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PHYSIOCHEMICAL CHARACTERIZATION OF *BAUHINIA PURPUREA* SEED OIL AND MEAL FOR NUTRITIONAL EXPLORATION

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Key words: *Bauhinia purpurea* oil, physiochemical characteristics, nutritional value

The present study deals with characterisation of *Bauhinia purpurea* seed oil and meal for the quality evaluation. The characteristics of the extracted oil and the seed residue (meal) were determined out for their possible utilizations. The extracted oil was also analysed for iodine value, saponification value, unsaponifiable matter, acid value, peroxide value, refractive index and colour values. Fatty acid composition analysis by gas chromatography has shown that linoleic (55.34%) and oleic (11.84%) acids were the predominant unsaturated fatty acids, while palmitic acid (17.47%) and stearic acid (11.40%) were the major saturated fatty acids. Three tocopherols and six phytosterols were identified and quantified; among these, α-tocopherol and β-sitosterol was predominant, respectively. The physiochemical characteristics has revealed that *Bauhinia purpurea* oil has nutritive and dietetic potentialities. While proximate analysis of meal has shown that it my be used in the manufacturing of poultry and animal feeds.

INTRODUCTION

The search for new good quality but economical sources of protein, oil and energy has emerging to be a major concern of governments and other institutions charged with the task for food and nutrition in many countries of the developing world [Karuppanan *et al*., 1997]. Hence, few research efforts were carried out to identify and evaluate underexploited legume crops as alternate food sources for protein and oil for the future [Egbe & Akinyele, 1990; Andersson *et al*., 1999; Rui *et al*., 2009]. The genus *Bauhinia* consisting of about 300 species, belongs to the family Leguminosae (Caesalpinioideae), cultivated in the tropical regions of the world. While, in Pakistan the trees are cultivated in plain and sub-mountainous tracks [Sarfraz *et al*., 2009; Ramadan *et al*., 2006]. The fresh and dried flower buds of *Bauhinia* are used as a food material while several *Bauhinia* species are utilized as folk medicines worldwide, including Africa, Asia, South America and Central America [Agbede, 2006]. The leaves, stems and roots are widely used in Pakistan and in other countries in the treatment of several diseases specially infections, pain, diabetes, antiulcer, jaundice, leprosy, cough and also used in several Ayurvedic medicine formulations [Kaletha *et al*., 1996; Rai & Pandey, 1997]. Phytochemical and pharmacological studies of the plant revealed that the ethanol extract of leaves used for antipyretic, anti-inflammatory, analgesic, antifungal, antispasmodic, antimicrobial activity and antitumor activity [Akhtar & Ahmad, 1995; Boonphong *et al*., 2007; Ali *et al*., 1995].

The main objective of the present study was to look into the detail of the characterisation and exploration of *B. purpurea* seed oil and meal for meeting the rising demand for vegetable oil for many applications and meal for the animal and poultry utilization.

MATERIALS AND METHODS

Samples

Approximately 2 kg seeds were collected from the *B. purpurea* plants grown in the campus of Sindh University, Pakistan, during mid February 2007 from two different locations. The average weight of single seed was 0.48 g. The plant was identified by Professor Dr. Tahir Rajput, Head of Botany Department and the voucher specimen deposited at the Herbarium of the Department of Botany University of Sindh, Jamshoro, Pakistan.

Reagents and standards

Reagents and chemicals used were of the highest purity (HPLC grade) and were purchased from Merck (Darmstadt, Germany). Pure standards of fatty acids methyl esters were obtained from Sigma Chemical Co (St. Llouis, MO, USA).

Oil extraction

Seeds of *B. purpurea* (50 g) were ground and oil extracted with *n*-hexane (b.p. 68–72°C) for quantitative determination in a Soxhlet apparatus following IUPAC method [IUPAC 1.122:1997]. The extraction was carried out on a water bath up to 6 h with 0.5 L of *n*-hexane. At the end of extraction the solvent was distilled under vacuum in a rotary evaporator, the recovered oil was oven dried at 75°C for 1 h. The oil was then transferred to a desiccator and allowed to cool before

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being weighed. The oil obtained was weighed to determine the extraction yields. Solvent-free residual meal and extracted oils were stored at 5°C under nitrogen atmosphere for further analysis.

