Journal of Applied Botany and Food Quality 83, 157 - 162 (2010)

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Bioactive lipids, fatty acids and radical scavenging activity of Indian *Celastrus paniculatus* oil

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(Received March 23, 2010)

Summary

Data concerning the exact composition of *Celastrus paniculatus* seed oil is scarce. In the present study lipid profile of Indian *Celastrus paniculatus* seed oil were determined. *Celastrus paniculatus* seeds are a rich source of oil (46%). The amounts of neutral lipids were the highest, followed by glycolipids and phospholipids, respectively. Oleic followed by palmitic and linoleic were the major fatty acids in *Celastrus paniculatus* oil and its' lipid classes. *Celastrus paniculatus* seed oil being characterized by a relatively high amount of phytosterols, wherein the sterol marker was β-sitosterol followed by campesterol and stigmasterol. γ-Tocopherol was the main tocopherol isomer whilst the rest being α-tocopherol. When *Celastrus paniculatus* oil and extra virgin olive oil were compared upon their radical scavenging activity **(RSA)** toward the stable 1,1-diphenyl-2-picrylhydrazyl **(DPPH)** radical *Celastrus paniculatus* oil exhibited stronger RSA. In consideration of potential utilization, detailed knowledge on the composition of *Celastrus paniculatus* oil is of major importance. The diversity of applications to which *Celastrus paniculatus* oil can be put gives this substance great industrial importance.

Introduction

Ayurveda, a traditional Indian medicinal system, employs many medicinal plants in the treatment of different diseases. Owing to their medicinal and curing properties as well as the health-boosting constituents, non-traditional plant oils are used in healthcare industry, both in the informal as well as professional versions of the health care. A popular alternative medicine therapy, aroma therapy or oil therapy can be used as an alternative to the many conventional pharmaceuticals and exposure to their side effects.

A genus *Celastrus* contain about 100 species of shrubs, distributed over tropical Asia, China, Japan, Australia and North America. Four species are confined to India of which *Celastrus paniculatus* Willd. belongs to family Celastraceae. *Celastrus paniculatus*, a plant known for centuries as the "Elixir of life", is a large woody, climbing shrub, height up to 10-18 m tall and common to all over the hilly parts of the India. Fruits are yellow when matured and seeds are reddish brown covered with scarlet aril (GODKAR et al., 2004, 2006).

Celastrus paniculatus is an Indian medicinal plant having a remarkable reputation. The plant exhibit varying degrees of therapeutic value some of which useful in the treatment of cognitive dysfunction, epilepsy, insomnia, rheumatism, gout and dyspepsia (RUSSO et al., 2001; GODKAR et al., 2004, 2006). *Celastrus paniculatus* is well known for its ability to improve memory. According to Ayurveda, *Celastrus paniculatus* may be employed as a stimulant nervine tonic, rejuvenant, sedative and diuretic (VAIDYARATNAM, 1997; KUMAR and GUPTA, 2002). Recently, a methanolic extract of *Celastrus paniculatus* was shown to have free-radical-scavenging effects, and was also capable of reducing hydrogen peroxide (H_2O_2)-induced cytotoxicity and DNA damage in human non-immortalized fibroblasts (RUSSO et al., 2001).

The seeds extracted with petroleum either yield dark brown oil known as Celastrus oil or Malkanguni oil. Early report on the seed oil using paper chromatography techniques stated that oil mainly contains palmitic, stearic, oleic, linoleic and linolenic acids (SENGUPTA and BHARGAVA, 1970). The oil also contains sesquiterpene alkaloids *viz.,* celapanin, celapanigin and celapagin (CSIR, 1999) which studied for many potential uses including analgesic and anti-inflammatory (AHMAD et al., 1994) and antianxiety activity (JADHAV and PATWARDHAN, 2003). Celastrus oil has hypolipidemic and antiatherosclerotic activities as well as antispermatogenic action. It also enhances learning and memory possibly by decreasing biogenic amines turnover or antioxidant effect. The oil acts as a powerful stimulant used for the treatment of scabies, body and rheumatic pains, wound eczema and beriberi (VAIDYARATNAM, 1997; KAPOOR, 2005). Moreover, *Celastrus paniculatus* seed oil has been reported to exert a number of additional pharmacological actions such as analgesic (AHMAD et al., 1994), anti-malarial (AYUDHAYA et al., 1987) and anti-spermatogenic (WANGOO and BIDWAI, 1988). Recently, *Celastrus paniculatus* oil exhibited significant anxiolytic activity and the oil was found to be safe and did not affect behavior or impair motor coordination up to dose of 5 g/kg in rats (KAPOOR, 2005).

