Basella alba seeds as a novel source of non-conventional oil with beneficial qualities

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Abstract

The search for new sources of oil with improved properties has focused our attention on the characteristics of seed oil from Basella alba, an important tropical leafy vegetable. The oil content of Basella alba seeds was 25.46 ± 0.67%. Physicochemical properties of extracted oil were as follow: specific gravity (0.91 ± 0.01), refractive index (1.47 ± 0.00), lovibond colour (10.3 ± 0.00), viscosity at 20 °C (35.65 ± 0.15 mPas), cloud point (- 1.7 ± 0.00 °C) acid value (3.74 ± 1.62 mg KOH/g), peroxide value (7.67 ± 0.58 meq O_2/kg), iodine value (107.16 ± 2.44 g I_2/100g), saponification value (195.42 ± 1.62 mg KOH/g). Biochemical and nutritive analysis have revealed the following assets: impurities (0.014 ± 0.00%), unsaponifiable matter (1.10 ± 0.02%), phosphorus (0.12 ± 0.01 mg/g), vitamin A (0.48 ± 0.01 mg/g) and vitamin E (0.20 ± 0.01 mg/g). Basella alba seed oil showed higher content of polyunsaturated fatty acids (PUFA) (49.22 ± 0.02%) made of linoleic and linolenic acids (46.10 and 3.12 ± 0.01%, respectively). All these interesting characteristics should arouse attention for the usage of Basella alba seed oil in food and pharmaceutical industries.

Keywords: *Basella alba*; seed oil; vitamin A; vitamin E; essential fatty acids.

Introduction

A large quantity of oils and fats, whether for human consumption or for industrial purposes, is presently derived from plant sources [1]. These vegetable fats and oils are extracted from seeds and fruits and are mainly composed of triacylglycerol (95 - 98%) and complex mixtures of minor compounds (2 - 5%) of a wide range of chemical nature [2]. These minor components include mono- and diglycerides, free fatty acids, phosphatides (or phospholipids), sterols, protein fragments, various resinous and mucilaginous materials and oxidative products [3].

More than 75% of the world vegetable fat production consists of liquid oils, which are used for retailing, frying, canning and preparation of emulsions or margarines [4]. There are numerous vegetable oils derived from various sources. These include the popular vegetable oils: soybean, cottonseed, olive, peanut, palm, palm kernel, coconut and sunflower oils [5]. Palm, olive, cottonseed, peanut, and sunflower oils are classified as oleic – linoleic acid oils seeing that they contain a relatively high proportion of the monounsaturated oleic acid and the polyunsaturated linoleic acid. Soybean oil is classified as linolenic acid oil since it contains the more highly polyunsaturated linolenic acid [6]. With the growing body of evidence that all fats and oils are not equivalent, interest in polyunsaturated fatty acid (PUFA) profiles has been emerging [7]. Among PUFA, the most important families are the well-known *n*-3 and *n*-6 fatty acids. These two families are similar as they both comprise a precursor, namely α-

linolenic acid (ALA) for the *n*-3 and linoleic acid (LA) for the *n*-6 family and terminal products obtained by a succession of elongations and desaturations during the metabolism. These compounds are said to be essential because the human body is unable to synthesize them, although it can metabolize them to longer-chain derivatives. So the diet must cover the organism need for these fatty acids [8].

To meet the increasing demand for vegetable oils, improvements are being made, with conventional crops in the one hand and great interest in newer sources of non-conventional edible oils has recently grown in the other hand. Indeed, no oil from a single source has been found to be suitable for all purposes because oils from different sources generally differ in their composition [1]. Several plants are now grown, not only for food and fodder, but also for a variety of products with applications in industry, including oils and pharmaceuticals. Among these plants, the specie *Basella alba* belongs to the family *Basellaceae* and is known as Malabar spinach or Cyclone spinach. This plant is a perennial vegetable that has short petioles, thick tender stems with circular to ovate leaves and protruding seeds of about 3 mm length [9]. In most countries of tropical Africa and particularly in Côte d'Ivoire, leaves of these plants are widely consumed as green vegetables due to their richness in polysaccharides, vitamins and minerals [10]. The underexploited seeds of this leafy vegetable have been reported in several studies for their traditional medicinal uses but there is no report to the best of our knowledge about the physicochemical and biochemical characteristics of oil extracted from these seeds. So the objective of this investigation is to explore the potentially nutritional, nutraceutical and industrial utility of *Basella alba* seeds as a new source of edible oil.