Physical and chemical parameters of extracted oil

The recommended analytical standard methods were applied to evaluate the quality of *B. purpurea* seed oil. IUPAC methods were used to determine: refractive index [IUPAC 2.102:1979], iodine value [IUPAC 2.205:1997], saponification value [IUPAC 2.202:1997], acidity [IUPAC 2.201:1997], and peroxide value [IUPAC 2.501:1997]. The colour was determined with a Lovibond Tintometer (Model AF710, The Tintometer, Ltd., Salisbury, UK) by using AOCS standard [AOCS Cc 13b-45:1993].

Fatty acid composition

FA composition was determined by GC analysis according to IUPAC standard method [IUPAC 2.301:1979]. Fatty acid methyl esters (FAMEs) were prepared and analysed on a Perkin Elmer gas chromatograph model 8700, fitted with a SP-2340 poly (bis-cyanopropyl siloxane) column (60 m x 0.25 mm), with 0.2 μm film thickness and an FID detector. Oxygen-free nitrogen was used as a carrier gas at a flow rate of 3.5 mL/min. Other conditions were as follows: initial oven temperature: 130°C; ramp rate: 4°C/min; final temperature: 220°C; injector temperature: 260°C; and detector temperature: 270°C. A sample volume of 1.0 μL was injected. FAMEs were identified by comparing their relative and absolute retention times to those of authentic standards of FAMEs obtained from Sigma Chemical Co (St. Louis, MO, USA). All of the quantification was done by a built-in data-handling program provided by the manufacturer of the gas chromatograph (Perkin Elmer). The FA composition was reported as a relative percentage of the total peak area.

Tocopherol analysis

Tocopherols (α, γ and δ) analysis was carried out using already developed HPLC method [Gliszczynska-Swiglo & Sikorska, 2004]. Stock and working standard solutions of tocopherol were prepared in 2-propanol and injected (20 μL) into the column. Peak areas *versus* concentration were plotted to generate the standard calibration curve. Similarly 0.12 g of *B. purpurea* oil was weighed and dissolved in 1 mL of 2-propanol. A 20 μL portion was injected onto Hitachi model 6200 HPLC unit equipped with Licrosorb octadecylsilane (ODS-2) column (25cm × 4.6 mm, 5-μm particle size), SGE (Austin, USA), a mobile phase acetonitrile-water (1:1; v/v) was used with a flow rate of 1 mL/min. The eluates were detected using a Hitachi F-1050 scanning florescence detector set at emission wavelength of 325 nm with an excitation at 293 nm. Tocopherols were identified by comparing their relative retention times with those of corresponding standards and were quantified on the bases of peak areas of the unknowns with those of pure standards (Sigma Chemica Co., St Louis, MO, USA). All quantitation was carried out by using CSW32 chromatographic integrator. All the experiments were repeated at least thrice when the variation on any one was routinely less than 5%.

