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Chemical Composition profile of *Acacia nilotica* Seed Growing Wild in South of Iran

KARIM ABBASIAN[1], JINOUS ASGARPANAH[2] and PARISA ZIARATI[1]*

[1]Department of Medicinal Chemistry, Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (IAUPS).
[2]Department of Pharmacognosy, Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (IAUPS).
*Corresponding author E-mail: ziarati.p@iaups.ac.ir

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ABSTRACT

Acacia Nilotica is a pioneer species, relatively high in bioactive secondary compound and are important for a variety of functions is economically used as a source of tannins, gums, timber, fuel and fodder. Babul plant is therapeutic used as Anti-cancer, anti tumours, Antiscorbutic, Astringent, anti-oxidant, Natriuretic, Antispasmodial, Diuretic, Intestinal pains and diarrhea, Nerve stimulant, Cold, Congestion, Coughs, Dysenter, Fever, Hemorrhages, Leucorrhea, Ophthalmia and Sclerosis. The aim of this study was determination of proximate composition, mineral elements (Calcium, Potassium, Iron, Zinc, Sodium, Selenium and Copper) contents in this endemic Iranian seed. A. *Nilotica* seeds (mature, dry) were collected in August 2014 from Sarkhun village, Bandar Abbas, Hormozgan Province, Iran. Specimen was identified by R. Asadpour and voucher was deposited in the Herbarium of Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS). The samples were analyzed by wet digestion method and analysis of mineral element contents analyzed by Atomic Absorption Spectrophotometer in Research Laboratory in Pharmaceutical Sciences Branch, Islamic Azad University. Obviously in *A. Nilotica* the contents of the some trace and essential mineral element are high in comparison by other seeds studied in other countries. The value of potassium, iron and Zinc, Copper and Manganese in *A. Nilotica* were 2.1, 203.1, 108.7, 322.7 and 1.09 (g/100g DW) based on dry weight respectively. The oil, crude protein and crude fiber contents in this edible seed were found to be 4.1, 25.3 and 28.4 % based on the fresh weight respectively. In this study, the nutritive value of *A. Nilotica* mature seed native to south of Iran was determined and results revealed that it is so rich in mineral elements especially Iron and Potassium. Traditionally the plant used widely for the treatment of various ailments, but scientifically few of them was screened out. Therefore the scientific studies should be conducted to investigate the unexploited potential of Acacia Nilotica (L.).Our results revealed that it could be recommended as a dietary supplement for people who need essential mineral elements.

Keywords: *Acacia Nilotica* L. Seeds, Mineral elements, Iran, Hormozgan Province.

INTRODUCTION

Acacia is a genus belonging to the Fabaceae family and comprises about 135 species of trees which are widely spread throughout the arid and semi-arid tropics[1,2]. "*Acacia Nilotica*" is a tropical tree with rapid growth and ten year period of yield which can grow extensively in the seashores of southern Iran[3]. *A. Nilotica* is a multipurpose tree with extensive distribution from Egypt to Mauritania and South Africa in Africa and from East Asia to India, Pakistan and Iran in Asia. *A. Nilotica* is a pioneer species, relatively high in bioactive secondary compound and is important for a variety of functions is economically used as a source of tannins, gums, timber, fuel and fodder[4-6]. It grows in semi-arid, hot and wet regions such as the Persian Gulf, Oman Sea and in Boushehr Province, Hormozgan Province and Sistan and Baluchestan Province (Chahbehar, Iranshahr and Nikshahre) as well as in deep loam soils. A. Nilotica plantation was started from 1984 in Chahbehar and Dashteyari[3]. A. *Nilotica* is a pantropical and subtropical genus with species abundant throughout Asia, Australia, Africa and America. A.nilotica occurs naturally and is imperative in traditional rural and agro-pastoral systems[7]. A. nilotica is recognized by the following names: Acacia, Acacia Arabica, Babhul - Hindi and Napalese, Babla - Bengali, Babool - Unani, Babool Baum - German, Babhoola - Sanskrit, Babul, Babul Tree, Huanlong Kyain - Burmese, Kikar, Mughilan - Arabian Indogom - Japenese and Ummughiion – Persian[8]. *A. Nilotica* is an imperative multipurpose plant that has been used broadly for the treatment of various diseases[9]. The plant is therapeutic used as Anti-cancer, anti tumours, Antiscorbutic, Astringent, anti-oxidant, Natriuretic, Antispasmodial, Diuretic, Intestinal pains and diarrhea, Nerve stimulant, Cold, Congestion, Coughs, Dysenter, Fever, Hemorrhages, Leucorrhea, Ophthalmia and Sclerosis[10]. In other studies it has been reported that Acacia species contains secondary metabolites including amines and alkaloids, cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, nonprotein amino acids, terpenes (including essential oils, diterpenes, phytosterol and triterpene genins and saponins), hydrolysable tannins, flavonoids and condensed tannins[11,12]. The plant is richer source of cysteine, methionine, threonine, lysine, tryptophan, Potassium, phosphorus, magnesium, iron and manganese[9]. As evident from literature, there was no phytochemical investigation on *A. Nilotica* seed growing wild in south of Iran. Hence the current study includes analyses of mineral and essential elements in *A. Nilotica* seed oil to evaluate its nutritive potential value as a new source of enriched seed for nutritional purposes.