Materials and Methods

Plant material

Mature *Basella alba* seeds were collected from market gardening of Abidjan district (Côte d'Ivoire) in June 2013. The plant was identified and authenticated by Professor Ake Assi (Botany Department of Félix Houphouët Boigny University – Abidjan). Voucher specimen (No AMPT09) of the plant was kept in the herbarium of National Center of Agronomic Research (CNRA) of Côte d'Ivoire. Seeds were rinsed thoroughly with distilled water to remove dirt and dried at 40 °C for 24 h in an electric oven (MEMMERT, GERMANY)

Chemicals

Analytical HPLC grade solvents, standards and reagents were used to perform analysis. Solvents (n-hexane, chloroform, acetic acid, diethyl-ether, ethanol, methanol and n-heptane) were from MERCK. Standards such as fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid), retinol palmitate (vitamin A), α-tocopherol acetate (vitamin E) and erucic acid were from SIGMA-ALDRICH. Wijs reagent was from PROLABO.

Seed oil extraction

Oil was extracted from 50 g crushed seeds (LABORATORY CRUSHER, CULATTI, FRANCE) with 300 mL of n-hexane (40-60 °C) in a Soxhlet extractor. Then the solvent was removed (vacuum-packed) at 40 °C with a rotary evaporator (HEIDOLPH, HEI-VAP, GERMANY). The extracted lipid was weighed to determine the oil content of the seed. Crude oils were stored at 4 °C in air tight brown sterile glass bottles until further use [11].

Determination of physicochemical characteristics

Specific gravity at 20 °C, refractive index at 20 °C and specific extinction (232 nm and 270 nm) were carried out following international chemical methods [12]. Colour and cloud point were determined by using a Lovibond colorimeter (LICO, LABOMAT, FRANCE) and a thermometric system (METLLER TOLEDO, SWITZERLAND), respectively. Viscosity was determined at different temperatures (20 – 80 °C) by using a viscometer apparatus (ANTON PAAR GMBH, AUSTRIA) equipped with a syringe filled with 1 mL of oilseed sample. Values of viscosities were automatically recorded after temperature programming. Visible and Near infrared spectrum (NIR) was determined by reading absorbance of oil sample in the range (400-2500 nm) by using infrared spectrophotometer (FOSS, DENMARK). pH value of oil sample was determined at 25 °C [13]. Acid, peroxide, iodine and saponification values were determined using official methods [14].

Determination of biochemical and nutritive characteristics

Unsaponifiable matter content

Unsaponifiable matter content of oil was determined following international chemical methods [12]. Oil sample (5 g) was saponified with 50 mL of 2 N KOH methanolic solution for 1 h. To the resulted mixture, 50 mL of distilled water was added. The unsaponifiable matter was extracted three times with 50 mL of diethyl-ether. Organic fractions were collected, washed three times with 50 mL of distilled water and then dried with sodium sulfate. Diethyl-ether was removed in a rotary evaporator (HEIDOLPH, HEI-VAP, GERMANY) to recover the unsaponifiable matter which was then weighed.

Phosphorus content

Phosphorus content of oil sample was determined following colorimetric method [12]. The test oil portion (5 g) was burned to ashes in the presence of magnesium oxide. The ashes obtained were dissolved in diluted nitric acid solution (65%). Absorbance was then measured at 460 nm using a spectrophotometer (PG INSTRUMENTS, ENGLAND) after adding an aqueous ammonium vanadate solution. A standard curve of phosphorus (1 mg/mL) was used as reference.

Vitamin A and vitamin E contents

The oil sample (1 g) was diluted in 10 mL of hexane. Thereafter, 200 µL of this mixture was transferred into a screw-capped tube where 800 µl of methanol were added. After being vortex-mixed and centrifuged (3000 rpm for 5 min), the samples were filtered through a 0.45 µm pore size filter and the overlay was used for high performance liquid chromatography (HPLC) analysis [15]. Separation by HPLC was carried out using a liquid chromatography system (ACQUITY WATERS, USA) equipped with an optical detector TUV system and a BEH C_{18} column (150 X 0.25 mm i.d., 1.7 µm particle size). The injection volume was 10 µL. The mobile phase was methanol-water (98:2, v/v) and the elution was performed at a flow rate of 2 mL/min. The analytical column was kept at 45 °C. Vitamin A of oil sample was detected at 325 nm and identified by comparing its retention time with this of authentic standard. Quantification of vitamin A identified in oil sample was done by using a

standard curve (concentration versus peak area) of retinol palmitate. Detection of vitamin E was done at 292 nm by using an optical detector TUV system. Vitamin E of oil sample was identified by comparing its retention time with this of authentic standard. Quantification of vitamin E identified in oil sample was done by using a standard curve (concentration versus peak area) of α-tocopherol acetate. All the data obtained were stored and processed by Empower software (WATERS, USA).

