Int. J. Drug Res. Tech. 2014, Vol. 4 (4), 55-61 ISSN 2277 - 1506

International Journal of Drug Research and Technology

Available online at http://www.ijdrt.com

Original Research Paper

ANALYTICAL CHARACTERIZATION OF *ADANSONIA DIGITATA* L. SEED OIL GROWN IN THE SIND REGION OF PAKISTAN

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ABSTRACT

Adansonia digitata L. (Malvaceae) is a one of the universal remedial plant having great medicinal and nutritional value. In this study we used fruit (seed and pulp) of this plant and evaluate its proximate composition, mineral and amino acid content. Seed oil analyzed for its fatty acid profile, sterol composition and tocopherol contents. All examined results are very promising and meet the recommended dietary allowances requirement. The present study of fruit of baobab could be the helpful in developing the new nutraceuticals from the region of Pakistan.

Keywords: *Adensonia digitata*, Fruit, Amino acid, Fatty acids.

INTRODUCTION

Malvaceae family consists of 88 genera and 2300 species, distributed in tropical, subtropical and temperate regions. Out of these only 19 genera and 94 specific and infra specific taxa are located in Pakistan.[1] Genus *Adansonia* have numerous resourceful plants including *Adansonia digitata* Linn., which is widely distributed throughout sub-Saharan Africa, Western Madagascar and Asia.[2] Commonly it is known as Baobab or monkey-bread tree while in local language it is known as Gorakh-imli. It's used not restricted to medicine other than that it is utilized as a food and beverages.[3] The different parts of the plant are used as a universal remedy against any type of disease but here precise documented uses are account: treatment of malaria, tuberculosis, fever, microbial infections, diarrhoea, anaemia, dysentery, toothache, immunostimulant etc.[4] Several types of compounds have been identified from the various parts of plant *viz.* including terpenoids, alkaloids, flavonoids, glycosides, sterols, vitamins, amino acids, minerals, carbohydrates, phenols, and lipids.[5] Different biological and pharmacological activities have been demonstrated from the leaves, root, stem, bark and seed and fruit pulp including antimicrobial, antinsecticidal, antioxidant antiviral, and analgesic, antipyretic, anti-inflammatory and hepatoprotective.[6] As a part of under developed country we have facing the problems regarding the cheap and good quality healthy food therefore we were search for natural origin food which can easily be reach to the every person of the nation. *Adensonia digitata* is one from them and in this study we are signify the detailed proximate, analytical and chemical characteristics of the fruit (seed and pulp) of this plant. To the best of our information no data has been published on the seeds and pulp of the plant from the Sind, Pakistan.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Gorakh Imli seeds were collected from the University of Karachi garden. After the identification of plant material by Prof. Dr. Surrya Khatoon, Department of Botany, University of

Karachi, voucher specimen #092 was deposited in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy.

Separation and Extraction of Pulp and Seed Oil

The knife was used to separate the fruit pulp from the seeds. The attached pulp to the seeds was then soaked in to the water up to 6 hours to remove the pulp from the seeds by gentle hand pressing and floating in water. The dried seeds were crushed in a hammer mill and then subjected to the Soxhelt's extractor fitted with the 1-L round bottom flask and condenser. The seeds were executed on a heating mantle for 8-9 h with 0.5 L n-hexane. To obtain the crude oil solvent was evaporated under reduced pressure at 40°C on the rotary evaporator (Eyela, Japan).

Chemicals

All the chemicals were used of Analytical grade and obtained from Sigma (St. Louis, MO) USA.

Proximate Composition

Ash, protein, total lipid, fiber and carbohydrate contents of baobab seeds, were analyzed by AOAC methods.[7]

Minerals Analysis

The samples were incinerated at 450 °C for 12 h in a muffle furnace and acid digest was prepared by oxidizing each sub-sample with a nitric/perchloric acid (2:1) mixture. Aliquots were used to estimate Na and K by flame photometer (Flame Photometer Model-EEL). The minerals, such as calcium, manganese, magnesium, zinc, iron and copper were determined with an atomic absorption spectrophotometer (Perkin–Elmer Model 5000) while phosphorus was determined by the phosphovanado-molybdate (yellow) method.[8] The samples were quantified against standard solutions of known concentration that were analyzed concurrently.

