-lobed seeds. Seeds are usually connected to the fruit rind by white acidic pulp called Aril. Many species belonging to the genus *Garcinia* have commercial value as medicines and cosmetics. *Garcinia indica* is used as a natural coagulant in the preparation of Tofu (Rekha and Vijayalakshmi 2010). Traditionally, Malabar tamarind has an important role as a

Published online: 12 December 2014

condiment and curing agent in meat and fish curries. The commercial value of Malabar tamarind is because of wide applications of its fruit rind. Generally seeds of *Garcinia cambogia* are a waste product of post-harvest operations (Abraham et al. 2006). Despite intensive studies on the chemical composition of the fruit rind of Malabar tamarind, there have been no reports of the chemical constituents in the seed oil. *Garcinia xanthochymus* a close species of *G.cambogia* has been evaluated for the chemical composition of the seed oil. The seed oil of *G.xanthochymus* showed nine fatty acids, oleic acid being the major fatty acid (Manohar et al. 2014).

Abraham *et al*. (2006) reported the authenticity of using seed oil for edible purposes some 100 years ago in South India. Later as the financial status of the farmers improved and as the availability of other edible oils became widespread, the usage of seed oil from Malabar tamarind reduced. However for the preparations of special dishes such as *holige* and *pancha kajaye* (local names of the dishes) the use of edible fat and oil of Malabar tamarind is still in practice (Abraham et al. 2006). The seeds of Malabar tamarind contain oleic acid rich edible fat which resembles Kokum butter obtained from *Garcinia indica*. Kokum fat is used in chocolate making to enhance its physical properties without affecting its taste. This property is due to 2-oleodistearin triglycerides (Hemshekhar et al. 2011). Also this seed's fat finds its application in cosmetic industry as an emollient.

There is a shortage of edible oil production in India to meet the current requirement. India is the third largest consumer of edible oils in the world with domestic consumption of 12.5 million tons, whereas the domestic production of edible oil is only 8.2 million tons. To meet the local demand for edible oils, India has become the world's largest importer of oils (about 40–50 %). In India, the requirement of edible oil will be 18.3 and 21.8 million tons by 2015 and 2020 respectively. The country needs to double the production of oil seeds to meet the requirement in the future. The technologies and strategies developed by the oil seed research network have not been completely successful due to the socioeconomic and cultural constraints of oilseed farmers (Choudhary et al. 2014). Hence for narrowing down the gap between the production and requirement of edible oil there is a need for exploration of other sources (Cardoso et al. 2012). To the best of our knowledge, until now a physicochemical characterization of the oil produced from the seeds of Malabar tamarind has not been reported. To bridge this gap we report herein the oil composition of seeds of *Garcinia cambogia* performed by GC-MS and NMR spectroscopy. The purpose of the study is to determine the profile of the components present in the seed oil of Malabar tamarind in order to provide basic information for using this as edible oil in the human diet.

Materials and methods

Materials

The authentic seed samples of Malabar tamarind were collected from National Bureau of Plant Genetic Resources (NBPGR) Regional station, Thrissur. All the chemicals and reagents, i.e., sulfuric acid, ethanol, soluble starch, petroleum ether, potassium hydroxide, methanol, phenolphthalein indicator and sodium thiosulphate were of analytical grade. These chemicals were purchased from HiMedia Laboratories Pvt. Ltd. India.

Oil extraction

Presently, there is no commercial production of Malabar tamarind seed oil in India. Hence the seed oil of Malabar tamarind is not available in the market. The extraction of oil from the collected seeds of Malabar tamarind is done at a laboratory scale by following the below mentioned procedure. The seeds are grinded to a fine powder and then dried for 2–3 h. The seed oil from Malabar tamarind is extracted by using soxhlet apparatus (Borosil Glass works Ltd.) and petroleum ether as a solvent (Schinas et al. 2009). The soxhlet apparatus is set to a temperature of 65–70 °C and the overall process carried out for 12 h. After the completion of the process, the seed oil is separated from petroleum ether by using rotary vacuum evaporator (Roteva-63 Rotary Evaporator), dried at 60 °C and weighed. The oil yield is calculated on a weight basis.

Properties of seed oil

Acid value

The acid value of seed oil was determined according to AOAC (Association of Official Agricultural Chemists) Official Method. (Ali and Anany 2012).