Fatty acids composition

The fatty acids were converted to their methyl esters (FAMEs) as follow: a quantity of 0.1 g of oil sample was mixed with 2 mL of n-heptane and 0.2 mL of a methanolic solution of potassium hydroxide (2 N). The whole mixture was shaken up for 30 s and allowed to settle for 5 min. The top layer containing the FAMEs was used for gas chromatography (GC) analysis [16]. FAMEs solution (1 µL) containing the internal standard (erucic acid) was injected into a gas chromatograph (SHIMADZU, GC-9A, JAPAN) equipped with a mass spectrometer (MS) and a RTX5 fused silica capillary column (30 m X 0.32 mm i.d. X 0.25 µm film thickness). The carrier gas was helium and the flow rate adjusted to 23 mL/min. Temperatures of detector and injector were 250 °C. The initial column temperature was fixed to 100 °C and programmed to increase by 5 °C per min intervals until 220 °C and, kept for 10 min at this temperature. The fatty acid methyl esters peaks were identified by comparing their retention times with those of standards. After adjusting areas with the internal standard (erucic acid), the yield of each fatty acid was calculated as follow: area of the fatty acid/areas of total fatty acids in the oil sample × 100 (%).

Statistical analysis

In the present experiment each test for the sample was analyzed in triplicate. Data were performed by using StatPlus 2009 (Analystsoft Inc) software and values were expressed as means ± standard deviation (SD).

Results and Discussion

Oil yield

The oil content of *Basella alba* seeds was 25.46 ± 0.67%. With regard to this oil yield, seeds of *Basella alba* are lipid-rich than most of conventional oilseeds such as cotton (13%), soybean (14%) and palm fruit (20%). Therefore, *B. alba* seeds could be used as an alternative source of oil for lipid industries [17, 18].

Physicochemical properties

The physicochemical parameters of *B. alba* seeds oil are shown in Table 1. The value of specific gravity was 0.91 ± 0.01 while the refractive index was about 1.45 ± 0.00. The specific gravity and refractive index of *B. alba* oilseeds are within the range of those reported for most conventional edible oils [19]. In view of specific extinction value (1.7 ± 0.00) at 270 nm, *B. alba* seed oil shows more oxidative stability than sunflower seed oil which extinction value at 270 nm is 1.872 [20]. Lovibond colour in red light (Lr) of *B. alba* seed oil was 10.3 ± 0.00. This parameter, generally related to carotenoids content and bleachability index of oil sample, is lower than that (20.4) of crude palm oil [4, 20]. The cloud point of *B. alba* seed oil was - 1.7 ± 0.00 °C. This parameter which is the temperature of first stage of sample crystallization indicates the liquid state and the unsaturated level of oil sample [4]. This unsaturated level of *B. alba* seed oil is also linked to the semi-drying state indicated by the

refractive index value (Rossell, 1991). With regard to this cloud point value (- 1.7 ± 0.00 °C), *B. alba* seed oil is more unsaturated than palm olein (4 °C) and sesame seed oil (0 °C) [21].

The food value of a greasy substance depends on its free fatty acids (FFA) content measured by the acid value. The acid value and the peroxide values of *B. alba* seed oil were 3.74 ± 1.62 mg KOH/g and 7.67 ± 0.58 meq O_2/kg, respectively. These values are lower than the limits recommended (4 mg KOH/g and 10 meq O_2/kg) for edible oils [19]. The relatively low peroxide value of this oilseed indicates that *B. alba* seed oil could be less liable to oxidative rancidity at ambient temperature [22]. Iodine value (107.16 ± 2.44 g I_2/100 g) determined in this study is higher than that of other non-conventional oilseeds such as *Coula edulis* (90-95 g I_2/100 g), *Dacroydes edulis* (60-80 g I_2/100 g) and *Canarium schweinfurthii* (71.1-94.9 g I_2/100 g) [23]. In view of the results above, the studied oilseed may consist predominately in polyunsatured fatty acids and could be nutritionally beneficial to patients suffering from most of lipid disorders [24]. In addition, *B. alba* seed oil could be recommended for soap making and in the manufacture of lather shaving creams due to its relatively high (195.42 ± 1.62 mg KOH/g) saponification value [25].

Table 1. Physicochemical properties of *Basella alba* seed oil

Parameters	**Value**
Specific gravity at 20 °C	0.91 ± 0.01
Refractive index at 20 °C	1.47 ± 0.00
Specific extinction at 232 nm	1.2 ± 0.00
Specific extinction at 270 nm	1.7 ± 0.00
Colour lovibond (Lr)	10.3 ± 0.00
Viscosity at 20 °C (mPas)	35.65 ± 0.15
Cloud point (°C)	- 1.7 ± 0.00
pH at 25 °C	5.47 ± 0.02
Acid value (mg KOH/g)	3.74 ± 1.62
Peroxide value (meq O_2/kg)	7.67 ± 0.58
Iodine value (g I_2/100 g)	107.16 ± 2.44
Saponification value (mg KOH/g)	195.42 ± 1.62

Results given as means ± standard deviation of triplicate analysis.

The effect of temperature on viscosity of *B. alba* seed oil is depicted in Figure 1. The viscosity of liquids as vegetable oil is commonly perceived as thickness, or resistance to

pouring [26]. The viscosity value (35.65 ± 0.15 mPas) at 20 °C of *Basella alba* seed oil was lower than the range (50-100 mPas) indicated for most vegetable oils [27]. This value decreases exponentially to 7.03 mPas when temperature increases of 20 to 80 °C.

