Journal of Medicinal Plants Research Vol. 6(18), pp. 3478-3481, 16 May, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.117
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Tocopherol and phytosterol profile of *Sesbania grandiflora* (Linn.) seed oil

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Accepted 22 February, 2012

Determination of the minor components in any botanical source is of great importance in establishing the extract of oil quality and its validity. *n*-Hexane extracted *Sesbania grandiflora* seed oil was investigated for its tocopherol and phytosterol contents. Total tocopherol content in seed oil is 258.21 mg/100 g, which shows its great potential as an antioxidant agent while occurrence of phytosterol shows that it have potentially anti-inflammatory, analgesic and antipyretic activities. Vegetable oils are the richest dietary source of phytosterols therefore presence of significant amount of sterols followed by maximum percentage of β-sitosterol (74.06 ± 2.61), indicates that it can be utilized as a raw material for the production of steroid hormones and manufacturing cosmetic products. We have also performed proximate analysis such as moisture content, ash value, protein and carbohydrate detections to establish the quality control of seeds.

Key words: Phytosterol, proximate analysis, tocopherol, *Sesbania grandiflora* Vegetable oil.

INTRODUCTION

Plants have gigantic significance due to their nutritive value and continue to be a major source of medicines that have been found throughout human history. Seed is an essential source of oils and fats having nutritional, industrial and pharmaceutical importance. Non-conventional seeds are under consideration because their constituents have unique chemical properties and may add to the supply of edible oils as well as a therapeutic agent (Bertrand et al., 2011). About 70% demand of natural oils and fatty acids consumed by the world, extracted from vegetable oils (Mendes et al., 2000; Bureau et al., 2003). They are mainly represented by triacylglycerol (95 to 98%) and complex mixtures of minor compounds (2 to 5%) of a wide range of chemical nature (Aluyor et al., 2009). Natural tocopherols have been intensively considered because of their medical, biological and physicochemical impact; Moreover possessing vital nutritional function for human beings a source of Vitamin E which is one of the important component of oils along with other fat soluble vitamins. It is the most efficient lipid soluble antioxidant present in nature in more than eight structurally related forms, including four tocopherols and is chiefly acquired from plant and they are rational to light, heat, alkali and contaminant metals (Tütem et al., 1997; Ruperez et al., 2001).

Phytosterols are wide occurrence in plants and they make up the largest nontriacylglycerol component of the refined vegetable fats (Itoh et al., 1973). The cholesterol-lowering consequence of phytosterol is supposed to be caused by an inhibition of cholesterol absorption resulting from the higher solubility of phytosterols than of cholesterol in bile salt micelles (Hirota et al., 2003). *Sesbania grandiflora* (Linn.) is one of the natural source which belongs to the family *Leguminosae* and commonly known as Agathi.

This tree occurs through India to tropical Australia, and is planted in other tropical countries including Pakistan. It contains plenty of natural constituents which includes sterols, saponins and tannins etc. The various parts of the plant possess different pharmacological properties. In

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Folk Medicine, it is applied as aperients, diuretic, emetic, emmenagogue, febrifuge, laxative, and tonic (Vijay et al., 2009). The active components of *Sesbania* seeds are leucocyanidin, cyaniding, saponin and sesbanimide while oleanolic acid and its methyl ester and kaemferol- 3-rutinoside are present in its flower.

The bark contains tannins and gum (Nadkarni, 1991; Kirtikar and Basu, 1995; Anon., 1980). The plant possesses anxiolytic, anticonvulsive (Kasture et al., 2002), anti ulcerative (Serti et al., 2001), antiurolithiatic, antioxidant (Doddola et al., 2008) and hepatoprotective properties (Pari et al., 2003). In addition, *S. grandiflora* is mentioned as a potent antidote for tobacco and smoking related diseases (Ramesh and Begum, 2008). The aim of this study was to calculate the concentrations of tocopherols and phytosterols from hexane extracted seed oil.

MATERIALS AND METHODS

Plant materials

The seeds of *S. grandiflora* Linn.were collected from the road side of the city of Karachi, Pakistan in 2010 and authenticated by a taxonomist Dr. Zamrud, Department of Botany, University of Karachi, Pakistan. Voucher specimen (No. 075) was deposited at herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan.

Oil content

500 g dried seeds of *S. grandiflora* were coarsely grounded in a grinding machine (Black and Decker, Germany) and extracted with 0.5 L of *n*-hexane (Analytical grade, Merck) on a heating mental by using Soxhelt's extractor fitted with 1-L round-bottom flask, extraction chamber and condenser for 8 h. After successive extraction solvent was evaporated through Rotary evaporator (Buchi, Switzerland) under reduced pressure and control temperature to obtain the oil. The yellow color oil was stored at room temperature in airtight glass bottle.

Proximate analysis

Moisture, ash, protein and carbohydrates were determined according to AOAC methods (Anon., 1990).