The study of *Celastrus paniculatus* seed oil for its' major and minor constituents is lacking. Natural fats and oils contain, apart from glycerides, a number of lipophilic materials with a very diverse chemical make up. Among the most interesting are the polar lipids, sterols and fat-soluble vitamins. In this work, lipid classes, fatty acids and fat-soluble bioactives of *Celastrus paniculatus* seed oil have been analyzed. The objective of this investigation was to obtain informative profile about the chemical nature of *Celastrus paniculatus* oil which will serve as a basis for further detailed chemical investigation and nutritional evaluation of the *Celastrus paniculatus* seeds. The results, furthermore, will be important as an indication of the potentially nutraceutical and economical utility of *Celastrus paniculatus* seeds as a new source of edible oils.

Material and methods

Materials

Celastrus paniculatus seeds were collected (October 2006) from the Khanapur forest Bidar district, Karnataka (India). Plant was identified with the help of the Flora of Gulbarga District (SEETHARAM et al., 2000) A voucher specimen (No. HGUG-503) was deposited at the Herbarium, Department of Botany, Gulbarga University (Gulbarga, India). Neutral lipid **(NL)** standards were from Sigma Chemical Co. (St. Louis, MO, USA). Standards used for glycolipids **(GL)** identification; monogalactosyldiacylglycerol **(MGD)**, digalactosyldi acylglycerol **(DGD)**, cerebrosides **(CER)**, steryl glucoside **(SG)** and esterified steryl glucoside **(ESG)** were of plant origin (plant species unknown) and purchased from Biotrend Chemikalien GmbH (Köln, Germany). Standards used for phospholipids **(PL)** identification; phosphatidylserine **(PS)**, phosphatidylethanolamine **(PE)**, phosphatidylinositol **(PI)** from Bovine liver and phosphatidylcholine **(PC)** from Soybean were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Standards used for sterols **(ST)** characterization were

purchased from Supelco (Bellefonte, PA, USA). Standards used for vitamin E (α-, β-, γ- and δ-tocopherol) were purchased from Merck (Darmstadt, Germany). Reagents and chemicals used were of the highest purity available.

Methods

Solvent extraction of total lipids (TL)

Seeds were fine powdered using high-speed mixer, and milled into a fine particle sized (*ca.* 2 mm) meal, which was sticky in consistency, then subjected to Soxhlet extraction using *n*-hexane for 14 h. Total lipids (**TL**) recovered (46% of fresh weight) were stored at 4 °C for further analysis.

Column chromatography (CC) and thin-layer Chromatography (TLC) of lipid classes

Fractionation of lipid classes and subclasses

Total lipids were separated into the different classes by elution with different solvents over a glass column (20 mm dia x 30 cm) packed with a slurry of activated silicic acid (70 to 230 mesh; Merck, Darmstadt, Germany) in chloroform (1:5, w/v). NL were eluted with 3-times the column volume of chloroform. The major portion of GL was eluted with 5-times the column volume of acetone and that of PL with 4-timed the column volume of methanol. The amount of the lipid classes obtained was determined by gravimetry. By means of thin-layer chromatography (**TLC**) on Silica gel F_{254} plates (thickness = 0.25 mm; Merck, Darmstadt, Germany) a further characterization of the GL and PL subclasses was carried out with the following solvent system chloroform/methanol/ammonia solution 25% (65:25:4, v/v/v). For the characterisation of NL subclasses silica gel F_{254} plates were developed in the solvent system *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v). For the detection of the lipids, the thin-layer plates were sprayed with the following agents: for the marking of all lipids with sulfuric acid (40%), for the marking of GL with α-naphthol/sulfuric acid and for the marking of PL with the molybdate-blue reagent (RAMADAN et al., 2006). Each spot was identified with lipid standards as well as their reported retention factor ($\mathbf{R}_f$) values. Individual bands were visualized under ultraviolet light, scraped from the plate and recovered by extraction with chloroform/methanol (2:1, v/v). Fatty acid composition of NL, GL and PL was determined by GLC/FID as described below.