MATERIALS AND METHODS

Plant material

A. nilotica seeds were collected in August 2014 from Sarkhun village, Bandar Abbas, Hormozgan Province, Iran: (27°23'34" N 56°23'59" E, 100m). Specimen was identified by R. Asadpour and voucher was deposited in the Herbarium of Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS) Tehran. The area is mountainous region that located among plains and hills. The region's geographical is located in the north of Bandar Abbas(figure 1). Plain part of the region includes much of the southern, eastern and northern part of the strip consisted of alkaline and saline soils contain large amounts of soluble salts such as chloride, sulfate and carbonate of Ca, Mg, sodium, and potassium[13,14].

Moisture Content

All grounded *A. nilotica* mature dry seeds samples were oven dried at 60°C for 36 hours until a constant weight were obtained. The moisture contents were expressed as loss in weights of the wet samples[15-18].

Fig. 1: Location of *A. nilotica* samples collection

Crude Fiber

Five grams of the grounded A. *nilotica* mature dry seeds samples were digested in 50 ml of 1.25% H_2SO_4. The solutions were boiled for 45 minutes and then were filtered and washed with hot distilled water. The filtrates were digested in 50 ml of 1.25% Sodium Hydroxide solutions. For 50 minutes these solutions were heated, filtered and washed with hot deionized water and over dried. The final oven-dried residues were ignited in a furnace at 550°C. The weights of the left after ignition were measured as the fiber contents and were expressed in term of the weights of the samples before ignition[19].

Crude Protein

The protein nitrogen in one gram of the dried samples were converted to ammonium sulphate by digestion with concentrated H_2SO_4 (Merck 96.5%) and in the presence of $CuSO_4$ and K_2SO_4 [20-21]. The solutions were heated and the ammonia evolved were steam distilled into Boric acid 2%. The nitrogen from ammonia were deduced from the titrations of the trapped ammonia with 0.1M HCl with Tashirus indicator (methyl red: methylene blue 2:1) until a purplish pink color were obtained. Crude proteins were calculated by multiplying the valve of the deduced nitrogen by the factor 6.25 mg[22-24].

Ash Content

One gram of the oven-dried samples in powder from was placed in acid washed crucible by known weight. They were ignited in a muffle furnace for 5 hours at 550 °C. After cooling crucibles they were weighed and the ash contents were expressed in terms of the oven-dried weight of the sample[19].

Zinc, Manganese, Copper and Potassium Determination

For Zinc, Manganese, Copper and Selenium concentration in *A. Nilotica* , powered seed samples were dried in oven for 48 hours at a temperature of 85°C. The samples were then ground and sieved through 0.5 mm sieve. The powdered samples then subjected to the acid digestion using concentrated nitric acid (65% Merck), Sulfuric acid (96.5% Merck) and per chloric acid (70% sigma). Analar grade hydrogen peroxide (about 30%) also was used for the digestion. Application of concentrated HNO_3 along with thirty percent hydrogen peroxide H_2O_2 (Merck) for mineralization of samples to the complete digestion of samples[25-27] following Environmental Protection Agency (EPA) Method 3052 was done.

