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Physico-chemical Characteristics of Oil and Seed Residues of *Bauhinia variegata* and *Bauhinia linnaei*

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Abstract

Physico-chemical characteristics of two *Bauhinia* seed varieties (*B. variegata* and *B. linnaei*), were evaluated for commercial exploration. Physico-chemical characteristics of the oils for both varieties were demonstrated and mean values found to be refractive index (40 °C) 1.4589 and 1.4588, peroxide value 1.9 and 2.4 (meq O_2 / kg of oil), iodine value 84.5 and 92.2 (g of I_2/100g of oil), saponification number 191.3 and 195.5 (mg of KOH /g of oil), free fatty acids 0.6% and 0.9%, unsaponifiable matter 0.9% and 1.2% and color (1 in. cell), 2.2-2.9R + 30.0-25.0Y, respectively. Linoleic 42.1 and 45.8 %, oleic 13.4 and 12.6%, stearic 17.5 and 18.8% and palmitic 22.1 and 16.8% were the main fatty acids in the crude seed oils. Minor amounts of palmitoleic, margaric, linolenic, arachidic, behenic, eicosapentaenoic and nervonic acid were also identified. The composition of defatted seed residue of *B. variegata* and *B. linnaei* were found as: protein 41.9% and 38.6%, oil 18.0%, and 17.4% ash 4.8% and 4.2%, moisture 6.7% and 6.3%, fiber 6.9% and 7.3% and total carbohydrate 28.4% and 33.8%, respectively. Proximate and fatty acid composition of both *Bauhinia* varieties were found to be almost similar. It was concluded that *Bauhinia* seed is a rich source of linoleic acid and could be explored for commercial uses.

Keywords: Bauhinia seeds; Physiochemical characteristics; Oil; Seed residues.

Introduction

Bauhinia is small evergreen medicinal tree belonging to the family Leguminosae (Caesalpinioideae), consisting of 300 species which are cultivated all over the world in the tropical regions. In Pakistan the trees are cultivated in plain and sub-mountainous tracks [1]. *Bauhinia* has been widely planted in garden, park and roadsides as ornamental plant in many warm temperate and subtropical regions [2]. Leaves makes good fodder and eaten by sheep, goats and cattle. The main uses of *Bauhinia* plant is as fuel calorific value is 4 800 kcal/kg [3]. The mature seeds and young pods of *Bauhinia* are eaten, cooked and pickled in the native countries [4]. The *Bauhinia* leaves extract are being used for medicinal purposes including anti-inflammatory, antifungal, antipyretic, analgesic, antispasmodic, antitumor and antimicrobial activities [5,6]. The stems, roots and leaves are also used for the treatment of several diseases especially in pain, diabetes, infections, ulcer, jaundice, leprosy and also utilized as folk medicines [7-9]. Phytochemical study of bark extract revealed the presence of flavanoids which have anti-carcinogenic activity [10-12]. The plant extract of *Bauhinia variegata* due to presence of β- sitosterol exhibited a significant hypolipidemic effect, reduced the obesity as well as decreased the levels of cholesterol, triglyceride, VLDL cholesterol (lipid profile) [13]. Lectins (glycol proteins) from *Bauhinia* seeds have been reported to possess antitumor activity [14]. The *Bauhinia* seeds are

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known to be good source of protein, vitamin A and minerals [15-17]. The objective of present study was to obtain knowledge about the physiochemical properties of oil and meal of *B. variegata* and *B. linnaei* seeds varieties grown in Pakistan for commercial exploration.

Experimental

Seed samples and reagents

The seeds of *B. variegata* and *B. linnaei*, harvested from three different locations (1kg of each variety) from plants grown in the campus of University of Sindh, Jamshoro, Pakistan. The samples were further identified by Professor Dr. Muhammad Tahir Rajput, Dean Faculty of Natural Sciences, University of Sindh Jamshoro. All chemicals and reagents used were of HPLC grade (highest purity) and purchased from Darmstadt, Germany (Merck). The standard of fatty acids methyl esters were obtained from Sigma Chemical Co (St. Louis, MO, USA).