Sterol analysis

Extraction and separation of total sterols (ST) was performed after saponification of the oil sample without derivatization [Ramadan & Morsel, 2003]. Oil (250 mg) was refluxed with 5 mL of ethanolic potassium hydroxide solution (6% w/v) and few anti-bumping granules for 60 min. The unsaponifiable was first extracted three times with 10 mL of hexane; the extracts were combined and washed three times with 10 mL of neutral ethanol/water (1:1 v/v), and then dried overnight with anhydrous sodium sulphate. The extract was evaporated in a rotary evaporator at 25°C under reduced pressure, and then dissolved in 1 mL of *n*-hexane. The qualitative and quantitative composition of the whole unsaponifiable fraction was determined on Agilent 6890 N gas chromatograph coupled with an Agilent MS-5975 inert XL mass selective detector and an Agilent autosampler 7683-B injector (Agilent Technologies, Little Fall, NY, USA). A capillary column HP-5MS (5% phenyl methyl polysiloxane) with dimension of 30 m x 0.25 mm i.d x 0.25 micron film thickness (Agilent Technologies, Palo Alto, CA, USA) was used for the separation of sterols. The initial temperature of 250°C was maintained for 2 min, raised to 310°C at the rate of 15°C /min, and kept at 310°C for 10 min. The split ratio was 1:50; helium was used as a carrier gas with a flow rate of 1.2 mL/min. The injector and detector temperatures were 240 and 260°C, respectively. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV; ion source temperature of 230°C; quadrupole temperature of 150°C; heated transfer line temperature of 270°C; the mass scan range of 50-550 m/z; and Em voltage of 1035 V. The identification of sterols was based on the comparison of their relative retention times with those of authentic standards. The sterols were also identified and authenticated using their MS spectra compared to those from the NIST mass spectral library. The quantification was done by Chemstation data handling software Agilent Technologies.

Residual meal characterisation

Standard AOCS methods were used to determine moisture [AOCS Ba 2a-38], ash [AOCS Ba 5-49] and crude fiber [AOCS Ba 6-61] in meal residue. The modified Kjeldahl method [AOCS Ba-4-38] was used to determine protein content. Carbohydrate content was determined by following formula: 100 – (moisture + protein + crude fat + ash + crude fiber).

Statistical analysis

All determinations were carried out in triplicate. Data of each parameter were put into OriginPro 7.5 program and reported as mean (n=2 x 3) ± SD.

RESULTS AND DISCUSSION

Physiochemical charateristics of *Bauhinia purpurea* seed oil

The seeds contain a higher percentage of total lipids (18.16%) compared to the reported value (12.45%). This disagreement in oil yield may be due to the differences

in natural soil texture and environmental effects [Leilah & Al-Khateeb, 2003]. However, the average oil content of *B. purpurea* seeds is more or less equivalent to the two conventional oil seed crops: cottonseed, and soybean [Pritchard & Rossell, 1991]. The main physicochemical characteristics of *B. purpurae* seeds oil was presented in Table 1. Refractive index is a characteristic parameter which may indicate the purity of particular oil. The determined value of refractive index of *B. purpurea* seed oil was found with a mean value of 1.4645 at 40°C. The colour of the extracted crude oils was golden yellow with 2.52 red and 50.5 yellow by the lovibond tintometere in 5.25 inch cell, which is in the normal range for the good quality of crude oil. The intensity of the colour of vegetable oils depends mainly upon the presence of various pigments like carotenoids and chlorophyll, which are effectively removed during the degumming, chemical refining and bleaching process. The iodine value (99.19 g of I_2/100 g of oil) and saponification value (189.02 mg of KOH/g of oil) suggested that the *B. purpurea* oils could be fine for soap making and in the manufacturing of lather shaving creams. As a result it could be explored for cooking and may find other uses as well as be a raw material in industries for the prepararion of vegetable oil-based ice-creams. The mean acid value (AV) was found to reach 0.16 g/100 g of oil. The acidity of the oil was significantly lower, and to some extent the nutritional value depends on oil's acidity. The AV of the non processed crude *B. purpurea* oil was within the range reported for edible oil [Rossell, 1991]. A very low acidity of *B. purpurea* oil indicates its good quality and stability [Norman, 1979]. Unsaponifiable matter was determined with a mean value of 1.81 g/100 g oil, which is comparable with that of olive oil [Ojeh, 1981], and not so significantly different from those of corn, soybean, and sunflower and safflower oil [Van Niekerk & Burger, 1985]. The peroxide value, which measures hydroperoxides present in the oil, was found to reach 0.5 (meq/kg of oil). The oils with high peroxide values are not so stable and easily become rancid with an undesireable odour.

TABLE 1. Physicochemical characteristics of *Bauhinia purpurea* seed oil.