Analysis of tocopherol contents

High performance liquid chromatography (HPLC) is used for the determination of tocopherols, using a solution of 250 mg of oil in 25 ml of n- heptane. The HPLC analysis was conducted using a Merck-Hitachi low-pressure gradient system, fitted with a L-6000 pump, a Merck-Hitachi F-1000 fluorescence spectrophotometer (detector wavelengths for excitation 295 nm, for emission 330 nm), and a D-2500 integration system.

The samples in the amount of 20 µl were injected with a Merck 655-A40 auto sampler onto a Diol phase of HPLC column; 25 cm × 4.6 mm ID (Merck, Darmstadt, Germany) using a flow rate of 1.3 ml/min. The mobile phase used was n-heptanes/tert-butyl methyl ether (99 + 1, v/v) along with pure standards of tocopherols for identification (Balz et al., 1992).

Analysis of sterol composition

The determination of sterols was made by following the official method of the Association of Official Analytical Chemists (AOAC,1990). Analysis was carried out on a Perkin Elmer gas chromatograph model 8700, equipped with methyl phenyl polysiloxane coated capillary column OV-17 (30 m × 0.25 mm, 0.20 µm film thickness) and a Flame Ionization Detector (FID). The column was isothermally operated at temperature of 255°C. Injector and FID temperatures were set at 275 and 290°C, respectively. Extra pure N_2 at a flow rate of 3 ml min^{-1} was used as a carrier gas. The internal standard used was α-cholestanol. Identification and quantification of unknown sterol components were made using a pure sterol standard mixture.

Statistical analysis

Analysis was performed in triplicate and values marked by the same letter in the same column of the same class were not significantly different ($p < 0.05$). Data were analyzed by using the "MSTATC" statistical computer package.

RESULTS AND DISCUSSION

Proximate, tocopherol and sterol content`s determination is an important measure during characterization of seed oil of plants and crops. These parameters indicate hidden potential and possibility of exploitation for commercial use of oils. The results of proximate analysis of *Sesbania* seeds are reported in Figure 1 which shows crude protein present in high amount that is 35%. All human beings necessitate a number of complex organic/inorganic compounds in diet to meet the requirement for their activities. The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water (Indrayan et al., 2005). Total tocopherol contents of seed oil are 258.21 mg/100 g. Soya bean and corn oils are the richest source of γ-tocopherol and the constitution of *Sesbania* oil showed that γ-tocopherol is present in significantly equal amount of these oils. The presence of other tocopherols complied by falling the occurrence of tocopherol as an α > δ > β – types (Table 1) (Sabria et al., 2006). The mixture of α, β, γ and δ isomers containing 60% wt of tocopherols, are widely used as an additive to many kinds of foods (Shimada et al., 2000).

Natural tocopherols are functional antioxidants exist in cereals and vegetable oils and different quantities of vitamin E present in both. Generally vitamin E supplement provides the high concentration of α-tocopherols which is inadequate and new discovery of research finding suggest that taking of γ-tocopherol is good for health because α tocopherol cause depletion of plasma level of γ-tocopherol but the taking of γ-tocopherol is increased the both (Jing et al., 2001). The main biochemical function of tocopherols is believed to reside in the protection of polyunsaturated fatty acids