Saponification value

The saponification value was determined according to AOAC Official Method (Ogunsina et al. 2011).

Peroxide value

The peroxide value was determined according to AOAC Official Method (Suja et al. 2004).

Iodine value

Iodine value was identified according to the standard International Organization for Standardization (Ogunsina et al. 2011).

UV-visible analysis

The absorbance and wavelength of the peaks were determined by wavelength scan between 200 and 800 nm. The UV-visible spectra were recorded on a Shimadzu UV-2401 PC UV-Vis spectrophotometer. The absorbance of the extracted seed oil was observed (Poiyamozhi et al. 2012).

Thin-layer chromatography

The oil sample was soluble in chloroform. TLC was run for the sample using ethyl acetate and petroleum ether as a mobile phase in the ratio 8:2. The R_f value was found to be 0.42 (Bansal et al. 2008).

FTIR (fourier transform infra-red) spectra

FTIR analysis was carried out on a Magna 750 FTIR spectrometer equipped with a DTGS (Deuterated Triglycine Sulfate) detector, Ni-Chrome source and KBr beam splitter. The spectrum of the sample was recorded in the range of 4000–500 cm^{-1} at a resolution of 4 cm^{-1}. The spectrum data was collected and treated using the Omnic software version 7.3.

Gas chromatography—mass spectrometry (GC-MS)

A GC-MS system consisting of a Perkin Elmer Technologies Model Clarus 680 GC equipped with Clarus 600 (EI) was used for qualitative and quantitative analyses (Perkin Elmer Technologies, Inc., Wilmington, DE). Separations of the volatile components were performed on a column (Elite-5MS 30.0 m, 0.25 mm ID, 250 μm df, Perkin Elmer Technologies, Inc., Wilmington, DE). One microliter of extract was injected at split (10:1) mode with an injector temperature of 250 °C. Helium gas (ultra high-purity grade, 99.99 %) was used as a carrier gas. GC oven conditions were initially at 60 °C and held for 2 min, then programmed at 10 °C/min until 300 °C, finally held for 6 min. Helium carrier gas flow was set at 30 cm/s. The MS conditions were as follows: ion source temperature, 240 °C; MS transfer temperature, 240 °C; electron multiplier, 1400 V; mass range, 50–600 Da; and scan rate, 2.91scans/s. The MS was run in the electron ionization (EI) full-scan mode, *m*/*z* 50–600.

NMR analysis

Nuclear Magnetic Resonance (NMR) spectroscopy was used to know the information about the structure of the prominent compounds present in the oil extract. ^{1}H NMR spectrum of *Garcinia cambogia* was obtained on a Bruker 500-MHz NMR spectrometer using a 5-mm broad band inverse probe head, equipped with shielded z-gradient accessories. The samples to be analyzed were dissolved in deuterated chloroform ($CdCl_3$) and transferred to the 5-mm NMR tube. The deuterated chloroform chemical shift peak at δ 7.25 ppm was used as internal reference. Typical parameters used were: spectral width, 4800 Hz; time domain data points, 296 K; flip angle, 90 °C; relaxation delay, 5 s; spectrum size, 32 K points; and line broadening for exponential window function, 0.3 Hz (Mazumdar et al. 2013).

Results and discussion

Properties of oil

Based on the oil extraction, the yield was 46.5 %. Some of the properties of the seed oil are given in Table 1.

The above parameters of Malabar tamarind seed oil were compared to sunflower oil (Campbell 1983), soy bean oil (Rehman et al. 2004) and olive oil values reported earlier. The acid value of Malabar tamarind seed oil (5.04 mg KOH/g) is high when compared to sunflower oil (3.09 mg KOH/g) and lower than olive oil (6.6 mg KOH/g). The peroxide value of Malabar tamarind seed oil (3.73 meq/kg) is lower than that of sunflower oil (12.6 meq/kg) and olive oil (20 meq/kg). However, peroxide value of Malabar tamarind seed oil shows higher value than soy bean oil (0.32 meq/kg). This value is an indication of the extent of oxidation suffered by oil. Similarly the saponification values for sunflower seed oil (197.43 mg KOH/g) show higher value than other oils. Lower saponification value of Malabar tamarind seed oil (145.36 mg KOH/g) indicates the presence of long chain fatty acids with high molecular weight. The oil sample containing relatively more of high molecular weight fatty acids will have low saponification value. This is because long chain fatty acids have a comparatively less number of carboxylic functional groups per unit mass. Iodine value for Malabar tamarind (131.0 g/100 g oil) and sunflower oils (131.6 g/100 g oil) is in the same range whereas it is lower for soybean (109 g/100 g oil) and olive oil (94 g/100 g oil). This is a measure of the amount of unsaturation in the given oil sample. Higher iodine