Quantitative determination of lipid subclasses

For the quantitative determination of NL subclasses individual bands were scraped from the plate and recovered by extraction with 10% methanol in diethyl ether, followed by diethyl ether. Data presented are the average of three gravimetrically determinations. For the quantitative estimation of GL subclasses, the acetone fraction obtained by CC was separated by TLC in the above given solvent system. The silica gel regions with the corresponding GL subclasses were scraped out followed by hexose measurement photometrically at 485 nm using the phenol/sulfuric acid in acid-hydrolyzed lipids (RAMADAN et al., 2006). The percent distribution of each component was obtained from the hexose values. From the extinction values the quantitative amount was determined and related to their portion of the GL fraction. The determined portion was set into relation with the amount of TL, which had been separated by CC into the main lipid fractions. For the determination of the PL, the methanol fraction from CC was also separated by TLC in the above given solvent system and after scraping out of the individual PL subclasses brought to reaction with the hydrazine sulfate/sodium molybdate reagent at 100 °C for 10 min and was photometrically analyzed at 650 nm. From the obtained extinction values *via* a calibration chart for phosphorus the amount of PL was calculated. The individual values were put into relation to the PL fraction (methanol fraction from CC) and to the amount of TL.

Gas chromatography (GC) analysis of fatty acid methyl esters

Fatty acids were transesterified into methyl esters (**FAME**) using N-trimethylsulfoniumhydroxide (Macherey-Nagel, Düren, Germany) according to the procedure reported by ARENS et al. (1994). FAME were identified on a Shimadzu GC-14A equipped with flame ionization detector (**FID**) and C-R4AX chromatopac integrator (Kyoto, Japan). The flow rate of the carrier gas helium was 0.6 mL/min and the split value with a ratio of 1:40. A sample of 1μL was injected on a 30 m x 0.25 mm x 0.2 μm film thickness Supelco SP™-2380 (Bellefonte, PA, USA) capillary column. The injector and FID temperature was set at 250 °C. The initial column temperature was 100 °C programmed by 5 °C/min until 175 °C and kept 10 min at 175 °C, then 8 °C/min until 220 °C and kept 10 min at 220 °C. A comparison between the retention times of the samples with those of authentic standard mixture (Sigma, St. Louis, MO, USA; 99% purity specific for GLC), run on the same column under the same conditions, was made to facilitate identification.

Gas chromatography (GC) analysis of sterols (ST)

Separation of ST was performed after saponification of the oil sample without derivatization according to RAMADAN and MÖRSEL (2003). TL (250 mg) were refluxed with 5 mL ethanolic potassium hydroxide solution (6%, w/v) and a few anti-bumping granules for 60 min. The unsaponifiables were firstly extracted 3-times with 10 mL of petroleum ether, the extracts were combined and washed 3-times with 10 mL of neutral ethanol/water (1:1, v/v) and then dried overnight with anhydrous sodium sulfate. The extract was evaporated in a rotary evaporator at 25 °C under reduced pressure, and then ether was completely evaporated under nitrogen. GLC analyses of unsaponifiable residues were carried out using a Mega Series (HRGC 5160, Carlo Erba Strumentazione; Milan, Italy) equipped with FID. The following parameters were performed: DB 5 column (J & W scientific; Falsom, CA, USA) packed with 5% phenylmethylpolysiloxan, 30 m length, 0.25 mm i.d., 1.0 μm film thickness; carrier gas (helium) flow 38 mL/min (split-splitless injection was used). Detector and injector were set at 280 °C. The oven temperature was kept constant at 310 °C and the injected volume was 2 μL. The repeatability of the analytical procedure was tested and the relative standard deviation of three repeated analyses of a single sample was <5%. Quantitative analyses were performed with a Shimadzu (C-R6A Chromatopac; Kyoto, Japan) integrator.