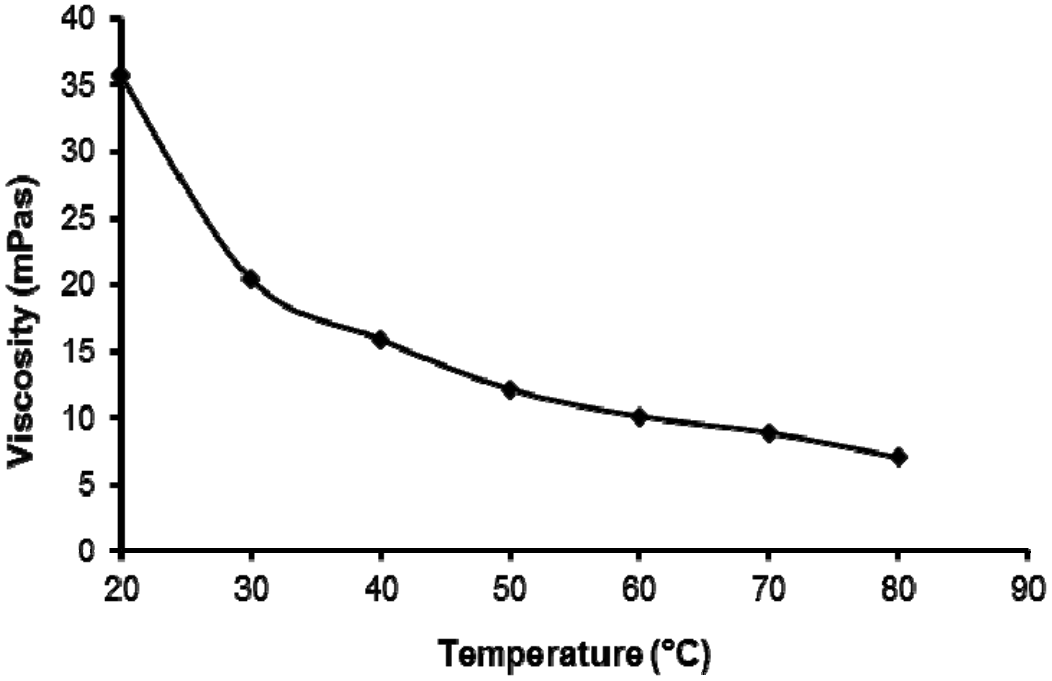

Figure 1. Effect of temperature (20-80 °C) on *Basella alba* seed oil viscosity

The visible and near infrared spectrum of *B. alba* seed oil is shown in Figure 2. In this spectrum, the wavelength range of visible domain was (400-800 nm) while that of near infrared was (800-2500 nm). The visible domain of this spectrum showed two (2) maximum absorbances (1.2 and 0.7) at 450 and 660 nm, respectively. The near infrared domain of this spectrum showed four (4) main maximum absorbances (0.9, 0.8, 2.8 and 1.5) at 1200, 1400, 1725 and 2150 nm, respectively. The maximum absorbances observed at 450 and 660 nm are related to carotenoids and chlorophyll compounds of the seed oil, respectively [28]. As concern the maximum absorbances observed at 1200, 1400, 1725 and 2150 nm, these are related to C-H stretching 2nd overtone (oil), C-H stretching 1st overtone (oil), CO stretching 1st overtone (oil), and C-H bending 2nd overtone (oil), respectively [29]. Free fatty acids (FFA) of the studied seed oil are characterized by their carboxylic acid, C=O, absorption (CO stretching 1st overtone) at 1725 nm whereas iodine value (IV) is characterized by absorption (vibration of C–H *cis*-unsaturation bonds) at 2150 nm [30]. The maximum absorption band at 1940 nm which is related to the moisture (water) content was not observed in the NIR spectrum of *B. alba* seed oil [31]. Compared to the NIR spectrum of rapeseed oil, *Basella alba* seed oil was showed more unsaturation due to the highest absorption at 2150 (iodine value) and more stability to deterioration due to the lowest absorption at 1940 nm (moisture content) [29].