Characteristics	Content*
Oil (%)	18.13±0.12
Iodine value (g of I_2 /100 g of oil)	99.19±0.79
Saponification value (mg of KOH/g oil)	189.02±1.39
Acidity (as oleic acid g /100 g)	0.16±0.02
Unsaponifiable matter (g /100 g)	1.81±0.34
Peroxide value (meq /kg of oil)	0.5±0.05
Refractive index (40°C)	1.4645±0.00
Color (red unit)	2.52±0.07
Color (yellow unit)	50.5±0.16

* values are means ± standard deviation of triplicate determinations.

Fatty acid profile of seed oil

According to the results presented in Table 2, fourteen fatty acids were identified, the analysis of FAMEs showed that *B. purpurea* seed oil contained a significant amount of saturated fatty acids (30.27%). Among individual saturated fatty acids, palmitic acid was found to predominate with a mean value of 17.47%, followed by stearic acid – 11.40%. The other saturated fatty acids, *i.e.* arachidic, behenic, and lignoceric were detected at levels lessers than 1%. The oil was found to contain a high level of unsaturated fatty acids up to 69.73%. Along with the content of monounsaturated fatty acid (MUFA), oleic acid was the major contributor – 11.84%, the other MUFAs were determined at the level lesser than 1%. In the case of polyunsaturated fatty acids (PUFA), linoleic acid C18:2 *n*-6 was the predominant fatty acid, *i.e.* 55.34% of the total fatty acids. The concentration of linoleic acid was relatively high, while that of other fatty acids like C16:0, C18:0, C18:1 of the oil investigated in the present study was lower than the reported values [Ramadan *et al*., 2006]. The stearic, oleic and linoleic acid contents of *B. purpurea* constituting about (78.58%) of the total fatty acids were corresponding to those of cottonseed, corn, and soybean oil [Pritchard & Rossell, 1991]. The arachidic acid (C20:0), behanic acid (C22:0), lignoceric acid (C24:0), nervonic acid (C24:1), eicosadienoic acid (C20:2), γ-linolenic acid (C18:3 *n*-6), α-linolenic acid (C18:3 *n*-3) and eicosapentaenoic acid (C20:5), were also determined in minor quantities (<1%). A good combination of SFA 30.27%, MUFA 12.79%, and PUFA 56.94% with a significant level of essential fatty acids was found in *B. purpurea* seed oil and thus it could be explored as special oil for nutritional applications and fuctional foods. For over two last decades, several physiological and clinical investigations have focused on the metabolism of polyunsaturated fatty acids (PUFAs). Their outcomes confirm the beneficial effects of these acids on both normal health and chronic diseases,

TABLE 2. Fatty acid profile of *Bauhinia purpurea* seed oil.

Fatty acid	Content* (%)
Myristoleic acid (C14:1)	0.18±0.02
Palmitic acid (C16:0)	17.47±0.98
Palmitoleic acid (C16:1)	0.16±0.01
Stearic acid (C18:0)	11.4±0.64
Oleic acid (C18:1)	11.84±0.97
Linoleic acid (C18:2)	55.34±0.72
Alpha linolenic (C18:3)	0.47±0.02
Gama linolenic (C18:3)	0.36±0.02
Arachidic acid (C20:0)	0.92±0.01
Eicosadienoic acid (C20:2)	0.36±0.01
Eicosapentaenoic (C20:5)	0.38±0.02
Lignoceric acid (C24:0)	0.14±0.02
Nervonic acid (C24:1)	0.51±0.04
Σ SFA	30.27
Σ MUFA	12.79
Σ PUFA	56.94

*values are means ± satandard deviation of triplicate determination.

TABLE 3. Tocopherol profile of *Bauhinia purpurea* seed oil.

Tocopherols	Content* (mg/100 g)
α-tocopherol	89.60±6.48
(β+γ)-tocopherol	64.49±3.98
δ-tocopherol	1.73±0.13

*values are means ± standard deviation of triplicate determination.

TABLE 4. Sterole profile of *Bauhinia purpurea* seed oil.

