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Proximate analysis, mineral contents, and GC-MS analysis of leaves, twigs, and thorns of *Acacia* *Etbaica* Schweinf

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Abstract

According to the results of a proximate study, the leaves of *Acacia etbaica* contained a considerable amount of crude protein (16.62%), total soluble amino acid (0.78 g/ml), total saccharides (135.33 mg/ml), crude fiber (40.28%), total ash (15.73%), and moisture (7.53%) contents. Compared to the national average, twigs had greater concentrations of crude protein (18.37%), total soluble amino acid (2.55 g/ml), total saccharide (76.90 mg/ml), crude fiber (50.31%), total ash (15.73%), and moisture (7.96%) contents. When compared to the leaves, the thorns had higher levels of crude protein (17.50%), total soluble amino acids (1.31 g/ml), and crude fiber (47.26%), but lower levels of total saccharides (89.33 mg/ml) and total ash (8.03%). Potassium (K), calcium (Ca), iron (Fe), copper (Cu), lead (Pb), bromine (Br), rubidium (Rb), strontium (Sr), and zirconium (Zr) are all present in varying proportions in the leaves, twigs, and thorns of the studied plant parts. The GC-MS analysis showed the presence of phosphine imide, 2-aminobenzophenone and 3-aminobenzophenone compounds in all plant parts. 1, 3, 5-triphenyl-1, 5-pentanedione and Benzimidazole compounds were detected only in the leaves. Benzimidazole is an important compound in medicinal chemistry that possesses many pharmacological properties. Several drugs with high pharmaceutical activity have been synthesized using 2-amino benzophenone.

Keywords: *Acacia etbaica*, proximate analysis, GC-MS analysis

Introduction

Acacia is the most important genus in the Leguminosae family, and it is first described by Linnaeus in 1773. It is estimated that there are roughly 1380 species of *Acacia* worldwide, *Acacia* species (31 species) predominate in Sudan and are of high importance not only because of the gum produced by some of them but also for their nutritive, medicinal and economical importance [1-3]. They represent about one third of the African species [4]. Many species of which are quite rich in proteins and minerals [5]. *Acacias'* leaves, twigs and pods contain fairly high concentrations of protein. The seeds in particular have high protein content, but normally, pass through the herbivore's system without being digested due to the hard covering. During the prolonged dry season of about 8 months in a year especially drought years, *Acacia* species serve as source of much needed nutrients to the domestic herbivores and wildlife since [6]. *Acacia etbaica* Schweinf is among the common woody browse plants that naturally grow in many arid and semi-arid rangelands in sub Saharan Africa [7, 8]. The tree is distributed commonly in eastern Sudan. Beside the woods and livestock traditionally leaves and bark of *Acacia etabica* are used since ancient time to treat many diseases including skin rashes, wounds, and stomach pain [9]. On the other hand, the fruits contained relatively high amounts of crude protein (135 g/kg DM), metabolizable energy (8.4 MJ/kg DM), and *in vitro* dry matter digestibility coefficient (0.6) and the amounts of acid detergent fiber (ADF), sulphuric acid solubilized lignin, and tannin (soluble and condensed) were high. Also, the fruits contained higher amounts of Na, K, Fe, and Zn contents [8]. In fact, currently applying biotechnological approaches to obtain plants as a source of nutritional and nutraceutical compounds with the global demand for chemical industries [10]. The aim of this study was to find out the nutritional value, minerals composition and chemical profile of one of the most popularly known tropical plants, *Acacia etbaica* collected from Erkowitz, eastern Sudan.

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Materials and Methods

Plant materials

The leaves, thorns and small twigs of *Acacia etbaica* were collected from Erkowit, eastern Sudan. Voucher specimens have been deposited at the Herbarium of the Department of Botany, Faculty of Science, University of Khartoum as reference materials. The plant materials were shade-dried then coarsely powdered separately in a hammer mill.

Proximate analysis

Leaves, thorns, and young twigs of *Acacia etbaica* were subjected to chemical analysis. The following parameters were analysed: crude protein, soluble amino acid, total saccharide, fibre, ash, moisture and mineral. The protein contents, fibre, ash and moisture content of the sample was determined according to the procedure of A.O.A.C [11]. Total saccharides and soluble amino acids were determined by the method described by Dubois *et al.* [12] and Yemm and Cocking [13], Respectively.

Mineral composition

Mineral elements were determined using an x-rays fluorescence (XRF) apparatus (MCA Canberra series 35 plus). It consisted of Cd-109 annular x-ray excitation source and Pop Top Si detector fitted with a Canberra Bin Power supply. Samples were ground to a fine homogenous powder using an electric grinder. Transparent thick pellets 4.9 cm in diameter were prepared using a pressing machine. Before analysis, energy calibration was done by using standard elements of known energy at certain channel. The background resulting from any interfering source was corrected and data were computed with help of microprocessor. Concentrations were expressed as ppm.