Fatty Acids Composition of the Oil

It is determined by ISO draft standard ISO/FIDS 5509.[9] One drop of the oil was dissolved in 1mL of n-heptane, 50 µl 2M sodium methanolate in methanol were added and the closed tube was agitated vigorously for 1 min. after addition of 100 µl of water, the tube was centrifuged at 4500 g for 10 min. and the lower aqueous phase was removed. After that 50 µl M HCl were added to the heptane phase the two phases were shortly mixed and the lower aqueous phase was rejected. About 20 mg of sodium hydrogen sulphate (monohydrate, extra pure, Merck, Darmstadt, Germany) were added and after centrifugation at 4500 g for 10 min. the top n-heptane phase was transferred into a vial and injected in a Varian 5890 gas chromatograph with a capillary column, CP-Sil88 (100 m long, 0.25 mm ID, film thickness 0.2 µm). the temperature programme was: from 155 °C cheated to 220 °C (1.5°C/min) 10 min isotherm; injector 250 °C; detector 250 °C; carrier gas 1.07 mL/min. hydrogen ; manual injection volume less than 1 µL. the integration software computed the peak areas and percentages of fatty acids methyl esters (FAME) were obtained as weight percent by direct internal normalization.

Amino Acid Analysis

Samples (300 mg), in triplicate from pulp, were hydrolyzed with 6 M HCl in an evacuated test tube for 24 h at 105°C. The dried residue was dissolved in citrate buffer (pH 2.2) after flash evaporation. Aliquots were analysed in an automatic amino acid analyser (Hitachi Perkin–Elmer Model KLA 3B), using the buffer system described earlier. Methionine and cystine were analysed separately after performic acid treatment and subsequent hydrolysis with HCl.[10] Tryptophan was determined after alkali (NaOH) hydrolysis by the colorimetric method.[11] Essential amino acids score was calculated with reference to the FAO/WHO reference amino acid pattern.[12]

Tocopherols Content in the Seed Oil

It can be analyzed by making a solution of oil (250 mg) in *n*-heptane (25 ml) via high performance liquid chromatography (HPLC), 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 autosampler onto a Diol phase

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-heptane / tert-butyl methyl ether (99:1, v/v) along with pure standards of tocopherols for identification.[13]

Sterol Composition

GC-FID was also used for the determination of sterols followed by the official methods of AOAC.[7] Analysis was carried out on Perkin Elmer gas chromatograph model 8700, equipped with column OV-17 (30m × 0.25mm, 0.20 µm film thickness) with Flame Ionization Detector (FID). Operation was conducted in isothermally methylphenyl polysiloxane coated capillary column in a temperature of 255 °C has been set with injector and FID temperatures in the 275 °C and 290 °C, respectively. Additional pure N_2 at a flow rate of 3 ml/min was used as a carrier gas. Internal standard was used α-cholestarol which was made to identify and estimate the amount of the components of unknown sterols by using a mixture pure sterols standard.

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

To the best of our knowledge, there is no previous report on compositional studies of *A. digitata* from Pakistan so fruit was firstly subjected to proximate analysis. The results is summarized in (Table -1) indicated the high amount of carbohydrates (70.45 ± 1.82), proteins (7.51 ± 1.71) and lipids (4.35 ± 0.05) were present, while fat content was in a low quantity. Proximate composition is an index of total energy content in a food and its analysis usually is the initial measure when estimating nutritional potential of any food stuff like seeds of crops. Our results agree with those reported earlier for *A. digitata* from the other parts of world.[14] The amount of carbohydrate was significantly high in the current study than reported from Saudia and Nigeria.[15,16] Relatively higher ash contents indicate that significant amount of minerals will be present. Therefore, mineral analysis was carried out. Mineral constituents shown in (Table – 2) potassium was constituted as the major mineral. Potassium content was present in highest concentration that is 2221.64 ± 4.08 which is higher than reported from the Saudia and other countries.[14] While magnesium is present in lowest that is 0.41 ± 0.06 content. These result revealed that *Adensonia* may provide a sufficient amount of minerals to meet the human mineral requirement (recommended Dietary Allowance).[17] A balanced amino acid profile is an indicator of quality of proteins and foods. The amino acid content of *A. digitata* are revealed in Table-3. Glutamic (12.20 ± 0.05) and aspartic acids (9.17 ± 0.04) were present in highest concentrations while methionine (1.18 ± 0.02) and cysteine 1.17 ± 0.06) were present in lowest concentrations. A similar amino acid pattern was reported from other countries. According to the FAO/WHO/UNU[12] for different age groups, the daily amino acids requirement can easily be fulfill by ghorak imli due to the presence of high amount of essential amino acids in it. Table - 4 shows the fatty acid composition. High content saturated fatty acids, steric acid while arachidic acid has been found in lowest amounts these results are similar to the data previously reported.[18] The composition of seeds oil of *A. digitata* can be consider an interesting point with regard to the further use of the seed oil as a raw material and therapeutic agent. The unidentified fatty acids most probably will be sterculic acid and other cyclic fatty acids, characteristics of *Malvacea* family oil. Thus the graph shows that seed oil contains 50% total saturates and 26% polyunsaturated fatty acids including ω-3 &ω-6. While monounsaturated fatty acids occupy the 24% of the oil. From Saudia the reported results were saturates 31.7%, polyunsaturated 31.7% and monosaturates 37%,[15] these results were not similar to our results and they were higher in unsaturated fatty acids % age. Moreover, γ-Tocopherol was found in highest amount in seed oil Table-5, while β-tocopherol was found in lowest amount. The similar pattern also seen from

