

Full Length Research Paper

Phytochemical and mineral content of the leaves of four Sudanese *Acacia* species

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The phytochemical and mineral composition of *Acacia nilotica*, *Acacia mellifera*, *Acacia albida* and *Acacia radiana* were investigated. Both freshly expended and older leaves of the plant species were used for the analysis. Averagely, the phytochemical constituent of the plants are as follows: Tannins (0.18 to 0.21 mg ml⁻¹), phenols (0.03 to 1.01 mg rnl⁻¹), saponins (0.11 to 0.23 mg ml⁻¹) and flavonoids (0.02 to 0.24 mg ml⁻¹). Alkaloids were found only in *A. albida* and *A. radiana* (0.04 to 0.06 mg ml⁻¹). The result also indicates that the leaves of the plant species contained in percentage, the following elements; phosphorous (0.50 to 1.04%), calcium (0.80 to 2.00%), magnesium (0.24 to 0.49%), potassium (1.05 to 1.54%), sodium (0.51 to 1.03%) and nitrogen (2.38 to 4.06%). The result obtained indicate that the leaves of the plant are good services of phytochemicals and minerals needed for maintainece of good health and can also be exploited in the manufacture of drugs.

Key words: *Acacia* species, leaves, phytochemical, mineral elements.

INTRODUCTION

The roles of plants in maintaining human health is well documented (Moerman, 2004). In Sudan, many of these indigenous spices and their extracts are used in traditional medicine (Okwu and Ekeke, 2003), many of these plants possess bioactive compounds that exhibit physiological activity against bacteria and other microorganisms. These species, hence are used in treatment or many diseases such as rheumatism, diarrhoea, dysentery, cough asthma, diabetes, malaria, elephantiasis, cold and others (Bartram, 1998; Burkill, 2000; Gill, 1999; Nnimh, 1998; Oliver, 2007).

The general assumption is that the active dietary constituents contributing to the protective effects of these plant materials are phytochemicals, vitamins and minerals. Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. These include spices, fruits, herbs and vegetables (Urquiaga, 2000). Thus, diets containing an

abundance of fruits and vegetables give protection against a variety of diseases, particularly cardiovascular diseases (Urquiaga, 2000). Many rare and useful herbs occur in Sudan, from which important drugs could be prepared or agents which may serve as starting materials for the partial synthesis of some useful drugs (Sofowora, 2005). The usefulness of these plant materials medicinally is due to the presence of bioactive constituents such as alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1998). These chemical are known to carry out important medicinal roles, in human body.

Alkaloids play some metabolic role and control development in living systems (Edeoga and Eriata, 2001). They are also involved in protective function in animals and are used as medicine especially the setriodal alkaloids (Stevens et al., 1997). Tannins are known to inhibit pathogenic fungi (Burkill, 2005). Saponin prevent disease invasion of plants by parasitic fungi (Bidwell, 1999), hence have some antifungal properties, they have anti-fertility effects on rats (Zhang et al., 1996). Studies revealed that flavonoids apart from their antioxidant protective effects, inhibits the initiation, promotion and progression of tumors (Kim et al., 2002;

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Okwu, 2004). *Acacia* species belong to the family Mimosaceae and they occur through out the tropics, the Sudanese species are trees with bright yellow flower and usually alternate leaflets (Keay et al., 1999). *Acacia nilotica* has distinct pickles on twigs and young branches.

The trees are about 3 m tall, with spreading crown, the fruits are dark brown and velvety when young, with short soft and often sticky pickles mostly surrounding the centre.

Acacia mellifera, trees are about 2.5 m tall. The fruit is flat and papery 6 to 9 cm across, densely velvet with short hairs. It becomes finely hairy, when ripe (Keay et al., 1999). *Acacia albida* is a water side tree very conspicuous during the flowering season. The trees are usually about 2.4 to 3.8 m tall, branching low down with staggling branches. The fruits are light brown, glabrous and 3.75 to 6.25 cm across, including the soft fleshy narrow wing which extends about three quarters way round the body with a corky and knobby flesh surrounding a hard woody shell (Keay et al., 1999). *Acacia radiana* trees are about 2 m tall having a compact rounded crown and drooping branches (Keay et al., 1999). The leaves of *A. nilotica* and *A. albida* aides have medicinal properties; they are used in treatment of skin disease such as eczema, candidiasis and acnes. The concoction made from their barks is also used in treating asthmatic patients (Gill, 1999). The leaves of *A. mellifera*, *A. albida* and *A. radiana* are used as vegetables in food preparation. *A. albida* leaves are used as fodder for feeding live stock, while the fruits of *A. mellifera* are edible (FAO, 2000). This study investigates the presence and the amount of some phytochemicals in the leaves of the *Acacia* species used in the investigation, thus determining their medicinal values.