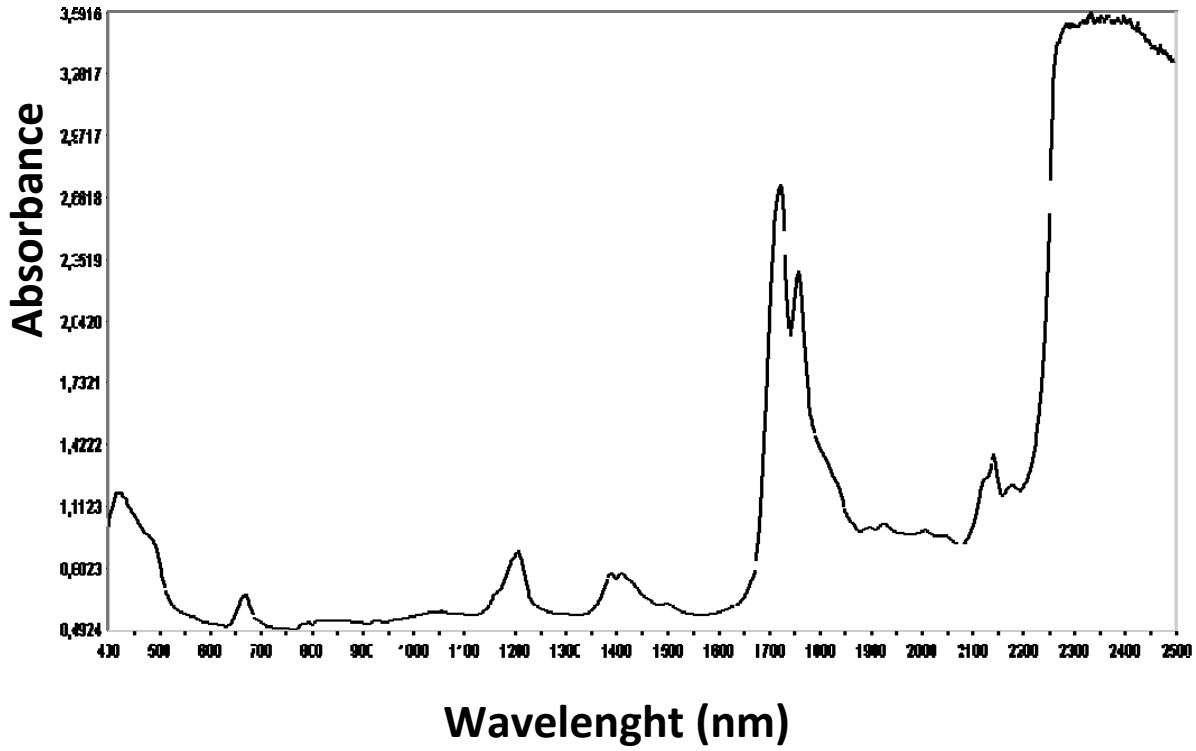

Figure 2. Visible and NIR spectrum of *Basella alba* seed oil

Biochemical and nutritive properties

The biochemical and nutritive properties of *B. alba* seed oil are shown in Table 2. unsaponifiable matter content of *B. alba* seed oil was 1.1 ± 0.02%. This intrinsic parameter value is higher than those reported for other high value oils such as cotton seed oil (0.52%), peanut oil (0.33%) and palm kernel oil (0.22%) [26]. Therefore *B. alba* seed could be used as a good source of stabilizers in cosmetic and food industry [4].

Table 2. Biochemical and nutritive properties of *Basella alba* seed oil

Parameters	Value
Unsaponifiable matter (%)	1.1 ± 0.02
Phosphorus (mg/g)	0.12 ± 0.01
Vitamin A (mg/g)	0.48 ± 0.01
Vitamin E (mg/g)	0.20 ± 0.01
Palmitic acid ($C_{16:0}$) (%)	20.54 ± 0.01
Stearic acid ($C_{18:0}$) (%)	11.38 ± 0.01
Oleic acid ($C_{18:1}$) (%)	18.85 ± 0.01
Linoleic acid ($C_{18:2}$) (%)	46.10 ± 0.01
Linolenic acid ($C_{18:3}$) (%)	3.12 ± 0.01

Results given as means ± standard deviation of triplicate analysis.

The chromatographic profiles of vitamin A and vitamin E in *B. alba* seed oil are given in Figure 3 and Figure 4, respectively. Vitamin A and vitamin E contents of *B. alba* seed oil were 0.48 ± 0.01 mg/g and 0.20 ± 0.01 mg/g, respectively (Table 2). In vegetable oils, vitamin A is provided by β-carotene which plays an important potential role in human health by acting as biological antioxidants protecting cells and tissues from the damaging effects of free radicals and singlet oxygen [32]. Vitamin A is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system, growth and development, maintenance of epithelial cellular integrity, immune function, and reproduction [33]. The content of vitamin A in *B. alba* seed oil is lower than that reported (1 mg/g) for palm oil [19]. Nevertheless, the consumption of this oilseed could cover vitamin A infant (0 to 6 months) needs, which are estimated at 0.375 mg per day [34]. Vitamin E content of *B. alba* seed oil was compared favourably with those (0.21 and 0.25 mg/g) of palm oil and corn oil which are high oxidative stability oils used in food and cosmetic industries [35]. The main biological function of vitamin E is the protection of the polyunsaturated fatty acids of cell membranes from free-radical damage in the oxidative stress [36].