Oil extraction

According to standard method ISO 659 [18], finely ground *Bauhinia* seeds (about 5g) (particle size =2 mm), were used to obtain oil by Soxhlet extraction using n-hexane for 6 h. The rotary evaporator was used to remove solvent at 40 °C. The oil was dried by nitrogen streaming and stored at – 20°C for further analysis.

Analysis of oil seed residue

Determination of moisture content

Moisture content of seed meal was determined by the AOCS method [19]. Five grams of test portion was taken in dish container and dried it in an oven at 130°C for 2h. Heated portion was allowed to cool in a desiccator to room temperature and loss of weight determined.

Determination of protein content

Kjeldahl digestion method (acid digestion and distillation) was used to determine total protein from seed residues as the nitrogen content of the sample multiplied by nitrogen factor. For the protein calculation nitrogen conversion factor (6.25) was used according to the official standard method AOCS [19].

Determination of crude fiber

According to the AOAC official standard method (1993) [19], fiber content was determined using 2.5g defatted seed meal. The meal residue for digestion was boiled with sulfuric acid solution (0.26 mol/L), followed by washing and separation of insoluble residue, after digestion the residue + sodium hydroxide (0.31mol/L), was boiled followed by washing and separation, with distilled water, and drying. The residue was dried, ashed at 600 °C in a muffle furnace and loss in mass was calculated.

Determination of ash content

Powdered seed samples about 0.5 g was ignited and incinerates at 550 °C for about 12 h in muffle furnace, and then ash content determined according to AOCS standard method [19].

Determination of carbohydrate content

By the difference of mean values the content of carbohydrate was estimated, i.e. Carbohydrate content = 100 - [%Lipids + %Proteins + %Ash + %Moisture].

Analysis of Extracted Oil

Physical and chemical parameters of oil Refractive index

AOAC standard [20] method no. 969.18 was used to measure the refractive index of oil at 40 °C.

Determination of peroxide value

Peroxide value defined as the milliequivlents of active oxygen per kilogram of oil (meq of O_2 kg^{-1}) expressed in the unit of milliequivalents, was determined, when potassium iodide reacted with a mixture of oil and chloroform/acetic acid in dark according to AOCS method Cd 8-5 [20].

Determination of saponification value

It is the number of KOH required to saponify 1 gram of oil. Saponification value through hydrolysis of ester under alkaline condition was determined according to AOCS method Cd 3-25 [20].

Determination of iodine value

The iodine value of oil was determined according to AOAC [20], Wijs method Cd 3d-63. In which the dissolved oil sample (CCl_4 used as solvent) was mixed with 25ml of Wij's (0.1mol/L) solution and reacted with freshly prepared (10%) potassium iodide solution. The standard potassium thiosulphate (0.1 M) was used for titration with liberated iodine from solution. Starch was used as an indicator in this procedure.

Determination of acid value

Acid value used to measure the free acids (total amount) found in a given quantity of fat. Number of milligrams of KOH (potassium hydroxide) utilized to neutralizing the free acids found in one gram of the oil sample were determined by AOCS method Cd 3d-63 [20].

Determination color of oil

Lovibond Tintometer (Tintometer Ltd., Salisbury, U.K.), with a 1″ in. cell was used to measure the intensity of the color of oil.

Determination of fatty acid composition

Standard IUPAC method no. 2.301 [21], was used for the preparation of fatty acid methyl esters and analyze by gas chromatograph (model 8700) Perkin Elmer, fitted with a capillary column SP-2340 polar (60 m x 0.25 mm), and FID (flame ionization detector). As a carrier gas nitrogen (oxygen free) was used at a flow rate of 3.5 mL/min. Injector temperature: 260°C; detector temperature: 270°C, initial oven temperature: 130°C; and final temperature: 220°C with ramp rate: 4°C/min. A sample volume of 2.0 µ L was injected. Fatty acid methyl esters quantification and identification was carried out by comparing the retention time of peak area with those of pure standards purchased from Sigma Chemical Co (St. Louis, MO, USA), under the same conditions. In lipid fraction the results were expressed as a percentage of individual fatty acids.