Normal phase high performance liquid chromatography (NP-HPLC) separation, identification and quantification of tocopherols

Procedure. NP-HPLC was selected to avoid extra sample treatment (e.g., saponification) according to RAMADAN and MÖRSEL (2002). Analysis was performed with a solvent delivery LC-9A HPLC (Shimadzu, Kyoto, Japan). The chromatographic system included a model 87.00 variable wavelength detector and a 250 mm x 4 mm i.d. LiChrospher-Si 60, 5 μm, column (Knauer, Berlin, Germany). Separation of tocopherol isomers was based on isocratic elution when the solvent flow rate was maintained at 1 mL/min at a column back-pressure of about 65-70 bar. The solvent system selected for elution was isooctane/ethyl acetate (96:4, v/v) with detection at 295 nm. 20 μL of the diluted solution of TL in the mobile phase were directly injected into the HPLC column. Tocopherol isomers were identified by comparing their retention times with those of authentic standards.

Preparation of standard curves. Standard solutions were prepared

by serial dilution to concentration of approximately 5 mg mL^{-1} of each tocopherol isomer. Standard solutions were prepared from a stock solution which was stored in the dark at -20 °C. Twenty µL was injected and peaks areas were determined to generate standard curve data.

Quantification. All quantitation was by peak area using Shimadzu C-R6A chromatopac integrator (Kyoto, Japan). Standard curves (concentration versus peak area) were calculated from six concentration levels by linear regression. Based on the established chromatographic conditions repeated injections of different concentrations of the standard tocopherols were made 3-times onto the HPLC system. Injections in triplicate were made at each concentration for both standards and samples. All work was carried out under subdued light conditions. All the experiments were repeated at least thrice when the variation on any one was routinely less than 5%.

Radical scavenging activity (RSA) of *Celastrus paniculatus* oil and extra virgin olive oil toward DPPH radical (spectrophotometric assay)

Different solvents were used to assay the RSA of *Celastrus paniculatus* oil and extra virgin olive oil, whereas the best results were achieved with toluene which was able to dissolve completely the hydrophobic and the hydrophilic compounds (RAMADAN et al., 2006). Therefore, the RSA of *Celastrus paniculatus* oil and olive oil was assayed with DPPH radical previously dissolved in toluene. Toluenic solution of DPPH radicals was freshly prepared at a concentration of 10^{-4} M. The radical, in the absence of antioxidant compounds, was stable for more than 2 h of normal kinetic assay. For evaluation, 10 mg of *Celastrus paniculatus* oil or olive oil (in 100 µL toluene) was mixed with 390 µL toluenic solution of DPPH radicals and the mixture was vortexed for 20 s at ambient temperature. Against a blank of pure toluene without DPPH, the decrease in absorption at 515 nm was measured in 1-cm quartz cells after 1, 30 and 60 min of mixing using UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA toward DPPH radicals was estimated from the differences in absorbance of toluenic DPPH solution with or without sample (control) and the inhibition percent was calculated from the following equation:

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of test sample}) / \text{absorbance of control}] \times 100.$$

All experimental procedures were performed in triplicate and their mean values (± standard deviation) were given.

Results and discussion

Keeping in view of high medicinal property of *Celastrus paniculatus* and wide spread distribution in India, the seeds are being used for different applications. For a plant to be suitable for oil production it must meet the following two criteria; (i) the oil content must reach the minimum for commercially viable exploitation and (ii) the plant must be suitable for high acreage cultivation (BOCKISCH, 1998). In the present investigation *Celastrus paniculatus* seeds were found to contain about 46% crude oil. This confirms that *Celastrus paniculatus* seeds are a rich source of oil.