Table 1 Specific properties of the extracted oil sample of *G. cambogia*

Parameter	Value[a]
Acid value (mg KOH/g)	5.04±0.02
Saponification value (mg KOH/g)	145.36±0.45
Peroxide value (meq/kg)	3.73±0.04
Iodine value (g/100 g oil)	131.0±0.14

[a] Values were expressed as mean±standard deviations of three (n=3) measurements

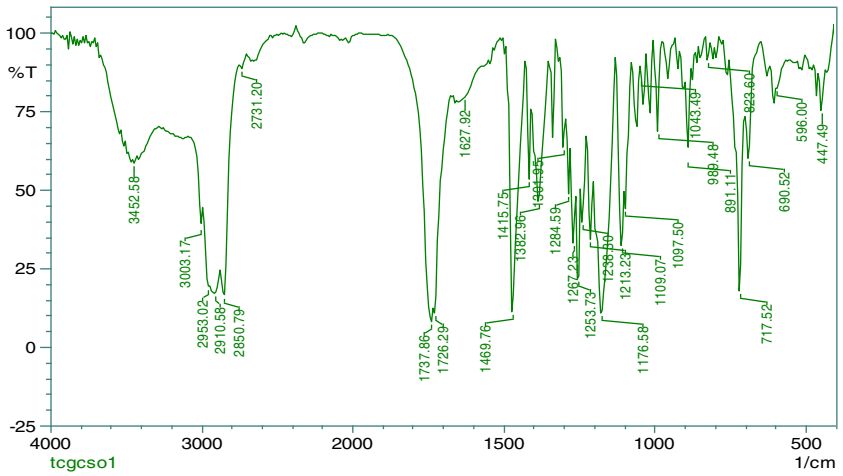

Fig. 1 FTIR spectrum of the extracted seed oil from Malabar tamarind

value for Malabar tamarind and sunflower seed oils indicates the presence of more double bonds in their fatty acid esters.

UV spectrum analysis

The UV-vis spectrum analysis of the extracted oil sample from Malabar tamarind shows hypsochromic shift. The absorbance of the sample at 269 nm refers to $n \rightarrow \pi^*$ and $p \rightarrow \pi^*$ transitions indicating the presence of unsaturated systems incorporating N or O groups (like C=O, C=N, S=O). The absence of absorption peak in the visible region (400–800 nm) clearly reveals that there are no conjugated aromatic ring systems in the extracted seed oil sample (Raaman 2006). The absorption bands between 200 and 300 nm are used to analyze the quality of the oil. The absorption peaks in this region may occur due to the presence of conjugated diene and triene systems. The maximum absorbance of this oil is comparable to the absorption of olive oil and soy bean oil (230–270 nm).

FTIR analysis

The IR spectrum of the extracted seed oil sample (Fig. 1) shows the characteristic peaks at 3003, 2950, 2850, 1737 and 1627 cm^{-1} for different functionalities (Table 2).

The peaks at 3003 and 2850 cm^{-1} indicate the presence of sp^3-CH, sp^2-CH and hybridized (alkane, alkene) carbons corresponding to aliphatic CH. The stretching frequency observed at 1737 cm^{-1} represents the presence of keto group attached to ester. The 1627 cm^{-1} stretch corresponds to the presence of unsaturated double bond which is not in conjugation with the ester bound keto group.

The FTIR result of this oil sample is compared to the IR spectrum obtained for corn oil, olive oil and sunflower oil (Rohman and Che Man 2012). The spectra for all the above mentioned samples correlate to the spectrum obtained for Malabar tamarind seed oil. The band at 3003 cm^{-1}, strong band absorptions in the range of 3000–2800 cm^{-1}, stretching vibrations of methyl groups at 2850 cm^{-1}, bending vibrations of methylene groups at 1469 cm^{-1} correlate with the previous reported data on FTIR analysis for corn oil, olive oil and sunflower oil.