Two gram of air-dried of each homogeneously *A. Nilotica* samples accurately weighed and 30.0 mL of the digestion mixture (3 parts by weight of nitric acid: 1 parts of Sulfuric acid & 3 parts by weight perchloric acid) and heated slowly by an oven and then rise the temperature. The remaining dry inorganic residues were dissolved in 30.0 mL of concentrated nitric acid and the solution used for the determination of trace and essential mineral elements. Blanks and samples were also processed and analyzed simultaneously. All the chemicals used were of analytical grade (AR). Standardized international protocols were followed for the preparation of material and analysis of heavy metals contents[28-32]. The samples were analyzed by Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene, flame temperature: 2800°C, acetylene pressure: 0.9–1.0 bar, air pressure: 4.5–5 bar, reading time: 1–10 sec (max 60 sec), flow time: 3-4 sec (max 10 sec), using at least five standard solutions for each metal and determination of potassium content was followed by FDA Elemental analysis[33] In order to verify of reliability of the measuring apparatus, periodic testing of standard solutions was performed . The accuracy was checked using quality control test for fungi and their substrate samples to show the degree of agreement between the standard values and measured values; the difference was less than 5%.

Iron Determination

The aliquot was passed through the atomic absorption spectrophotometer to read the iron concentration. Standards were prepared with a standard stock of 10 mg/L using ferrous ammonium sulphate where 3 - 60 ml of iron standard solution (10 mg /L) were placed in stepwise volumes in 100 ml volumetric flasks. 2 ml of hydrochloric acid were added and then brought to the volume with distilled water. The concentration of iron in the aliquot was measured using the atomic absorption spectrophotometer in mg/L. The whole procedure was replicated three times[34, 35].

Calcium, Sodium and Magnesium Determination

5 ml of the aliquot were placed in a titration flask using a pipette and diluted to 100 ml with distilled water and subsequently 15 ml of buffer solution, ten drops of Eriochrome black T indicator and 2 ml of triethanolamine were added. The mixture was titrated with Ethylene-Diamine-Tetra-Acetate (EDTA) solution from red to clear blue[36].

Selenium Determination

Stock standard solutions for selenium were 1000 g /mL solution. All reagents and standards were of analytical grade (Merck, Germany) .The palladium matrix modifier solution was prepared by the dilution (10 g/ L) $Pd(NO_3)_2$ and iridium AA standard solution, 1000 g/ mL in 20% HCl , 0.1 % V/V nitric acid prepared by dilution trace pure 65 % nitric acid and 0.1 % Triton X-100 were used. Doubly distilled water was used in all operations. The samples were analyzed by Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan). The analyze performed according by Analytical Method ATSRD[30,35,36].

RESULTS

The mean content of trace and essential mineral elements (g/100g DW) in the mature dry seed of *Acacia Nilotica* samples is shown in table 1. The samples were analyzed by wet digestion method and standardized international protocols were followed for the preparation of material and analysis of mineral contents and analyzed by Atomic Absorption Spectrophotometer in Research Laboratory in Pharmaceutical Sciences Branch, Islamic Azad University.

The order depending on the contents of trace metal and essential elements (g/ 100 g) in A. *Nilotica* samples in Hormozgan-Iran studied regions was:

Table 1: The Mean content (g/100g DW) composition of the mature dry seeds of *A. Nilotica* from Hormozgan Province, Iran

Minerals	Mean content ± SD* (g/100 g)	Minerals	Mean content ± SD* (g/100 g)
Sodium	0.0421 ± 0.0001	Manganese	1.0911± 0.0111
Potassium	2.1006 ± 0.0036	Phosphor	8.6778 ± 0.2719
Calcium	0.6340 ± 0.0091	Iodine	0.9871 ± 0.1206
Magnesium	0.4602 ± 0.0026	Cobalt	0.1208 ± 0.0021
Iron	203.11 ± 25.067	Sulphur	2.4350 ± 0.1111
Copper	322.73 ± 20.56	Fluorine	0.0052± 0.0052
Selenium	0.003 ± 0.001	Lithium	0.002 ± 0.0005
Zinc	108.703 ± 23.778	Molybdenum	0.001± 0.0

*SD = Standard Deviation

Cu > Fe > Zn > K> Mn> Co > Ca >Mg > Na > Se > Li > Mo

And the order of non-metals:

P > S > I > F

Proximate composition and physicochemical characteristics of the samples has shown in table 2 , based on the fresh weight.