Data analysis

The mean values (means ±SD) were calculated from replicates of each experiment. Significant differences among means were determined by the analysis of variance (ANOVA) and comparison between means ($P<0.05$) was carried out by statistical package Statistica 7.1 (StatSoft, Inc., Tulsa, OK, USA) software.

Results and Discussion

Proximate composition of bauhinia seed meal

(Table 1) shows the proximate compositions of *B. variegata* and *B. linnaei* seed meal. The results revealed that high amount of protein content of the seeds ranging from 41.9-38.6%, where as fiber, moisture, ash and carbohydrates content were found to be 6.9-7.3%, 6.7-6.3%, 4.8-4.2% and 28.4-33.8%, respectively. The protein content of *B. variegata* 41.9% was higher as compared to *B. linnaei* (38.6%) and closely comparable to the previous reported data [15]. This analysis showed, that the meal of *Bauhinia* seeds varieties with other essential nutrients (fiber, ash and carbohydrets) could be an excellent source of protein, which can be added to the chicken diets as a source of energy (calories) and it is a good substitute of (sunflower and soybean) meal for the local poultry feed industry. The oil content (Table 1) of *B. linnaei* and *B. variegata* seeds was in the range of 17.4-18.0 %. *B. variegata* contained 18.0% of oil which was higher than those reported in previous study data [15]. Such type of variations in the concentrations of nutrients within the country between varieties and species may be associated to the probable changes in agroclimatic regions (climatic and geographical differences) where the seeds had been grown [22]. The average oil contents of *B. variegata* and *B. linnaei* seed in the present study were found to be comparable with those of two conventional oilseed crops: of cotton (15.0-24%) and soybean (17.0-21.0%) grown in the Asian and European countries [23].

Table 1. **Proximate composition of *B. variegata* and *B. linnaei* seeds.**

Constituents	B. variegata	B.linnaei
Oil content (%)	18.0 ± 0.9	17.4 ± 0.6
Moisture (%)	6.7± 0.46	6.3 ± 0.4
Protein (%)	41.9 ± 1.6	38.6 ± 1.7
Ash (%)	4.8± 0.1	4.2 ± 0.3
Fiber (%)	6.9 ± 0.8	7.3 ± 0.6
Carbohydrates (%)	28.4 ± 1.6	33.8 ± 1.0

Values are means ± SD for triplicate determination.

Physical and chemical analysis of bauhinia seed oil

(Table 2) shows the results of physicochemical characteristics of extracted oils (*B. variegata* and *B. linnaei*). The refractive indices (40 ˚C) of the oils from *B. variegata* and *B. linnaei*, found in the present study 1.4589-1.4588 were comparable with those of olive oil [24]. Peroxide value and free fatty acids (FFA) are the measure of oil quality. The levels of FFA 0.6-0.9%, and peroxide value 1.9-2.4 (meq O_2/kg of oil) were found to be comparable with those commonly suggested level for commercial vegetable oils [25]. The results regarding to the lower concentration of peroxide value and free fatty acids content indicate that *B. variegata* and *B. linnaei* seed oils could be used for edible purposes. The iodine values of these two species were in the range of 84.5-92.2 (g of I_2/100g of oil), lower iodine value confers, to *B. variegata* oil, more stability and comparable with the iodine value of olive oil [26]. Iodine value correlated with the degree of unsaturation present in the oil of both varieties. The saponification value, found in the range of 191.3-195.5 (mg of KOH/g of oil*)*, were in close agreement with those of olive oil and canola oil [26], indicating the presence of very high proportion of low molecular weight triacylglycerols in *B. variegata* and *B. linnaei* oils. The unsaponifiable matters of both varieties, ranged 0.9-1.2%, were in close agreement with those of corn, olive, sunflower and soybean [25]. Color of extracted crude oil from both varieties exhibited red unit 2.2-2.9 and yellow unit 30.0-25.0, respectively. The red and yellow units of investigated oil were found to be comparable with those of good quality commercial vegetable oils [25].