Sterols	Content* (mg/100 g)
Compesterol	77.0±5.5
Stigmasterol	178.3±7.4
β-sitosterol	662.4±11.3
Δ5-avenasterol	40.5±4.7
Δ7-avenasterol	16.2±2.4
Δ7-stigmasterol	25.3±5.8

*values are means ±standard deviation of triplicate determination.

such as regulation of lipid levels, cardiovascular and immune functions [Finley & Shahidi, 2001; Mori *et al*., 2000; Connor, 2000; Hwang, 2000; Lombardo & Chicco, 2006].

Tocopherol profile

The nutritionally important components, such as tocopherols (vitamin E) are the major fat-soluble membrane-localized antioxidant in humans and also contribute the stability of the oil [Kallio *et al*., 2002]. Alpha tocopherol has the highest vitamin E activity; it prevents cardiovascular disease, cancer, infection, inflammation, and decreases the risk of degenerative diseases [Brigelius-Flohe & Traber, 1999]. Results of HPLC separation of tocopherol standards mixture (A) and tocopherols present in *B. purpurea* seed oil (B) are shown in Figure 1. Levels of different tocopherols present in the *B. purpurea* seed oil are summarized in Table 3. Three isomers of tocopherols were identified in *B. purpurea* seed oil, *i.e.* α-tocopherol, γ-tocopherol, and δ-tocopherol constituting 58%, 41%, and 1% of the total tocopherols, respectively. The level of predominant tocopherols (α-tocopherol and γ+β-tocopherol) investigated in *B. purpurea* oil indigenous to Pakistan was greatly different as compared to the values reported for β-tocopherol – 72.2% and δ-tocopherol – 27.8% of the total tocopherol contents [Ramadan & Morsel, 2006]. In the presnt study, levels of α- and γ-tocopherol were significantly higher than these reported for soybean, groundnut, cottonseed, and sunflower [Rossell, 1991].

Sterol composition

The levels of phytosterols in vegetable oils have been used for the identification of oils, oil derivatives and also for the determination of oil quality [De-Blas & Del-Valle, 1996; Nyam *et al*., 2009]. The composition of sterols in *B. purpurea* oil was determined by the GC-MS (Table 4). The total sterol fraction of the oil mainly consisted of six sterols, with β-sitosterol (662.4 mg/100 g of oil) and stigmasterol (178.3 mg/100 g of oil) predominating. These two major components constituted 84% of the total sterols. Among other determined sterols were compesterol and Δ5-avenasterol (12% of the total sterols). The Δ7--stigmasterol and Δ7-avnasterol were at a lower levels 4%, while brassicasterol, lanosterol, sitostenol and Δ5, 24-stigmastadinol were not detected in *B. purpurea* sterol fraction. The contents