Levels of lipid classes and subclasses

A suitable combination of chromatographic procedures on silica gel was used to obtain major lipids classes and subclasses of *Celastrus paniculatus* seed oil. The proportion of lipid classes and subclasses presented in *Celastrus paniculatus* seed oil as well as R_f values of these subclasses are shown in Tab. 1. Among the TL present in the seeds, the level of NL was the highest (*ca.* 99% of TL), followed by GL (0.55% of TL) and PL (0.34% of TL), respectively. Subclasses of NL in the crude oil contained triacylglycerol (TAG), free fatty acids (FFA), diacylglycerol (DAG), esterified sterols (STE) and monoacylglycerol (MAG) in decreasing order. Significant amount of TAG was found (*ca.* 97.1% of total NL) followed by FFA (*ca.* 1.08% of total NL), while DAG and STE were recovered in lower levels. Subclasses of GL in the crude oil were sulphoquinovosyldi acylglycerol (SQD), digalactosyldiglycerides (DGD), cerebrosides (CER), sterylglycosides (SG), monogalactosyldiglycerides (MGD) and esterified sterylglycosides (ESG) as presented in Tab. 1. SG, ESG and CER were the prevalent components of the total GL. The average daily intake of GL in human has been reported to be 140 mg of ESG, 65 mg of SG, 50 mg of CER, 90 mg of MGD and 220 mg of DGD (SUGAWARA and MIYAZAWA, 1999). Therefore, it is worthy to point out that *Celastrus paniculatus* crude seed oil could be a good source of GL in diet. PL subclasses in oilseed extract were separated into four major fractions *via* TLC. Phosphorimetry of the TLC fractions (Tab. 1) revealed that the predominant PL subclasses were PC followed by PE, PI and PS, respectively. About a half of total PL was in PC and a quarter was in PE, while PI and PS were isolated in lower quantities.

Fatty acid profile of seed oil and its lipid classes

Fatty acid profiles of TL and lipid classes (NL, GL and PL) are presented in Tab. 2. According to the results shown in the table six

Tab. 1: Levels of lipid subclasses (g/kg TL) in *Celastrus paniculatus* crude seed oil

Neutral lipid Subclass	R_f values x 100[a]	g/kg TL	Glycolipid Subclass	R_f values x 100[b]	g/kg TL	Phospholipid Subclass	R_f values x 100[b]	g/kg TL
MAG	14	3.87 ± 0.08	SQD	6	0.22 ± 0.02	PS	4.7	0.17 ± 0.01
DAG	39	7.55 ± 0.11	DGD	17	0.58 ± 0.04	PI	11	0.54± 0.01
FFA	56	10.2 ± 0.18	CER	29-35	1.01 ± 0.07	PC	20	1.90 ± 0.03
TAG	79	915 ± 4.09	SG	41	1.71 ± 0.05	PE	30	0.64 ± 0.07
STE	95	5.53 ± 0.07	MGD	64	0.16 ± 0.03			
			ESG	76	1.60 ± 0.06			

[a] Solvent system used in TLC development: *n*-hexane/diethyl ether/acetic acid (60:40:1, v/v/v).
[b] Solvent system used in TLC development: chloroform/methanol/ammonia solution 25% (65:25:4, v/v/v).
Results are given as the average of triplicate determinations ± standard deviation.
Abbreviations: TL, total lipids; **MAG**, monoacylglycerols; **DAG**, diacylglycerols; **TAG**, triacylglycerols; **FFA**, free fatty acids; **STE**, sterol esters. **SQD**, sulphoquinovosyldiacylglycerol; **DGD**, digalactosyldiacylglycerol; **CER**, cerebrosides; **SG**, steryl glucoside; **MGD,** monogalactosyldiacylglycerol; **ESG**, esterified steryl glucoside; **PS,** phosphatidylserine; **PI,** phosphatidylinositol; **PC,** phosphatidylcholine; **PE,** phosphatidylethanolamine.

Tab. 2: Fatty acid profile of *Celastrus paniculatus* oil and its' lipid classes.