Table 2: Proximate analysis of A. *Nilotica* from Hormozgan Province, Iran

Nutrient	Percentage (%)
Dry Matter(DM)	91.3
Crude Protein (CP)	25.3
Crude Fiber (CF)	28.4
Ash	13.1
Fat	3.4

DISCUSSION

Obviously in *A. nilotica* the amount of the some mineral element contents such as copper, iron, zinc and calcium are high in comparison by other seeds studied in other countries.

Copper has the role of assisting in the formation of haemoglobin, helping to prevent anemia as well as being involved in several enzymes. Iron is the central metal in the haemoglobin molecule for oxygen transport in the blood and is portion of myoglobin located in muscles. Manganese is one of the co-factors in a number of enzymes as is molybdenum. Selenium has several roles such as regulating the thyroid hormone as well as being part of an enzyme that protects against oxidation[37], Selenium has also been reported as assisting in deactivating heavy metals. Calcium is responsible for strong bones and teeth and accounts for ninety percent of the calcium in the body whereas the other one percent is circulating in fluids in order to ionize calcium. The metal's function is related to transmitting nerve impulses; contractions of muscles; blood clotting; activation of some enzyme reactions and secretion of hormones Magnesium has many roles including supporting the functioning of the immune system; assists in preventing dental decay by retaining the calcium in tooth enamel; it has an important role in the synthesis of proteins, fat, nucleic acids; glucose metabolism as well as membrane transport system of cells. Magnesium also plays a role in muscle contraction and cell integrity. Potassium and sodium work together in muscle contraction nerve transmission. Sodium is important in muscle contraction and nerve transmission Sodium ions are the main regulators of extra cellular fluid and volume (37) .Zinc is an essential trace element and plays an important role in various cell processes including normal growth, brain development, behavioral response, bone formation and wound healing. Zinc deficient diabetics fail to improve their power of sensitivity and it cause loss of sense of touch and smell[14, 38, 39].

This is obviously seen in the crude protein content of the samples of Iranian *Acacia nilotica* examined in this study which reached 25.3 and this value is significantly higher and superior than other *Acacia nilotica* reported in other countries especially in Africa samples. Rubanza et al., in 2005 stated that the Acacia nilotica seeds had protein (19%) and contain more fibre[40] and Abdalla et al in 2014 in Sudan reported 21.4% crude protein and the crude fiber content of Acacia nilotica fruit in this study is 30.12%, fat (ether extract) is 24.77%, DM is 93.71% and the ash content is 11.76% in A. *Nilotica* grown in Sudan[41]. Our results show that *Acacia nilotica* seeds from Hormozgan :south province in Iran have more crude protein and ash and less crud fiber and fat. The protein content for this seed is high and it could be used as dietary supplement for people who need a lot of protein and most importantly for those who require plant protein especially people suffering from hypertension. They can also be incorporated into animal feed to increase the protein content. The Recommended Dietary Allowance (RDA) for protein is 0.8 g/kg body weight for adults, set by the Institute of Medicine, and is based on the consumption of good-quality protein (U.S. Department of Health and Human Services 2006). According to the most recent statistics from the American Cancer Society, more than 1.5 million new cancer cases are diagnosed annually (American Cancer Society 2010).

The "*ash content"* is a measure of the total amount of minerals present within a food, whereas the "*mineral content"* is a measure of the amount of specific inorganic components present within a food, such as Ca, Na and K. Determination of the ash and mineral content of foods is important for a number of reasons such as nutritional labeling: The concentration and type of minerals present must often be stipulated on the label of a food and quality: The quality of many foods depends on the concentration and type of minerals they contain, including their taste, appearance, texture and stability[19].

CONCLUSION

In this study, the nutritive value of *A. Nilotica* mature seed native to south of Iran was determined and results revealed that it is so rich in crude protein and some trace and essential mineral elements especially Iron, Zinc, Copper and Potassium. Traditionally the plant used widely for the treatment of various ailments, but scientifically few of them was screened out. Therefore the scientific studies should be conducted to investigate the unexploited potential of *Acacia Nilotica* (L.).Our results revealed that it could be recommended as a dietary supplement for people who need essential mineral elements. In conclusion the present study revealed that the seed oil of *A. Nilotica* growing in south of Iran could be a new source of high protein and mineral elements and its full potential should be exploited. The use of this seed is of potential economic benefit to the poor native population of the areas where it is cultivated. Hence the seed protein and minerals of *A. Nilotica* could be a new source of edible vegetable after the future toxicological studies.

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