MATERIALS AND METHODS

Sample collection

The leaves of *A. nilotica*, *A. mellifera*, *A. albida* and *A. radiana* were collected from (Central of Sudan) Elgazira State. They were identified by the taxonomy unit of the Forestry Department College of Natural Resources and Environmental Management University of Khartoum. The leaves were separated into freshly expanded and older and air dried in the laboratory for one week. The leaves were separated due to the fact that the freshly expanded leaves are the only ones used for phytochemical screening.

Preparation of sample for analysis

The dried leaves were ground to a fine powder using Thomas-Willey Milling Machine. The milled sample bottles at room

temperature in the laboratory of the Biological Sciences Department, University of Khartoum.

Phytochemical analysis (qualitative analysis for presence of phytochemicals)

Alkaloid determination

The presence of alkaloid was determined using the Mayer and Wagner's test as described by the Harbone, 1998. 2 g of each portion of the powdered sample were put into a conical flask and 20 ml of dilute sulphuric acid in ethanol was added into it and then heated in water bath to boil for 5 min. The mixture was filtered and the filtrates were separated and treated with 2 drops of Mayer and Wagner reagents in test-tubes. Development of an orange colouration indicated positive result.

Saponins determination

The froth test and emulsion test as described by Harbone 2001 were used to determine the presence of saponins. 20 ml of water was added to 0.25 g of the powdered sample in 100 ml beaker and boiled was filtered and then used for the test.

Froth test: 5 ml of the filtrate was diluted with 20 ml of water and shaken vigorously. A stable froth (foam) up on standing indicates the presence of saponins.

Emulsion test: 2 drops of olive oil was added to the frothing solution and shaken vigorously the formation of emulsion indicates the presences of saponins.

Tannin determination

The presence of tannins was carried out using the Harbone method (Harbone, 2001). 1.0 g of the powdered sample was boiled with 50 ml of water, filtered and the filtrate used to carryout the ferric chloride test. Few drops of ferric chloride was added to 3 ml of the filtrate in a test tube. A greenish black precipitate indicates the presence of tannins.

Flavonoids determination

The presence of flavonoids in the samples was determined using the Harborne and Sofowora methods (Sofowora, 2005; Harbone, 2001). 10 ml of ethyl acetate was added to 0.2 g of the powdered sample and heated in a water bath for 5 min. The mixture was cooled filtered and the filtrates used for the test.

Ammonium test: About 4 ml of filtrate was shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and the yellow colour in the ammoniacal layer indicates the presence of flavonoids.

Aluminum chloride solution test: 1 ml of 1% aluminum chloride solution was added to 4 ml of the filtrate and shaken. A yellow coloration indicates the presence of flavonoids.

Quantitative analysis of phytochemical

Alkaloid determination

The quantity of alkaloids in the sample was determined using Harbone method (Harbone, 2001). 5 g of the powdered sample was extracted with 10 ml of petroleum ether. The petroleum ether was removed using aspirator. 1.0 g of the extract was suspended in 10 ml of double distilled water and the pH adjusted to 7.6, after shaking for one hour, the suspension was centrifuged. 1 ml of the supernatant was diluted to 50 ml with phosphate buffer. The absorbance was measured spectrophotometrically at 580 nm wavelength.

Saponins determination

Saponins determination was carried out using Harborne (2001) method. 0.1 g of the sample boiled with 5 ml of double distilled water for 5 min decanted filtered while still hot. 2 ml of olive oil was added to it and shaken for 30 s. The absorbance was measure at 620 nm wavelength.

Tannin determination

Okeke and Elekwa (2003) method was used for tannin determination 0.5 g of the sample was shaken with 10 ml of 2 M HCl in a test tube for 5 min. The contents were then transferred into a volumetric flask made up to 50 ml and then filtered. 5 ml of the filtrates, was introduced into a test tube and 3 ml of 0.1 M FeCl_3 in 0.1 HCl and 3 ml of 0.008 M of potassium ferro cyanide ($\text{KFe}(\text{CN})_3$) were added. The absorbance was read at 720 nm within 10 min.

Flavonoids determination

Flavonoids determination was done using Boham and Kocipai method (Boham and Kocipai, 1997). 10 g of the plant material was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper number 42 (125 mm). The filtrate was then transferred into a crucible and evaporated into dryness over in a water bath and weighed to a constant weight.