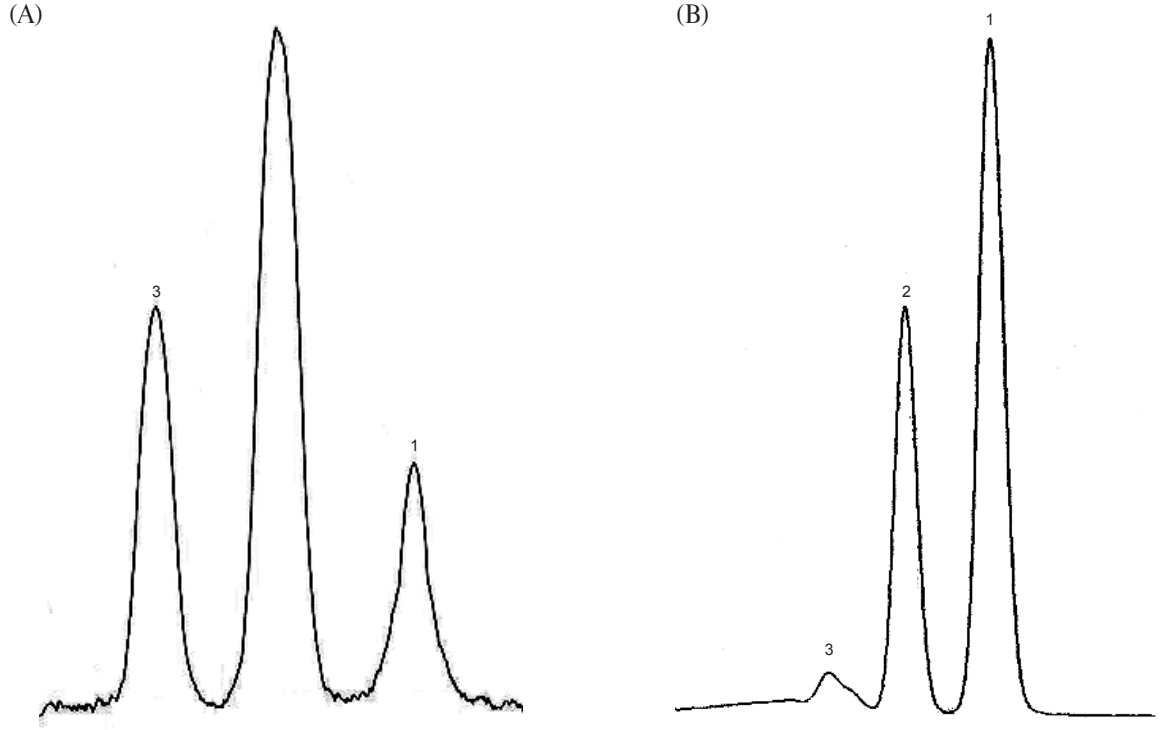

FIGURE 1. HPLC separation of tocopherol standards mixture (A) and tocopherol isomers present in *B. purpurea* seed oil (B) with peak identity: 1 – α-tocopherol; 2 – (β+γ)-tocopherol; and 3 – δ tocopherol.

TABLE 5. Proximate composition of *Bauhinia purpurea* seed meal.

Constituent	Content* (%)
Moisture	5.69±7.9
Protein	43.72±10.1
Fiber	8.04±4.3
Ash	5.78±7.2
Carbohydrates	33.93±15.2

*values are means ± standard deviation of triplicate determination.

of major sterols, β-sitosterol and stigmasterol, of the investigated oil were comparable whereas the level of compesterol and Δ5-avenasterol Δ7-stigmasterol and Δ7-avenasterol varied to some extent with the reported values [Ramadan & Morsel, 2006]. The sterol composition of the major fraction of *B. purpurea* oil was greatly different from most of the conventional edible oils [Rossell, 1991]. Many beneficial effects have been shown for the sitosterol as described by Yang *et al*. [2001]. Plant sterols due to their antioxidant activity and impact on health have been added to edible oils as a successful functional food [Nyam *et al*., 2009; Ramadan & Morsel, 2006].

Characterization of *B. purpurea* seed meal

The proximate analysis of *B. purpurea* seed residue (Table 5) after oil extraction (meal) revealed high protein content (43.72 g/100 g). In terms of percentage, carbohydrates (33.93 g/100 g) were found the major contributor after protein. While, the mean value of moisture, fiber, and ash contents were 5.69, 8.04 and 5.78 g/100 g, respectively. The results determined for protein, fiber and ash contents have clearly shown that *B. purpurea* seed meal could serve as a good source of protein, for the manufacturing of poultry and animal feeds.

CONCLUSIONS

1. The present study provides quantitative and qualitative nutritional data of *B. purpurea* oil and meal, which assess their potential for the useful applications.

2. The results of analyses of the extracted oil showed a reasonable ratio of saturated and unsaturated fatty acids. After the extraction of oil, the seed residue (meal) could be used as a source of protein in the manufacturing of poultry and animal feeds.

3. In view of the important findings, we stand to recommend the commercial cultivation of *B. purpurea* tree for multipurpose applications and to control the environmental pollution as well as to enhance the natural beauty as it has beautiful purple flowers.

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