	Total lipids	Neutral lipids	Glycolipids	Phospholipids
Fatty acid		*Relative content (%)*		
C14:0	0.40± 0.88	0.39 ± 0.82	0.50 ± 0.03	0.46 ± 0.03
C16:0	26.1 ± 0.02	26.0 ± 0.03	26.5 ± 0.33	26.7 ± 0.33
C18:0	2.75 ± 0.36	2.55 ± 0.32	2.90 ± 0.55	2.84 ± 0.55
C18:1	54.2 ± 0.39	54.3 ± 0.42	54.0 ± 2.30	54.0 ± 2.30
C18:2	11.2 ± 1.25	11.3 ± 1.36	10.9 ± 1.22	10.9 ± 1.22
C18:3*n*-3	5.35 ± 0.09	5.46 ± 0.02	5.20 ± 0.05	5.10 ± 0.05

Results are given as the average of triplicate determinations ± standard deviation.

fatty acids were identified in *Celastrus paniculatus* seed extract, wherein the analysis of FAME gave the proportion of oleic followed by palmitic and linoleic as the major fatty acids, which comprising together more than 90% of total FAME. α-Linolenic acid (**ALA**, C18:3*n*-3) was also estimated in a relatively high amounts. The results are totally not in line with the early study (SENGUPTA and BHARGAVA, 1970) used paper chromatography techniques which stated that oil mainly contains palmitic (31.2%), stearic (3.5%), oleic (22.5%), linoleic (15.7%), linolenic acids (22.2%). A striking feature of the *Celastrus paniculatus* seed oil was the relative high level of monounsaturated fatty acids (**MUFA**), which accounted for 54% total fatty acids. From the health point of view, MUFA have been shown to lower "bad" LDL cholesterol (low density lipoproteins) yet retain "good" HDL cholesterol (high density lipoproteins). This is in fact the major benefit of olive oil over the highly polyunsaturated seed oils, wherein the polyunsaturated fatty acids (**PUFA**) reduce both the "bad" as well as the "good" serum cholesterol levels in our blood. Fatty acids in neutral lipids and polar lipids were not differed significantly from each other, wherein oleic followed by palmitic and linoleic were the main fatty acids. The ratio of unsaturated fatty acids to saturated fatty acid, however, was higher in neutral fractions than in the corresponding polar fractions (GL and PL). Concerning saturated fatty acids (especially palmetic and stearic), GL resemble PL in the higher content of saturates, while saturated fatty acids were detected in relatively lower levels in the corresponding NL. In general, the fatty acid composition and high amounts of MUFA makes the *Celastrus paniculatus* seed oil a special component for nutritional applications.

Tab. 3: Levels of sterols and tocopherols (g/kg) in *Celastrus paniculatus* crude seed oil.

Compound	g/kg
Brassicasterol	nd[a]
Campesterol	1.66 ± 0.08
Stigmasterol	1.44 ± 0.10
Lanosterol	nd
β-Sitosterol	4.90 ± 0.52
Δ5-Avenasterol	0.15 ± 0.01
Sitostanol	nd
Δ5, 24-Stigmastadinol	nd
Δ7- Stigmastenol	0.43 ± 0.04
Δ7-Avenasterol	0.22 ± 0.02
α-Tocopherol	0.52 ± 0.02
β-Tocopherol	nd
γ-Tocopherol	1.04 ± 0.07
δ-Tocopherol	nd

[a] Not detected.
Results are given as the average of triplicate determinations ± standard deviation.

Sterol (ST) analysis and composition

Amounts of phytosterols (**ST**) in vegetable oils are used for the identification of oils, oil derivatives and for the determination of the oil quality Furthermore, the concentration of ST has been reported to be little affected by environmental factors and/or by cultivation of new breeding lines (HOMBERG, 1991; RAMADAN et al., 2006, 2007). *Celastrus paniculatus* seed oil being characterised by a relatively high amount of unsaponifiables (16 g/kg TL), of which *ca.* 55% were phytosterols. Six compounds were detected, wherein the sterol marker was β-sitosterol which comprised *ca.* 64.5% of the total ST content (Tab. 3). The next major components were campesterol and stigmasterol and these three major components were accounted for more than 90% of total ST. Other components, e.g., Δ7-Avenasterol, Δ5-avenasterol and Δ7-stigmastenol, were found in lower levels. Brassicasterol, lanosterol, sitostanol and Δ5, 24-stigmastadinol were not detected in the *Celastrus paniculatus* unsaponifiables. Among the different plant sterols, sitosterol has been most intensively investigated with respect to its physiological effects in man. Many beneficial effects have been shown for the sitosterol (YANG et al., 2001). Phytosterols, in general, are of interest due to their antioxidant activity and impact on health. Recently, phytosterols have been added to vegetable oils as an example of a successful functional food.