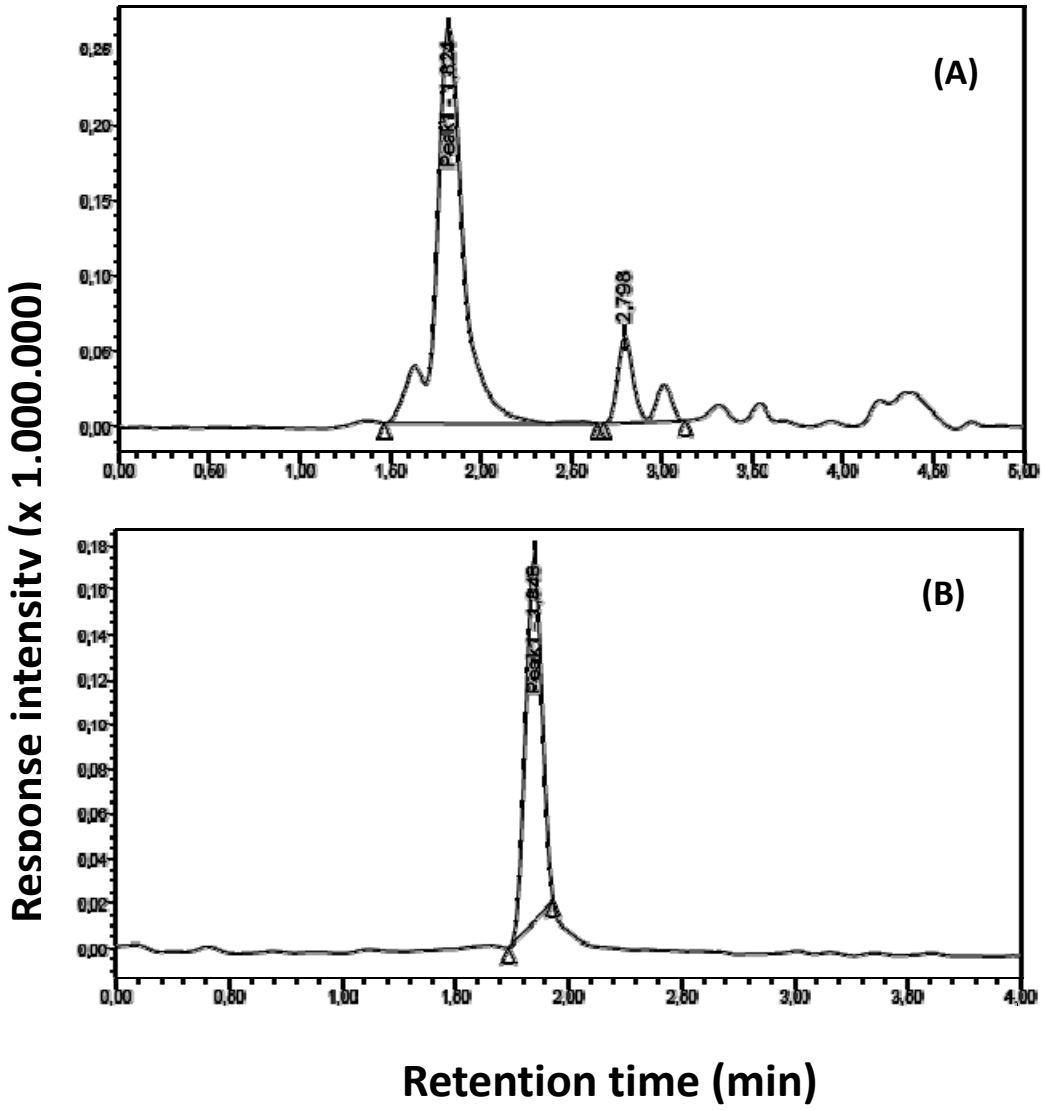

Figure 3. Chromatographic profile of vitamin A of *Basella alba* seed oil. (A): oil sample; (B): Standard vitamin A (retinol palmitate)