Total phenol determination

The total phenol determination was done using Harborne (2001) method. The fat free sample was boiled with 50 ml of ether for the extraction of phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask and 10 ml of distilled water was added 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were added and made up to mark and left to react for 30 min for color development. The absorbance of the solution was read at 505 nm wavelength using a spectrophotometer.

Mineral element determination

The levels of the mineral elements calcium, phosphorus, sodium, magnesium, potassium and nitrogen were determined using the wet

digestion extraction methods as described by Ojuwale (1998), Andrew (1999) and Nivozamsky et al. (2007). 0.2 g of the samples were weighed into a 15 ml flask. 5 ml of the extraction mixture (H_2SO_4 -Selenium salicylic acid was added to the sample and allowed to stand over night. The mixture was heated initially at 20°C for 3 h and 5 ml of concentrated perchloric acid (HClO_4) added. This was then heated vigorously until digestion was completed. The solution was allowed to cool and filtered using an acid washed filtered paper into 50 ml volumetric flask and finally made up to mark with distilled water. The potassium and sodium content were determined using the flame photometer method, phosphorus by the vanado-molybdate yellow method using the spectrophotometer method. Calcium and magnesium determined by the versenate EDTA complexometric titration method and nitrogen by the semi micro distillation method using the Markham apparatus.

RESULTS

The phytochemical composition of the *Acacia* species determined is summarized in Table 1. The freshly expanded and older leaves of the *Acacia* species contain tannins, phenols, saponins and flavonoids. Alkaloids were present in the leaves of *A. albida* and *A. radiana* and not in *A. nilotica* and *A. mellifera*. The quantitative estimation of the phytochemicals content of the leaves of the *Acacia* species is summarized in Table 2. *A. radiana* had more alkaloid content (0.24 and 0.06 mg ml^{-1}) when compared with that of *A. albida* (0.04 and 0.06 mg ml^{-1}). Tannins were relatively fairly distributed in all the *Acacia* species used. However, older leaves of *A. nilotica* had the highest amount (0.22 mg ml^{-1}) followed by those of *A. radiana* (0.21 mg ml^{-1}). *A. mellifera* contained more phenols and saponins than other species, its phenols content is 0.06 mg ml^{-1} and 1.01 mg ml^{-1} , respectively for fresh and older leaves and saponins is 0.23 mg ml^{-1} and 0.23 mg ml^{-1} for fresh and older leaves. *A. radiana* had the least amount of phenol and saponins (0.06 and 0.08 mg ml^{-1}) respectively. The freshly expanded leaves of all the *Acacia* species had more flavonoids content when compared with the older leaves.

The mineral element content of the *Acacia* species determined is summarized in Table 3. The leaves of all the *Acacia* species investigated contained phosphorus, calcium, manganese, potassium, sodium and nitrogen in varying quantities. Generally, the freshly expanded leaves of the *Acacia* species have more phosphorus when compared with the older leaves. The phosphorus content of freshly expanded leaves of *A. nilotica*, *A. mellifera*, *A. albida* and *A. radiana* are 0.84, 0.74, 1.04 and 1.02% while that of the older leaves are 0.38, 0.50, 0.10 and 0.75% in that order. *A. radiana* and *A. albida* had more phosphorus when compared to those of other species. The calcium content of the species ranged from (0.80 to 2.00%), magnesium (0.24 to 0.49%), potassium

Table 1. Qualitative analysis of the phytochemical in the leaves of the four *Acacia* species investigated.

<i>Acacia</i> species	Alkaloid	Tannins	Phenols	Saponins	Flavonoids
<i>Acacia nilotica</i> (fresh)	-	+	+	+	+
<i>Acacia nilotica</i> (old)	-	+	+	+	+
<i>Acacia mellifera</i> (fresh)	-	+	+	+	+
<i>Acacia mellifera</i> (old)	-	+	+	+	+
<i>Acacia albida</i> (fresh)	+	+	+	+	+
<i>Acacia albida</i> (old)	+	+	+	+	+
<i>Acacia radiana</i> (fresh)	+	+	+	+	+
<i>Acacia radiana</i> (old)	+	+	+	+	+

+ = Presence; - = absence.

Table 2. The alkaloids, tannins, phenol, saponins and flavonoids contents mg ml⁻¹ of the *Acacia* species investigated.