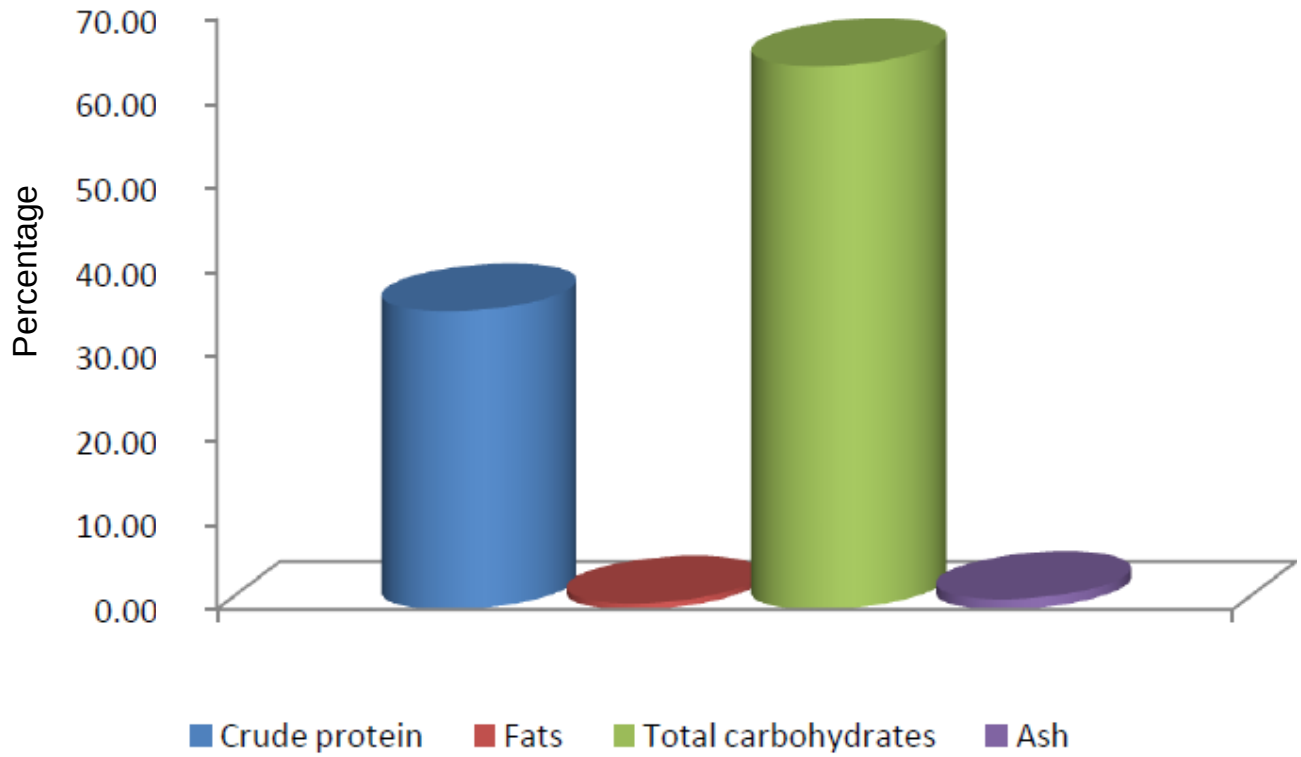

Figure 1. Proximate analysis of seed oil of *S. grandiflora.*

Table 1. Tocopherol contents (mg/100g) of *S. grandiflora* seed oil.

Tocopherol contents (mg/100 g)	Alpha (α)	Beta (β)	Gamma (γ)	Delta (δ)
Sesbania oil	47.04±1.43[b]	2.09±0.65[d]	201.06±2.48[a]	8.02±1.25[c]
Soybean oil	0.9-35.2	ND-3.6	8.9-230.7	15.4-93.2
Sunflower oil	40.3-93.5	ND-4.5	ND-3.4	ND-0.7
Corn oil	2.3-57.3	ND-35.6	26.8-246.8	2.3-7.5

Data are expressed as means ± standard deviations; values having different letters differ significantly ($p<0.05$); ND: Non detectable.

against peroxidation (Beringer and Domper, 1976; Kamal-Eldin and Andersson 1997) as well as they also avoid the oxidation of vitamin A, β- carotene and essential fatty acids (Ferrari et al., 1996). Due to this great potential of tocopherols they are able to prevent the number of diseases which includes cancer, cardiovascular and cataracts (Stampfer et al., 1993). They are also used in food, cosmetics and pharmaceutical industries (Chu et al., 2002). Investigated sterol profile of seed oil of *S. grandiflora* is given in Table 2 which showed β-sitosterol significantly in high amount 74.06% as compared to other vegetable oils including soya bean, sunflower and corn oil 47.0 to 60.0%, 50.0 to 70.0%, 54.8 to 66.6%, respectively (Sabria et al., 2006). The analysis of sterols is valuable in detecting the adulteration of oil, e.g. of butter fat with vegetable oils. More recent is the concern in the nutritional value of sterols and the monitoring and exact quantification of oxysterols (Bosinger et al., 1993). The high amount of β-sitosterol supports phytosterols to act as an important precursor in the production of commercial steroid hormones. They have hypocholesterolemic, anti- carcinogen properties. On this basis, they are used in manufacturing of progesterone, corticoids, estrogens, contraceptives, diuretics, male hormones and vitamin D. They are, also, used in cosmetics, foods like margarine, salad oils and dressings (Balazs, 1987; Chu et al., 2002).

Present exploration revealed that *S. grandiflora* seed oil has a very good potential for edibles and industrial purposes as well as mounting a nutritional balance same like other commonly used vegetable oils. These analytical findings will supply the database of this valuable cultivar

Table 2. Sterol contents of *S. grandiflora* seed oil (%).

Sterols	Campesterol	Δ^7- avenasterol	Stigmasterol	β-sitosterol	Δ^5 –avenasterol	Unidentified
Sesbania oil	9.21±1.21[b]	2.06±0.15[c]	3.02±0.72[c]	74.06±2.61[a]	7.65±1.01[b]	4.00±0.96[c]
Soybean oil	15.8-24.2	1.0-4.6	14.9-19.	47.0-60.0	1.5-3.7	ND-1, 8
Sunflower oil	6.5-13.0	3.0-7.5	6.0-13.0	50.0-70.0	ND-6.9	ND-5.3
Corn oil	16.0-24.1	0.3-2.7	1 4.3-8.0	54.8-66.6	1.5-8.2	ND-2.4

Data are expressed as means ± standard deviations; values having different letters differ significantly ($p<0.05$); ND: Non detectable, defined as ≤ 0.05%.

which has not been explored so far.

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