ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal
www.orientjchem.org
Est. 1984

ISSN: 0970-020 X
CODEN: OJCHEG
2015, Vol. 31, No. (4):
Pg. 2311-2318

Chemical Composition Profile of Wild *Acacia oerfota* (Forssk) Schweinf Seed Growing in the South of Iran

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http://dx.doi.org/10.13005/ojc/310459

(Received: August 01, 2015; Accepted: September 13, 2015)

ABSTRACT

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. There was no phytochemical investigation on *A. Oerfota* (Forssk) Schweinf seed growing wild in south of Iran. Hence the current study includes analyses of Chemical composition of *A. Oerfota* seed to evaluate its nutritive potential value as a new source of enriched seed for nutritional purposes. *A.Oerfota* seeds were collected in August 2014 from Sarkhun village, Bandar Abbas, Hormozgan Province, Iran. The samples were analyzed by standardized international protocols in Research Laboratory in Pharmaceutical Sciences Branch, Islamic Azad University. Obviously in *A. Oerfota* the order of amount of the some mineral element contents such as zinc copper, iron, and calcium are different in comparison by other Acacia seeds studied in Iran in recent studies. The crude protein content of Iranian *Acacia oerfota* examined in this study which reached 25.06 and this value is significantly higher and superior than other *A. oerfota* reported in other countries especially in Africa samples and even higher to A. *Nilotica.* . Therefore due to the seed protein, Tanin and minerals of *A. Oerfota* could be a new source of edible vegetable after the future toxicological studies.

Key words: *Acacia oerfota* seed*,* Chemical Composition , Tanin, Mineral elements, Iran.

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]. Previous studies have shown that some trees and shrubs in Asia and Africa were found to be of great nutritive value as fodder for animals[1-4]. *Acacia oerfota* (Forssk) Schweinf is a shrub growing up to 5 meters tall. The plant has short, thick thorns about 2cm long and often pointing backwards[5]. A. *oerfota* Seed requires pre-soaking or scarification. To scarify, the seed coat should be nicked at the distal (cotyledon) end using a sharp tool like a scalpel, knife or nail clipper. Large quantities of seeds should be treated by pouring a small amount of almost boiling water on them (so that the water cools down quickly enough not to cook the seeds) in a vessel. Then the seeds should be left for 24 hours in the water as it cools.

Because of their widespread distribution in the province of Hormozgan in the south of Iran , Acacia family offer a potentially suitable source of supplemental protein for ruminant livestock which would improve their intake and digestibility of native pasture[2]. This should result in improved animal performance[6,7] . Their use has been limited by the scarcity of information relating the amount and types of tannins they contain which would affect animal performance. The toxic or anti-nutritional effects tend to occur in times of stress when a very large proportion of the diet is tanniniferous. With a better understanding of their tannin properties and proper management, they could become invaluable sources of protein for strategic supplementation.

A. oerfota 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. Oerfota* 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[8-10]. 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. Oerfota plantation was started from 1984 in Chahbehar and Dashteyari[3]. A. *Oerfota* is a pantropical and subtropical genus with species abundant throughout Asia, Australia, Africa and America. A.oerfota occurs naturally and is imperative in traditional rural and agro-pastoral systems[11]. A. oerfota 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[12]. *A. Oerfota* is an imperative multipurpose plant that has been used broadly for the treatment of various diseases[13]. 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[13,14]. The plant is richer source of cysteine, methionine, threonine, lysine, tryptophan, Potassium, phosphorus, magnesium, iron and manganese[13]. As evident from literature, there was no phytochemical investigation on *A. Oerfota* seed growing wild in south of Iran. Hence the current study includes analyses of chemical composition of *A. Oerfota* seed to evaluate its nutritive potential value as a new source of enriched seed for nutritional purposes.

MATERIALS AND METHODS

Plant material

A.Oerfota 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[14,16]. Bandar Abbas city is the capital of Hormozgan province, situated in south of Iran and north of the Straits of Hormoz at 27°13´N, 56°22´E. This coastal city lies in the northeast of the Persian Gulf with about 45 km^2 area and average elevation of 10 m a.s.l. Average minimum temperature in coldest month (January) is 12.3°C and average maximum temperature in the hottest month (July) is 38.4°C. Totally, it has two seasons; hot and wet conditions between May and October, and temperate season between November and April[17].

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[18,19].

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[22-25].

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[18].

Zinc, Manganese, Copper and Potassium Determination

For Zinc, Manganese, Copper and Selenium concentration in *A. Oerfota ,* 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 (26- 29) following Environmental Protection Agency (EPA) Method 3052 was done.

Two gram of air-dried of each homogeneously *A. Oerfota* 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[30-32]. 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].