Table 2. **Physiochemical characteristics of *B. variegata* and *B. linnaei* seed oils.**

Constituents	B. variegata	B. linnaei
Refractive index (40 ºC)	1.4589 ± 0.001	1.4588 ± 0.001
Iodine value (g of I_2/100g of oil)	84.5 ± 1.6	92.2 ± 1.2
Free fatty acids (%)	0.6 ± 0.1	0.9 ± 0.6
Saponification values (mg of KOH /g of oil)	191.3 ± 1.9	195.5 ± 2.1
Peroxide value (meq O_2 / kg of oil)	1.9 ± 0.6	2.4 ± 0.9
Unsaponifiable matter (%)	0.9 ± 0.4	1.2 ± 0.1
Color (1″ cell) Red unit	2.2 ± 0.5	2.9 ± 0.4
Yellow unit	30.0 ± 1.1	25.0 ± 1.8

Values are means ± SD for triplicate determination

Fatty acid composition of bauhinia seed oil

Fatty acids composition of *Bauhinia* varieties (*B. variegata* and *B. linnaei*) is shown in (Table 3). The representative GC-FID chromatogram *Bauhinia variegata* seed oil was presented in (Fig 1). Thirteen fatty acids were identified in *Bauhinia* varieties; in which the linoleic acid was the predominant fatty acid 42.1% for *B. variegata* and 45.8% for *B. linnaei* seed oils. The dietary fat (lipid), rich in linoleic acids are beneficial in alleviating the cardiovascular disorders, arteriosclerosis, high blood pressure and coronary heart diseases [27]. The linoleic acids derivatives are the precursors of some metabolic regulatory compounds and also serve as constituent of the plasma membrane [27]. The content of total saturated fatty acids present in both varieties including palmitic (C16:0), stearic (C18:0), arachidic (C20:0), behenic (C22:0) and nervonic (C24:1) acids in the oil were 41.7-37.9% for *B. variegata* and *B. linnaei*. The palmitic acid (22.1-16.8%) was the dominant saturated fatty acid. The total monounsaturated fatty acids (C18:1n-9) were found ranged from 15.1-14.7%, whereas palmitoleic C16:1, eicosapentaenoic C20:5 and nervonic C24:1 acids were also identified in both the varieties with concentration (<1) while arichidic acid (1.3-1.2%). The linolenic acid (C18:3 n-3, n-6) was also found in lower

concentration (<1) in both the varieties. The results of fatty acid composition of *B. variegata* were found to be quite comparable with the results of previous study [15]. The major fatty acids were linoleic, oleic, stearic and palmitic acids in seeds oil of *Bauhinia* in which *B. linnaei* contributing to 45.8%, 12.6%, 18.8% and 17.3% of the total fatty acids and showed relatively high percentage 47.4% of polyunsaturated fatty acids as compared to the *B. variegata* about 43.2% respectively. The fatty acid composition of *Bauhinia* seed oils (*B.variegata and B.linnaei*) shows that the oil is a good source of nutritionally important essential fatty acids. Both varieties contained high level of polyunsaturated fatty acids. Interest in health-promoting nutrients such as polyunsaturated fatty acids has expanded dramatically in recent years, and a rapidly growing literature illustrates their benefits [28]. Results revealed that with this special fatty acid composition the *Bauhinia* (*B. variegata* and *B. linnaei*) seeds oil can be explored for edible uses.