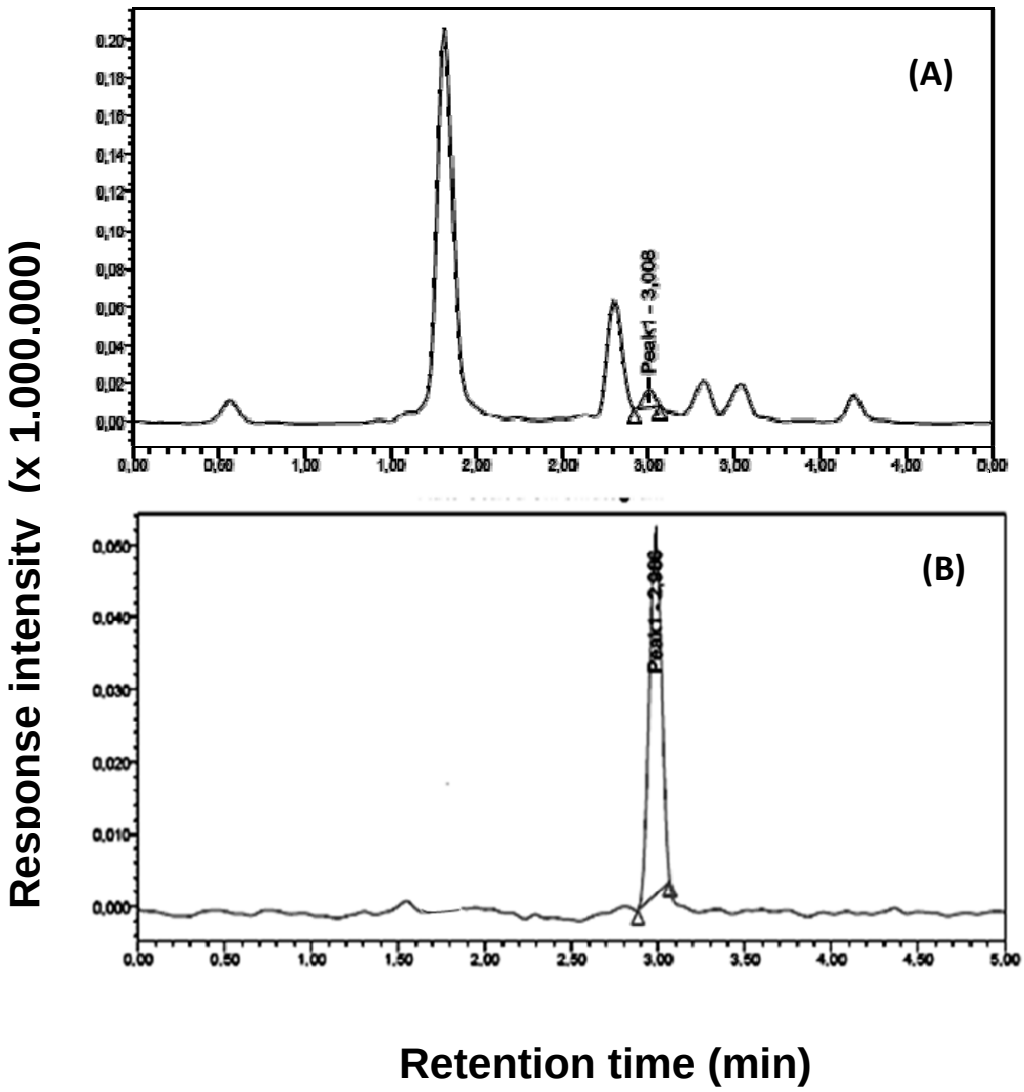

Figure 4. Chromatographic profile of vitamin E of *Basella alba* seed oil. (A): oil sample; (B): Standard vitamin E (α-tocopherol acetate)

Chromatographic profiles of fatty acids composition and their relative amounts in *B. alba* seed oil are given in Figure 5 and Table 2, respectively. Fatty acid proportions of the studied oilseeds highlighted the presence of five compounds namely palmitic (20.54 ± 0.01%), stearic (11.38 ± 0.01%), oleic (18.85 ± 0.01%) , linoleic (46.10 ± 0.01%) and linolenic (3.12 ± 0.01%) acids (Table 2). Polyunsaturated fatty acids (PUFA) of the studied oilseed were essentially made up of linoleic and linolenic acids while palmitic and stearic acids was the saturated fatty acids (SFA). The proportions of PUFA and SFA were 49.22 and 31.92%, respectively. Total unsaturated fatty acids (UFA) represented 68.07% of total fatty acids. Polyunsaturated fatty acids (PUFA) amounts of *B. alba* seed oil is higher than those reported for most of non-conventional oilseeds as shea butter (6.9%), avocado (15.5%), *Dacroydes edulis* (25.2%) and *Canarium schweinfurthii* (28.8%) [37]. The higher content of total PUFA observed in the studied oilseed may confer flexibility, fluidity and selective permeability to cellular membranes and may also be beneficial for reducing cardiovascular

disease risk [38]. In view to the fatty acids profile, *B. alba* seed oil could be considered as a linoleic oil. Moreover, the highest content of linolenic acid of *B. alba* seed oil than that of most common conventional linoleic oils such as safflower (0.3%) and cotton (0.2%) oilseeds, is an advantageous for anti-inflammatory, anti-thrombotic, anti-hypertensive and anti-arrhythmic actions in human nutrition [39]. Nevertheless, this amount of linolenic acid which is above 1% constitutes an unfavourable property for using this oil in food frying [17] . Therefore, *B. alba* seed oil could be used in human nutrition for salad seasoning. The relatively higher linoleic acid content of *B. alba* seed oil could also be useful in cosmetic industries to decrease trans-epidermal water loss and to eliminate scaly lesions common in patients with essential fatty acid deficiency [40].