Tocopherols profile

The nutritionally important components such as tocopherols (vitamin E) improve stability of the oil. Tocopherols are the major lipid-soluble, membrane-localized antioxidants in humans. Vitamin E deficiency in man causes defects in the developing nervous system of children and hemolysis in man. Eipdemiologic studies suggest that people with lower vitamin E and other antioxidant intake and plasma levels may be at increased risk for certain types of cancer and for atherosclerosis (RAMADAN et al., 2006). Tocopherols in vegetable oils, moreover, are believed to protect PUFA from peroxidation. Data about the qualitative and quantitative composition of vitamins E are summarized in Tab. 3. Two of the four tocopherol isomers were present, wherein γ-tocopherol constituted 66.6% of the total analytes and the rest being α-tocopherol (*ca.* 33.3 %). α- and γ-Tocopherols proved to be the major tocopherols in vegetable oils and fats. Wheat germ oil was found to be a good source of α-tocopherol, followed by sunflower and olive oils. γ-Tocopherol occurred in highest concentrations in camelina, linseed, cold-pressed rapeseed and corn oil (CHOO et al., 2005, SCHWARTZ et al., 2008). α-Tocopherol is the most efficient antioxidant of tocopherol isomers, while β-tocopherol

has 25-50% of the antioxidative activity of α-tocopherol, and γ-isomer 10-35% (KALLIO et al., 2002). Despite general agreement that α-tocopherol is the most efficient antioxidant and vitamin E homologue in *vivo*, however, studies indicate a considerable discrepancy in its absolute and relative antioxidant effectiveness in *vitro*, especially when compared to γ-tocopherol (KAMAL-ELDIN and APPELQVIST, 1996). Levels of tocopherols detected in *Celastrus paniculatus* seed oil may contribute to the stability of the oil toward oxidation.

Radical scavenging activity (RSA) of *Celastrus paniculatus* oil

Interest has increased in the past few years in the free radical theory of disease causation, particularly in vascular diseases and certain forms of cancer. These developments have led to the investigation on dietary agents, the antioxidant nutrients (mainly vitamins A, C, and E), in a possible prophylactic, even curative, role in the disease process. Closely related to this probable benefit of natural antioxidants is their role in controlling free radicals as they may lead to pathological effects such as vascular diseases and cancer. A free radical is defined as any chemical species that has one or more unpaired electrons. This often results in very reactive compounds. Oxidation is a natural and needed reaction in metabolism. Highly reactive hydroxyl radicals, • OH, results. These can attack DNA, protein and polyunsaturated fatty acids residues of membrane phospholipids, among others. With the latter, a peroxyl radical is formed. Antioxidants quench this radical. If the supply of antioxidants is inadequate, a chain reaction takes place that may lead to damaged tissue. The evidence in the literature begins to make an overwhelming case for the existence of a relationship between high blood levels of antioxidant nutrients and a lowered incidence of disease.

Natural antioxidants, on the other side, allow food processors to produce stable products with clean labels and tout all-natural ingredients. The tests expressing antioxidant potency can be categorized into two groups: assays for radical scavenging ability and assays that test the ability to inhibit lipid oxidation under accelerated conditions. However, the model of scavenging stable free radicals is widely used to evaluate the antioxidant properties in a relatively short time, as compared to other methods. Pervious study on radical scavenging properties of vegetable oils had used different solvents to dissolve the oils and the free radicals). Hence, the results were difficult to compare because the reactions were occurred under different conditions. In contrast, our simple experiment (RAMADAN and MÖRSEL, 2003, 2006; RAMADAN et al., 2006, 2007) has been performed using the same solvent (toluene) to dissolve the fat or oil samples and the free radicals. This allowed us to characterize and compare the RSA of all samples under the same conditions.