Gas Chromatography Mass Spectrometry (GC/ MS) analysis

Petroleum ether extract of all plant parts was subjected to chemical analysis using GC/ MS Model GC-MS-QP2010-Shimadzo-Japan series produced by Thermo equipped with Selective Detector Mass Spectroscopy Model SSQ 7000 produced by Finnigan. This equipment was interfaced via HP chemstation version A 02.12 software (Hewlett-Packard, Avondale, PA). The gas chromatography was equipped with DB-5 (5%- phenyl) Methylpolysiloxane 25 μ capillary column, Rtx50- length 30cm, diameter 0.25 μ m, thickness 0.025mm. The operating conditions for gas chromatography were as follows: injector temperature 280 °C, carrier gas: helium at 30 cm/sec, measured at 150 °C, oven temperature 60 °C for 4 min, 150 °C for 4 min and held at 280 °C until the chromatography was completed. The detector temperature was 280 °C. Mass spectroscopy operating parameters were electron ionization at 70 ev, accelerating voltage 10 KV and scan M/Z range from 50 to 500. Identification of the constituents was carried out by comparing retention times, or peak-matching using Wiley and NBS libraries and available literatures.

Statistical analysis

The obtained results for each assay were expressed as the mean \pm standard deviation (SD). Significant differences among the groups were determined by one-way analysis of variance (ANOVA) and Duncan test as post-hoc, $P < 0.05$ using SPSS 16 software.

Results and Discussion

The nutritive value of dry leaves, twigs, and thorns of *Acacia*

etbaica, moisture, fiber, total saccharide, soluble amino acid, crude protein and mineral contents were determined (Table1). It was clear that the leaves, twigs and thorns contained fairly high concentrations of crude proteins (16.62-18.37%). The occurrence of high levels of protein in all parts seems to be nutritionally significant, it was comparable with that of (17.1%) alfalfa hay [14]. Al-Soqeer [14] who studied 14 *Acacia* species reported that the crude protein content among tested *Acacia* spp. ranged from 8.0 to 16.7 %. The three parts were significantly varied in their amino acid contents. The highest level of amino acid content was detected in the twigs (2.55 μ g/ml); it was three times that of the leaves (0.78 μ g/ml) and nearly two times that of the thorns (1.3 μ g/ml). Previous studies on other *Acacia* species showed that the plants contained various amounts of amino acids [15, 16]. The highest level of total saccharides was detected in the dry leaves (135.35 mg/ml) followed by thorns (89.38 mg/ml) then dry twigs (76.92 mg/ml). It was clear that the twigs and thorns were not significantly different in their total saccharide contents. Several authors have reported that the *Acacia* species were rich in total carbohydrates [17-19]. The detected amount of crude fiber in the dry leaves, twigs and thorns were 40.34, 50.33 and 47.3%, respectively. Mokoboki *et al* [20] found that the fiber content of the foliage of seven *Acacia* species was in the range of 488 to 622 g/kg dry matter (DM). These results supported previous studies on the fiber contents of *Acacia* plants [21]. It was observed that the amount of ash content in thorns (15.73%) equal to that of Leaves. twigs showed significantly low content of ash (8.04%). The obtained value was nearly three times higher than the result recorded on other *Acacia* species [19, 21]. Generally, the thorns of *A. Etbaica* were a good source of minerals. The moisture content of leaves, twigs and thorns were 7.4, 8.0 and 8.5%, respectively. No significant differences between twigs and thorns in their moisture contents. Verma *et al.* [22] reported that the moisture content in different parts of *Acacia catechu* ranged from 5.4 to 7.43%.

Data related to the mineral elements analyzed in the present investigation are given in the (Table 2). All plant parts were rich in potassium, calcium and iron elements. Of all the macro elements, calcium was the most abundant, ranging from 1070 ppm in the thorns to 17100 ppm in leaves. Potassium was high in the leaves (5320 ppm) and low in twigs (3740 ppm). Among the microelements, iron concentration ranged between 273 ppm in twigs and 781 ppm in leaves; copper ranged between 2.41 ppm in leaves and 4.73 in thorns. Almahy and Nasir [23] have reported that the leaves of several *Acacia* species contained phosphorus, calcium, manganese, potassium, sodium, and nitrogen in varying quantities. The detected amounts of iron and selenium in previously studied *Acacia* species were in the range of 132 to 459 and 13 to 100 mg/g, respectively [24]. Table (3) shows the GC-MS spectral analysis of petroleum ether extract of *A. Etbaica* leaves, twigs, and thorns. The identification of components were based on comparison of their mass spectra with those of Wiley and NBS libraries and those described by Adams as well as comparison of their retention indices. 2-Aminobenzophenone, 1, 3, 5-triphenyl-1, 5-pentanedione, 2-aminobenzophenone, Benzimidazole, phosphine imide compounds were detected in leaf extract. Twigs and thorn extracts contained only three compounds, 2-aminobenzophenone, 3-aminobenzophenone and phosphine imide. Benzimidazole is a heterocyclic aromatic organic compound. It is an important pharmacophore and a privileged structure in medicinal chemistry. This compound is bicyclic