Tanin determination

3 gram of *A. Oerfota* seed sample was extracted with distilled deionized water into 250 ml volumetric flask during 5 hours at room temperature and the sample was filtered. The analyses of Tanin content was performed according to the international Pharmacopeia[37,38] and AOAC method , after some modification. 25 ml of the infusions were measured into 1 liter conical flask then 25 ml of *Indigo* solution and 750 ml deionized water was added. 0.1 N aqueous solution of Potassium permanganate was used for titration until the blue colored solution changed to green color. The few drops were added until solution became golden yellow. 6 g *Indigo* carmine was dissolved in 500 ml of distilled deionized water by heating, after cooling 50 ml of 96% H_2SO_4 was added. The solution is diluted to 1 Liter the filtered. The blank tests by titration of a mixture of 25 ml *Indigo* Carmine solution and 750 ml distilled deionized water were carried out. All samples were analyzed twice at least.

RESULTS

The mean content of trace and essential mineral elements (g/100g DW) in the mature dry seed of *A. Oerfota* 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.

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

Minerals	Mean content ± SD* (g/100 g)	Minerals	Mean content ± SD* (g/100 g)
Sodium	0.1086 ± 0.016	Manganese	148.06 ± 10.22
Potassium	118.42 ± 9.03	Phosphor	8.6778 ± 0.2719
Calcium	0.4092 ± 0.052	Iodine	0.9871 ± 0.1206
Magnesium	0.2308± 0.011	Cobalt	0.1208 ± 0.0021
Iron	203.11 ± 25.067	Sulphur	1.9867 ± 0.098
Copper	322.73 ± 20.56	Fluorine	0.0076± 0.0023
Selenium	0.005 ± 0.001	Lithium	0.001 ± 0.0005
Zinc	354.86 ± 12.46	Molybdenum	0.001± 0.0002

*SD = Standard Deviation

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

DISCUSSION

Obviously in *A. Oerfota* the order of amount of the some mineral element contents such as zinc copper, iron, and calcium are different in comparison by other Acacia seeds studied in Iran in recent studies[1, 2]. The order depending on the contents of

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

Nutrient	Percentage (%)
Dry Matter(DM)	38.82
Crude Protein (CP)	25.06
Crude Fiber (CF)	27.89
Ash	8.23
NFE	50.88
Tanin (g/100 mg)	3.11

trace metal and essential elements (g/ 100 g) in A. *Oerfota* samples in Hormozgan-Iran studied regions was:

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

And the order of non-metals

P > S > I > F

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

While in A. *Nilotica* samples studied by abbasian et. *al* in 2015 in Hormozgan-Iran , the order was: Cu > Fe > Zn > K> Mn> Co > Ca >Mg > Na > Se > Li > Mo.

Our finding reveals that A. *Nilotica* is a rich resource of Copper and Iron, but *A. Oerfota* seed is a good source of zinc and the profiles of non-metal contents of these two Acacia are the same approximately.

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[39], 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[39]. 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[17,40,41].

This is obviously seen in the crude protein content of the samples of Iranian *Acacia oerfota* examined in this study which reached 25.06, Crude Fiber (CF): 27.89 and this value is significantly higher and superior than other *Acacia oerfota* reported in other countries especially in Africa samples and even higher than A. *Nilotica*. . Our results show that *Acacia oerfota* 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]. The implication of food tannins on human health is a public concern, but it has preventive benefits to health also. They have been considered to be cardio-protective, antiinflammatory, anti-carcinogenic and anti-mutagenic, among others. These protective effects are related to their capacity to: (a) act as free radical scavengers; (b) activate antioxidant enzymes[42-45]. The protective properties of these compounds mean that it is important to study the principal mechanisms of action of selected polyphenols. Furthermore, advances in this research may lead to the development of nutritional products (i.e. food supplements) and semisynthetic analogs that retain substantial protective capacity but produce minimal adverse side effects.

CONCLUSION

In this study, the nutritive value of *A. Oerfota* mature seed native to south of Iran was determined and results revealed that it is so rich in crude protein, Tanin and some trace and essential mineral elements especially Zinc, Copper Iron, and Potassium. Traditionally the plant used widely for the treatment of various ailments, but scientifically few of them was screened out. Tannins in these fruits thus serve as a natural defense mechanism against microbial infections. The antimicrobial property of tannic acid can also be used in food processing to increase the shelf-life of certain foods, such as catfish fillets. Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immunoresponses. The dosage and kind of tannins are critical to these effects. The results of this research by revealing high Tanin content in Acacia *Oerfota* needs more surveys on human health. Therefore the scientific studies should be conducted to investigate the unexploited potential of *Acacia Oerfota* (L.).Our results suggested 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. Oerfota* 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. Therefore due to the seed protein, Tanin and minerals of *A. Oerfota* could be a new source of edible vegetable after the future toxicological studies.

ACKNOWLEDGEMENTS

Authors are thankful to Mrs Mahtab Alimardan, Amin Azariun and Parviz Raoufi , for their technical assistance. Financial Supports from Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS) is gratefully acknowledged.

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