GC-MS analysis

GC-MS analysis was performed to measure the fatty acids present in the extracted seed oil (Fig. 2).

The components of the oil were identified by matching their recorded mass spectra with the standard mass spectra from National Institute of Standards and Technology (NIST05.LIB) libraries provided by the software of the GC-MS system (TurboMass ver 5.4.2) and literature data. The results were confirmed by analyzing the mass spectra of the produced compounds and also by comparing their retention indices to the indices reported in the literature. From GC-MS analysis, the major compound is Methyl 16-methyl heptadecanoate (54.57 %), a methyl ester of margaric acid. Occurrence of this margaric acid is reported in butterfat, mutton fat and shark liver oil (Hansen et al. 1957). The presence of margaric acid is also reported in certain vegetable seed oils such as tomato (Botinestean et al. 2014) and okra (Sami et al. 2013) at low levels. The second major compound (E) - methyl octadec-13-enoate (39.02 %) is structurally

Table 2 Characteristic peaks in FTIR spectrum of extracted seed oil sample

S. No	Group	Structural unit	Frequency (cm^{-1})
1	Carbonyl group bound to ester	O-C = O	1737
2	Olefin group	C = C	1627
3	Alkane C-H	Sp^3-CH	2850
4	Alkene C-H (Aliphatic)	Sp^2-CH	3003
5	Carbonyl single bond stretch	Sp^2 C-O	1213

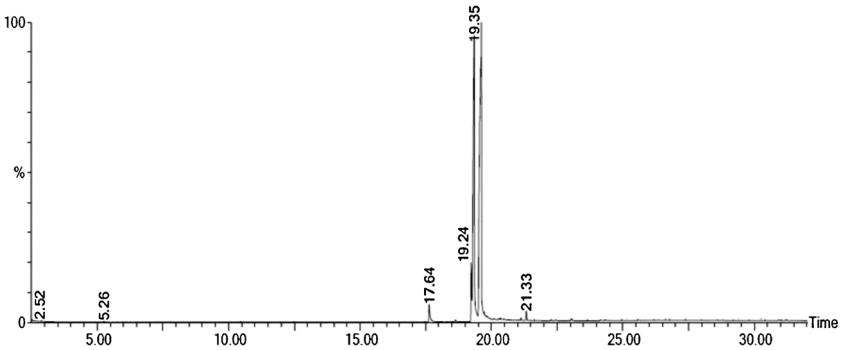

Fig. 2 Fatty acid profile of Malabar tamarind seed oil

similar to oleic acid of olive oil except for the position of a double bond and a methyl group substitute. The structures of these compounds are represented in Fig. 3. The mass spectra of the compounds identified by NIST libraries showed the characteristic molecular ion peaks, their respective daughter peaks and base peaks. Generally the compounds containing ester moieties undergo Mc Lafferty rearrangement during mass fragmentation. Here in our results also, Mc Lafferty molecular rearrangement with β-carbon atom is observed in mass fragmentation of all the compounds (Mc Lafferty 1959; Kingston et al. 1974) (Table 3). The mass fragmentation pattern of the compounds is in line with the mass spectra generated.

NMR studies

^{1}H NMR analysis

^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm)): 0.87 (s, 3H, $-CH_3$), 1.25 (s (br), 13H, $(-(CH_2)_{13})$, 1.60 (s, 3H, $-OCH_3$), 2.0 (s, 2H, $-CH_2$), 2.3 (s, 2H, $-CH_2$), 4.20 (dd, 1H, $-CH=\underline{CH}\text{-}CH_2$), 5.29 (d, 1H, J=31.2Hz, −CH=CH);

In order to study the chemical composition of the isolated Malabar tamarind oil, ^{1}H NMR spectroscopy was studied (Fig. 4). A peak was observed as a singlet at δ 0.87 ppm relative to methyl ($-CH_3$) group. A broad singlet peak at δ 1.25 ppm corresponding to the long chain fatty acids was observed. The characteristic peak of methoxy ($-OCH_3$) protons was observed as a singlet at δ 1.6 ppm, which was attributed to methyl esters. The other singlet peaks were observed at δ 2.0 and δ 2.3 ppm of methylene ($-CH_2$) protons. A doublet signal at δ 5.29 ppm of –CH=CH, a doublet of doublet signal at δ 4.20 ppm for $-CH=CH\text{-}CH_2$ were observed.