in nature which consists of the fusion of benzene and imidazole [25]. Nowadays, is a popular substance with numerous pharmacological properties. Several drugs possessing high pharmaceutical activity, such as chloridiazepoxide (clinical psychosis), proquazone, and amfenac (anti-inflammatory agents) have been prepared from 2-aminobenzophenone [26]. GC-MS analysis of chloroform

extract of *Acacia nilotica* L. leaves revealed the presence of various compounds like 2,4 dimethyl-butylphenol, palmitic acid, linolenic acid, stearic acid, 2- methylresorcinol acetate, 1, 3, 4 eugenol, megastigmatrienone, neophytadiene, myristic acid, lariciresinol, 3,4,7-trimethylquercetin, δ -5-avenasterol, and arachidonic acid [27].

Table 1: Proximate composition and nutritional data of *Acacia etbaica* leaves, twigs and thorns

Parameters	Leaves	Twigs	Thorns
Crude protein (%)	16.62 \pm 0.01 ^c	18.37 \pm 0.04 ^a	17.50 \pm 0.01 ^b
Total soluble amino acids (μ g/ml)	0.78 \pm 0.01 ^c	2.55 \pm 0.06 ^a	1.31 \pm 0.01 ^b
Total saccharides (mg/ml)	135.33 \pm 0.02 ^a	76.90 \pm 0.01 ^c	89.33 \pm 0.01 ^b
Crude fiber (%)	40.28 \pm 0.07 ^c	50.31 \pm 0.01 ^a	47.26 \pm 0.05 ^b
Ash (%)	15.73 \pm 0.01 ^a	15.73 \pm 0.01 ^a	8.03 \pm 0.02 ^b
Moisture (%)	7.53 \pm 0.32 ^c	7.96 \pm 0.05 ^b	8.10 \pm 0.04 ^a

Results are mean of three replicates with standard errors (Means \pm SD.) Different letters in the same raw are significantly different, P < 0.05.

Table 2: Mineral content (ppm) of leaves, twigs and thorns of *Acacia etbaica* using X-ray fluorescence (XRF) technique

Element	Leaves	Twigs	Thorns
Potassium (K)	3740	5320	4440
Calcium (Ca)	17100	15600	1070
Iron (Fe)	781	273	352
Copper (Cu)	2.41	3.78	4.73
Lead (Pb)	0.626	1.47	1.60
Bromine (Br)	4.49	1.72	3.38
Rubidium (Rb)	4.61	3.70	2.41
Strontium (Sr)	619	114	45.3
Zirconium (Zr)	1.62	0.919	0.982

Table 3: Chemical profile of leaf, twig and thorn extracts using GC-MS technique

Extract	No	RT (min)	Name of the compound	MW	Area %
Leaves extract	1	35.85	2-Aminobenzophenone	197	1.59
	2	36.44	1, 3, 5-triphenyl-1, 5-pentanedione	328	0.49
	3	38.23	3-Aminobenzophenone	197	5.27
	4	41.61	Benzimidazole	208	0.29
	5	47.43	Phosphine imide	277	93.36
Twigs extract	1	35.85	2-Aminobenzophenone	197	1.67
	2	38.23	3-Aminobenzophenone	197	3.73
	3	47.42	Phosphine imide	277	93.02
Thorn extract	1	35.86	2-Aminobenzophenone	197	1.40
	2	38.23	3-Aminobenzophenone	197	1.83
	3	47.41	Phosphine imide	277	96.76

Conclusion

The results of nutritional composition of *Acacia etbaica* suggest that, this plant are important feed source for livestock in the Eastern Sudan. Based on the nutritive values, leaves, twigs and thorn were found to have considerable amount of sugars, soluble amino acids, crude proteins and minerals. Moreover, results of minerals content study suggest that *Acacia etbaica* might be considered as good sources of natural minerals and chemical compounds which are essential in the human diet that could help in the prevention of some diseases such as rheumatism, diarrhoea, dysentery, cough asthma, diabetes, malaria, elephantiasis. Thus, this study may provide valuable information on the potential application of *Acacia etbaica* in the nutritional and pharmacological industries.

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