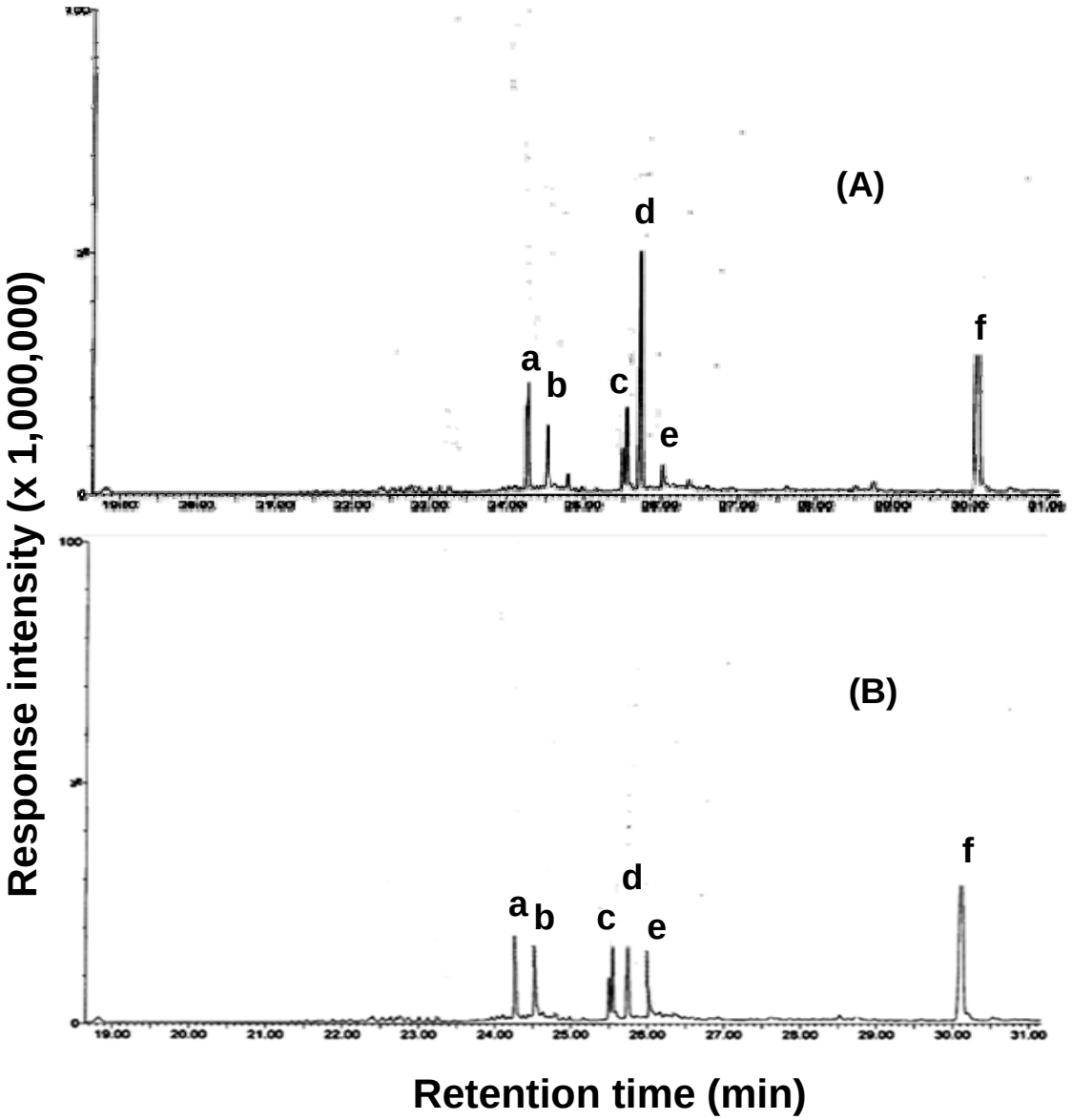

Figure 5. Gas chromatographic profile of fatty acids of *Basella alba* seed oil. (A): oil sample; (B): standard fatty acids. (a): palmitic acid; (b): stearic acid; (c): oleic acid; (d): linoleic acid; (e): linolenic acid; (f): erucic acid (internal standard)

Conclusions

It could be concluded in view of the results of the present investigation that *Basella alba* seeds may be developed for oil production. The oil extracted from this plant, used as leafy vegetable, is predisposed to human consumption due to its low content in acid and peroxide values. Saponification value and physical properties of this oil make the studied seed oil suitable in cosmetic industries for skin care products as soaps and lather shaving. As concern biochemical and nutritive properties, *B. alba* seed oil is a suitable source of vitamin A and vitamin E. In addition, the relatively higher content of polyunsaturated fatty acids (PUFA) predominantly composed of linoleic acid confers to this oil, good edible, cosmetic and dietetic values. In view of all these potentialities and qualities, *B. alba* seeds may be considered as new source of non-conventional oils which could be use in pharmaceutical, cosmetic and food industries.

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