Apart from the oxidative stability of vegetable oils and fats depends on the fatty acid composition, the presence of minor fat-soluble bioactives and the initial amount of hydroperoxides. Antiradical properties of the *Celastrus paniculatus* oil and extra virgin olive oil (as a standard crude oil with respective high levels of nutritive antioxidants and bioactives) were compared using stable DPPH free radicals. Fig. 1 shows that *Celastrus paniculatus* oil has higher RSA than extra virgin olive oil. After 1 h incubation, 24% of DPPH radicals were quenched by *Celastrus paniculatus* oil, while extra virgin olive oil was able to quench only 9.40%. ESR measurements showed also the same pattern (data not shown). Regarding the composition of *Celastrus paniculatus* oil and extra virgin olive oil, they have different pattern of fatty acid and lipid-soluble bioactives. It could be said that the RSA of oils and fats can be interpreted as the combined action of different endogenous antioxidants. However, when polar fractions, which contain mainly polar lipids and in low level phenolics, are found in high levels, strong RSA of these components can be expected as well as synergistic activity with primary antioxidants (RAMADAN et al., 2006, 2007). The significantly stronger antiradical action of *Celastrus paniculatus* oil compared to extra virgin olive oil may be due to (i) the differences in content and composition of polar lipids and unsaponifiables (ii) the diversity in structural characteristics of potential phenolic antioxidants present, (iii) a synergism of polar lipids with other components present, and (iv) different kinetic behaviors of potential antioxidants. All these factors may contribute to the radical quenching efficiency of oils and fats.

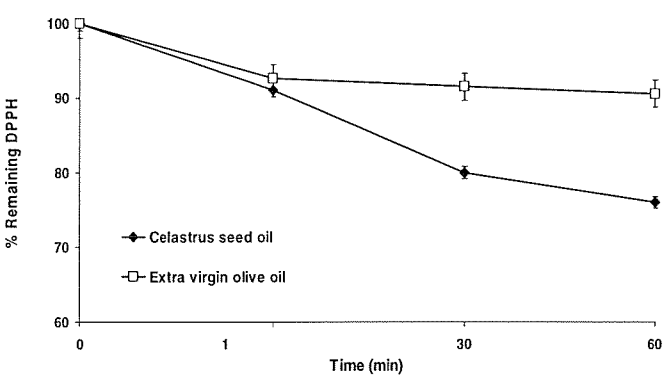

Fig. 1: Scavenging effect at different incubation times of *Celastrus paniculatus* oil and extra virgin olive oil on DPPH radical as measured by changes in absorbance values at 515 nm.

Conclusions

Folk medicines have been widely employed for centuries, and they remain one important source for the discovery of new bio-active compounds. Ayurveda, an ancient traditional system of medicine that has been practiced in India since 200 B.C. employs a large number of medicinal plants used in the prevention and treatment of a wide number of diseases. One of these includes the plant *Celastrus paniculatus* a plant known for centuries as "the elixir of life".

Improved knowledge on the composition, analysis and properties of *Celastrus paniculatus* seeds would assist in efforts for industrial application of this plant. Data about *Celastrus paniculatus* seed oil are very few; on the other hand, there are not reports in literature about detailed composition of *Celastrus paniculatus* seed oil. In concluding this study on *Celastrus paniculatus* seed oil, it could be said that the *Celastrus paniculatus* seeds give considerable yield of oil and the oil seem to be a good source of essential fatty acids and lipid-soluble bioactives. The high oleic acid content makes the oil nutritionally valuable. Tocopherols and sterols at the level estimated may be of nutritional importance in the application of the seed oil. *Celastrus paniculatus* seeds could be nutritionally considered as a new non-conventional supply for pharmaceutical industries and edible purposes.

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