other countries[14] . High amounts of tocopherols can be interesting for the stabilization of fats and oils against oxidative deterioration and for applications in dietary, pharmaceutical biomedical products.[19&20] β - sterol was the major constitute of sterol profile of seed oil Tabel-6. Sterols are perhaps the most important class of the minor components and comprise major portion of the unsaponifiable matter of most of the vegetable oil. The occurrence of the Δ^5avenasterol in the seed oil is interesting because this compound is known to act as an antioxidant and as an antipolymerization agent in frying oils.[21]

CONCLUSION

Finally, the results were shown that Ghorak imli is one of the rich sources of energy as well as protein and minerals. It contains both essential and non essential amino acids and fulfills the daily requirement of the nutrition. Balanced fatty acids profiles also give advantages in the dietary pattern of the control diet. Fruit pulp can also be used as a nutrient supplement. The differences in the results from different region of the world may be due to the soil, climate and strain conditions.

Table 1: Proximate Composition of Seeds (%)

Parameter	% ± S.D
Ash	5.95±0.19c
carbohydrate	70.45±1.82a
crude fiber	11.74±1.7b
Crude lipid	4.35±0.05c
Protein	7.51±1.171c

Data are expressed as means ± standard deviation on dry weight basis values having different letters differ significantly ($p<0.05$)

Table 2: Mineral contents of *Adensonia digitata* L.

Minerals	**Mg/100mg**	**NRC/NAS (patteren for infants (1989)**
Calcium	261.21±4.73b	600
Copper	1.05±0.04g	0.6-0.7
Iron	6.34±0.52f	10
Magnesium	216.71±2.05c	-
Manganese	0.41±0.06g	0.3-1
Phosphorus	182.71±3.08d	500
Potassium	2221.64±4.08a	500-700
Sodium	17.6±1.33e	113-200
Zinc	1.6±0.11g	5

Data are expressed as means ± standard deviation 0n dry weight basis values having different letters differ significantly ($p<0.05$).

Table 3: Amino acid composition of the Baobab

Amino acids	Percentage ± SD	2-5 years	10-12 years
Alanine	5.05 ± 0.11c		
Arginine	6.22± 0.03 b		
Aspartic acid	9.17±0.04 b		
Cysteine	1.17±0.06d	2.5[a]	2.2[a]
Glutamic acid	12.20±0.05a		
Glycine	6.34±0.04b		
Histidine	2.01±0.17d	1.9	1.9
Isoleucine	3.58±0.03c	2.8	2.8
Leucine	5.29±0.02c	6.6	4.4

Lysine	4.89±0.03c	5.8	4.4
Methionine	1.18±0.02d		
Phenylalanine	5.36±0.19c		
Prolamine	3.45±0.25c		
Proline	7.90±0.27b		
Serine	6.93±0.03b		
Threonine	2.26±0.15d	3.4	2.8
Tryptophan	3.18±0.07c	1.1	0.9
Tyrosine	8.72±0.36b	6.3[b]	2.2[b]
Valine	5.01±0.05c	3.5	2.5