^{13}C NMR analysis

^{13}CNMR (100 MHz, $CDCl_3$) (δ, ppm): 22.94, 22.96, 25.25, 25.30, 27.13, 27.20, 27.27, 27.35, 27.40, 27.44, 27.56, 27.61, 27.70, 27.84, 29.98, 30.00, 32.12, 32.27, 66.95, 127.75, 128.09, and 171.35.

The spectrum of ^{13}C NMR of the extracted Malabar tamarind seed oil is shown in Fig. 5. The signal at 171.35 ppm was assigned the carbonyl carbon (−C = O) of the methyl ester group present in the compound. The chemical shift of this signal falls into the region for carbonyl carbons in general and for esters in particular. The signals at 128.09 ppm and 127.75 ppm may correspond to the unsaturation in the compound. The peak at 66.95 ppm corresponds to the

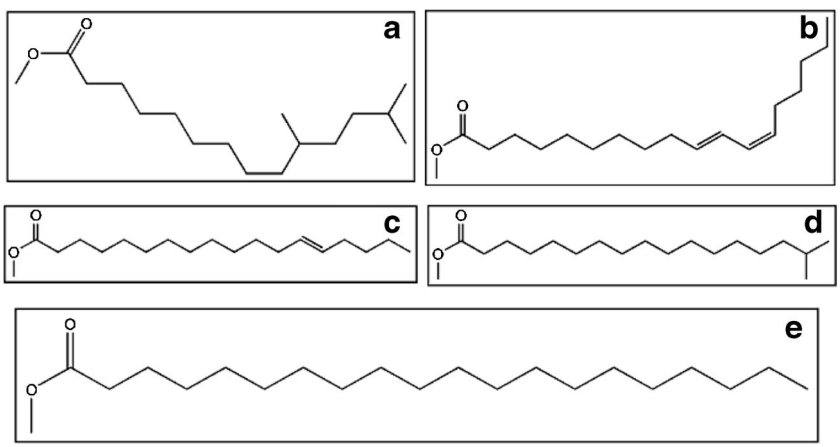

Fig. 3 Structures of the compounds identified by mass spectrometry with reference to NIST libraries (**a**) 10, 13-trimethyl myristate and (**b**) Methyl linoleate (**c**) Methyl oleate (**d**) 16-dimethyl margarate & (**e**) Methyl arachidate

Table 3 Chemical composition of the Malabar tamarind seed oil

Peak	RT (min.)	Peak area (%)	Fatty acid methyl ester	Fatty acid	C number	EI mass (*m/z*)	Mol. formula	Pubchem CID/CAS	IUPAC name	Class of compound
1.	17.64	1.79	10,13-trimethyl myristate	Myristic acid	14:0	270 (M^+), 242, 227, 143, 74	$C_{17}H_{34}O_2$	267650-23-7	Tetradecanoic acid, 10,13-dimethyl-, methyl ester	Saturated
2.	19.24	3.95	Methyl linoleate	Linoleic acid	18:2	294(M^+), 264, 234, 109, 67	$C_{19}H_{34}O_2$	900336-44-2	Methyl 10-trans,12-cis-octadecadienoate	Poly-unsaturated
3.	19.35	39.02	Methyl oleate	Oleic acid	18:1	296(M^+), 266, 223, 83, 55	$C_{19}H_{36}O_2$	900336-41-6	Methyl 13-octadecenoate	Mono-unsaturated
4.	19.62	54.57	16-dimethyl margarate	Margaric acid	17:0	298(M^+), 255, 241, 143, 87	$C_{19}H_{38}O_2$	900336-38-6	Methyl 16-methyl-heptadecanoate	Saturated
5.	21.33	0.65	Methyl arachidate	Arachidic acid	20:0	326(M^+), 283, 269, 227, 74	$C_{21}H_{42}O_2$	1120-28-1	Methyl eicosanoate	Saturated

RT Retention time, *C number* Carbon number, *EI* Electron ionization, *m/z* mass to charge ratio, *CID* Compound identifier, *CAS* Chemical Abstracts Service, *IUPAC* International Union for Pure and Applied Chemistry

methoxy group (O-Me) attached to the ester keto group (Amelio et al. 2013).