Table 3. **Fatty acid composition of *B. variegata* and *B. linnaei oil.***

Fatty acids	B.variegata	B. linnaei
Palmitic C16:0	22.1 ± 1.5	16.8 ± 0.9
Palmitoleic C16:1	0.4 ± 0.1	0.5 ± 0.03
Margaric C17:0	0.3±0.04	0.5±0.02
Stearic C18:0	17.5 ±1.7	18.8 ± 1.2
Oleic C18:1 cis 9	13.4 ± 0.8	12.6 ± 1.3
Oleic C18:1 cis 7	0.5 ± 0.1	0.7 ± 0.2
Linoleic C18:2	42.1± 1.8	45.8 ± 1.4
Linolenic C18:3 n-3	0.6 ± 0.4	0.9 ± 0.3
Linolenic C18:3 n-6	0.5 ± 0.1	0.7 ± 0.2
Archidic C20:0	1.3 ± 0.6	1.2 ± 0.4
Behenic C22:0	0.5 ± 0.2	0.6 ± 0.3
Eicosapentaenoic C20:5 EPA	0.2 ± 0.4	0.4 ± 0.5
Nervonic C24:1	0.6 ± 0.6	0.5 ± 0.7
∑SAFA	41.7	37.9
∑MUFA	15.1	14.7
∑PUFA	43.2	47.4

All values are means ± SD, analyzed individually in triplicate. ∑SAFA, total saturated fatty acids; ∑MUFA, total monounsaturated fatty acids; ∑PUFA, total polyunsaturated fatty acids

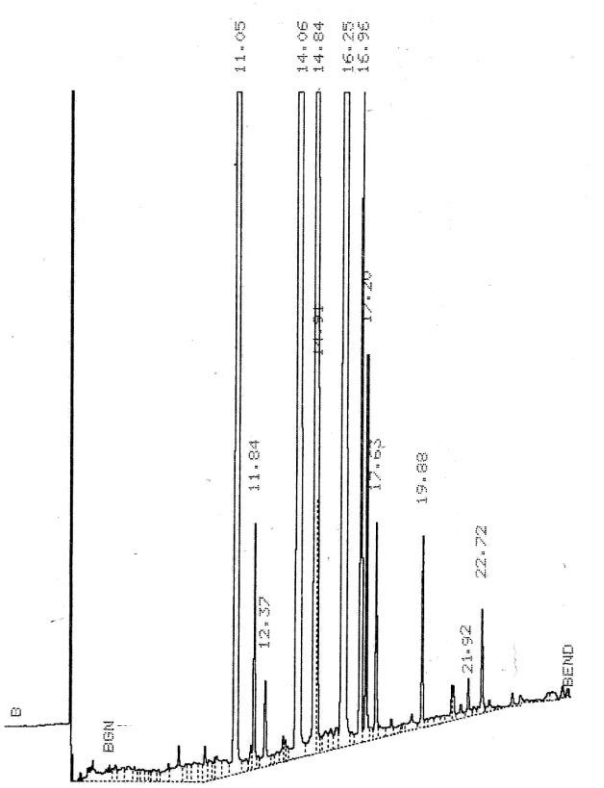

Figure 1. **Representated GC-FID chromatogram of fatty acids methyl esters for *Bauhinia variegata* oil. Elution order of fatty acids with respect to retention time of fatty acids: C16:0, C16:1, C17:0, C18:0, C18:1 cis 9, C18:1 cis 7, C18:2, C20:0, C18:3 n-3, C18:3 n-6, C22:0, C20:5, C24:1. Retention time. 11.05, 11.84, 12.23, 14.07, 14.82, 14.90, 16.25, 16.95, 17.20, 17.62, 19.86, 21.92, 22.72.**

Conclusion

The results of present study indicated that both *Bauhinia* seed varieties contained significant amount of oil which is comparable to soybean and cotton seeds. The presence of appreciable level of essential fatty acids and other favorable physiochemical characteristic make the *Bauhinia* oil nutritionally viable for human health. The high protein content of *Bauhinia* seed meals could be suggested for their potential application in poultry and animal feed formulations as a good source of vegetable protein.

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