Data are expressed as means ± standard deviation 0n dry weight basis values having different letters differ significantly ($p<0.05$).
[d] Patterns of amino acids requirements for different age groups, [b]=Tyr + phe [a] = cys + meth

Table 4: Fatty acids composition of the baobab seed oil

Fatty acids	Percentage ± SD
Myristic acid ($C_{14:0}$)	1.01±0.07d
Palmatic acid ($C_{16:0}$)	29.57±1.03b
Palmitoleic acid($C_{16:1}$)	0.27±0.06d
Stearic acid($C_{18:0}$)	36.28±0.81a
Oleic acid($C_{18:1}$)	31.41±0.53b
Linoleic acid($C_{18:2}$)	27.31±0.16b
α-linolenic acid($C_{18:3}$)	6.65±0.42c
Arachidic acid($C_{20:0}$)	0.14±0.04d
Gadolic acid($C_{20:1}$)	0.20±0.02d
Unidentified	6.97±0.37c

Data are expressed as means ± standard deviation 0n dry weight basis values having different letters differ significantly ($p<0.05$).

Table 5: Tocopherol profile

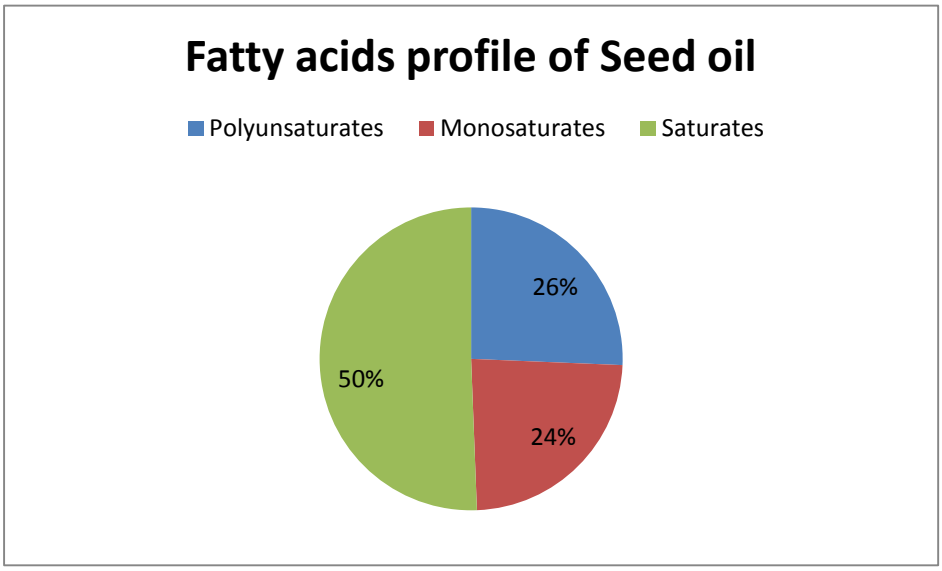
Component	Content (mg/Kg) ± SD
Alpha (α)	27.32 ± 1.41c
Beta (β)	4.04 ± 0.09 d
Gamma (γ)	204.31±0.78b
Delta (δ)	20.07±0.65c
Total	255.74±2.93a

Data are expressed as means ± standard deviation 0n dry weight basis values having different letters differ significantly ($p<0.05$).

Table 6: Sterol composition % of seed oil

Sterols components	Percentage ± SD
Cholestrol	1.81 ± 0.32 [ec]
Campesterol	5.67±1.02 [bb]
Stigmasterol	1.30±0.17 [cc]
β-sitosterol	78.61±1.66 [aa]
Δ^5 – Avenasterol	2.15±0.07 [dc]
Δ^7 – Stigmasterol	4.26±0.17 [eb]
Δ^7- Avenasterol	6.20±0.35 [eb]

Data are expressed as means ± standard deviation 0n dry weight basis values having different letters differ significantly ($p<0.05$).



Graph 1: Percentages of SFAs, MUSFAs and PUFAs in Seeds oil

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Cite This Article: Muhammad, Ayaz; Ghazala, H Rizwani; Huma, Shareef; Muhammad, Zia-ul-Haq and Tayyaba, Mumtaz (2014), "Analytical characterization of adansonia digitata l. seed oil grown in the sind region of pakistan", ***International Journal of Drug Research and Technology***, Vol. 4 (4), 55-61.

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