Based on the results, a good correlation among UV analysis, IR spectrum and proton NMR data was observed. From UV analysis, the absorption peak at 269 nm indicates the presence of unsaturated systems in the sample which is evident from the compounds methyl linoleate and methyl oleate identified in GC-MS analysis. From IR analysis, peak at 1737 cm^{-1} indicates the presence of carbonyl group attached to ester, which is justified as all the components identified were fatty acid methyl esters and peak at 1627 cm^{-1} corresponds to the presence of unsaturated double bond(C = C) as in methyl linoleate and methyl oleate. From ^{1}H NMR analysis, singlet peak at δ 0.87 ppm—methyl group, broad singlet peak at δ 1.25 ppm—long chain fatty acids, singlet peak at δ 1.6 ppm—methoxy protons of methyl esters, doublet signal at δ 5.29 ppm of –CH = CH, doublet of doublet signal at δ 4.20 ppm for –CH = CH-CH_2 are clearly observed in all the

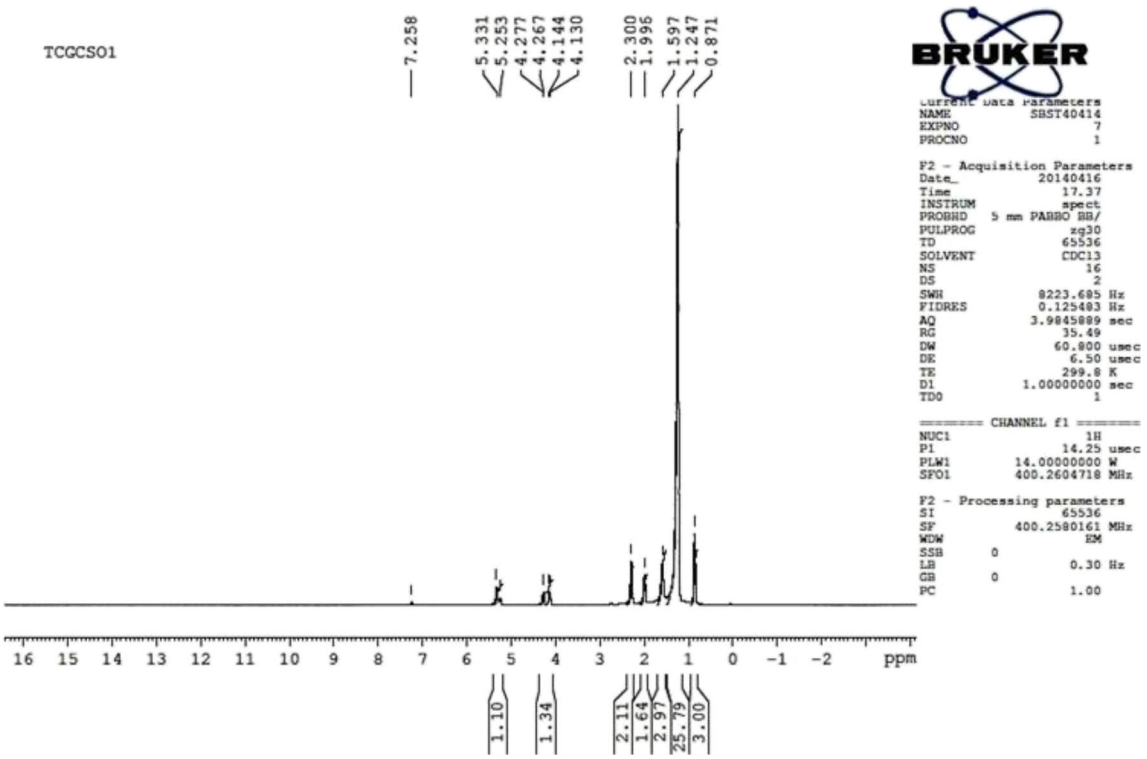

Fig. 4 ^{1}H NMR spectrum of Malabar tamarind seed oil dissolved in $CDCl_3$

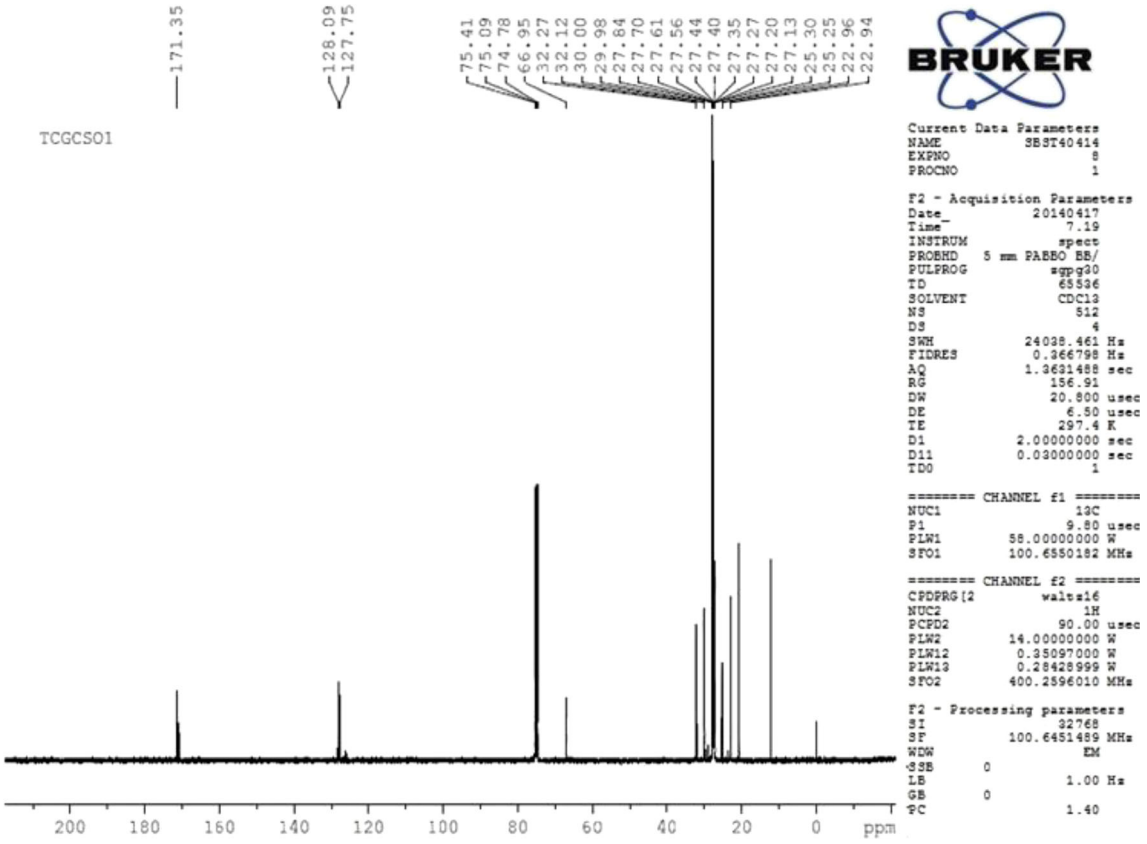

Fig. 5 ^{13}C NMR spectrum of Malabar tamarind seed oil

identified fatty acid esters. From ^{13}C NMR analysis, signal at 171.35 ppm – carbonyl carbon of the methyl ester, signals at 128.09 and 127.75 ppm—unsaturation(C = C) in the compound, 66.95 ppm—methoxy group bound to ester keto group are also found in the identified compounds of GC-MS. The fatty acid composition of Malabar tamarind seed oil contains a mixture of both saturated and un-saturated fatty acids.

Conclusion

In this paper, we provide information on the extraction, physicochemical properties and the chemical composition of the Malabar tamarind seed oil which has not been reported so far. The properties of Malabar tamarind seed oil such as acid value, iodine value, peroxide value and saponification value were found to have values similar to those of the domestic oils used for cooking purposes. Malabar tamarind seed oil, based on its high margarate content (54.57 %) can be a valuable source of margaric acid. This margarine with no trans-fatty acids is extensively used in making sweets in India. Oleic acid is the second most abundant fatty acid (39 %) in Malabar tamarind seed oil next to margaric acid. Therefore, use of separation techniques such as fractionation, counter-current distribution and hydrophilization will help in obtaining high oleic acid component from the oil with increased health benefits. Hence, the production of oil from Malabar tamarind seeds provides a viable source for cooking oil.

Acknowledgments Authors thank the Director, NBPGR, New Delhi and NBPGR Regional station, Thrissur for providing the *Garcinia cambogia* fruit samples for our studies and SIF VIT-DST-FIST, VIT University for providing GC-MS analysis and NMR spectroscopy instrumentation facilities.

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