<i>Acacia</i> species	Alkaloid	Tannins	Phenols	Saponins	Flavonoids
<i>Acacia nilotica</i> (fresh)	0.000	0.210	0.960	0.220	0.240
<i>Acacia nilotica</i> (old)	0.000	0.215	0.025	0.150	0.020
<i>Acacia mellifera</i> (fresh)	0.000	0.210	0.645	0.230	0.230
<i>Acacia mellifera</i> (old)	0.000	0.208	0.010	0.228	0.020
<i>Acacia albida</i> (fresh)	0.060	0.180	0.470	0.120	0.200
<i>Acacia albida</i> (old)	0.050	0.200	0.470	0.160	0.150
<i>Acacia radiana</i> (fresh)	0.024	0.210	0.047	0.140	0.180
<i>Acacia radiana</i> (old)	0.055	0.212	0.080	0.110	0.040

Table 3. The percentage of some mineral elements in the leaves of the *Acacia* species investigated.

<i>Acacia</i> species	P	Ca	Mg	K	Na	N
<i>Acacia nilotica</i> (fresh)	0.84	1.50	0.37	1.45	0.70	3.36
<i>Acacia nilotica</i> (old)	0.38	2.00	0.30	1.54	0.85	3.85
<i>Acacia mellifera</i> (Fresh)	0.74	1.00	0.49	1.18	0.70	3.92
<i>Acacia mellifera</i> (old)	0.50	0.80	0.24	1.52	0.86	2.38
<i>Acacia albida</i> (fresh)	1.04	0.80	0.49	1.05	1.03	3.15
<i>Acacia albida</i> (old)	0.10	0.37	0.37	1.15	0.82	3.36
<i>Acacia radiana</i> (fresh)	1.02	1.32	0.36	1.24	0.51	3.68
<i>Acacia radiana</i> (old)	0.75	1.60	0.31	1.43	0.53	4.06

(1.05 to 1.54%), sodium (0.51 to 0.86%) and nitrogen (2.38 to 4.06%). *A. nilotica* and *A. radiana* had the highest calcium content while *A. albida* the least calcium content. The nitrogen content of the leaves of the *Acacia* species was higher than that of the other minerals element.

DISCUSSION

The fully expanded and older leaves of the *Acacia* species were found to have saponins, tannins, phenols and flavonoids. Alkaloids were present only in *A. albida* and *A. radiana*. These phytochemicals are known to have antimicrobial activity (Ebana et al., 2009). The presence

of phenolic compounds in the leaves of these plants indicates that they may act as antimicrobial agents. Phenols and phenolic compounds are extensively used in disinfections and remain the standard with which other bacteriacides are compared. Thus, the presence of phenolic compounds in the *Acacia* species may be the reason for the therapeutic, antiseptic, antifungal or bactericidal properties of the plants (Gill, 1999). The presence of flavonoids in the leaves of all the *Acacia* species indicates their medicinal value. Flavonoids are antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect the cell against all stages of carcinogenesis (Salah et al., 2002; Okeke and Elekwa, 2003). Flavonoids in intestinal-tract lower the risk of heart disease (Okwu,

2005). *Acacia* species have been used in the treatment of arthritis in herbal medicine (Stray, 1998). The leaves of the *Acacia* species are found to contain saponins. The freshly expanded leaves of *A. mellifera* are of high economic value due to their high saponins content. Saponins is useful in medicine and pharmaceutical industry due to its foaming ability that produces frothy effects in the food industry (George, 2002). Saponins are also used in the manufacture of shampoos, insecticides, various drug preparation and synthesis of steroidal hormones (Sodipo and Akiniyi, 2000).

A. albida and *A. radiana* leaves had very little amount of alkaloids others lacked alkaloids. Alkaloids are known to exhibit marked physiological activity when administered to animals (Okwu, 2004). Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for analgesic antispasmodic and bactericidal effects (Stray, 1998). The high concentration of tannins detected in the older leaves of *A. nilotica* and *A. radiana* makes them of high demand in the world market. Tannins have been found to possess astringent properties, hasten the healing of wounds and inflamed mucous membranes (Morton, 2001; Kozioc and Marcia, 2004). The *Acacia* species studied were known to contain calcium, potassium, magnesium, nitrogen and sodium. These elements are very important in human nutrition. They are required for repair of worn out cells, strong bone and teeth, building of red blood cells, maintaining osmotic balance and for body mechanisms (WHO, 2008). The *Acacia* species, contained high quantity of nitrogen which is an essential constituent of protein.

In general, there are indications that *Acacia* leaves constitute rich sources of mineral elements. The low percentage of sodium in the samples might be an added nutritive advantage due to the direct relationship of sodium intake and hypertension in human (Dahl, 2006). This investigation has revealed that the *Acacia* species studied are of high medicinal value due to their phytochemical and mineral contents which can be utilized in the treatment of many diseases and also be exploited for use in pharmaceutical and cosmetic industries. As a result of their high phytochemical and mineral content, the *Acacia* species are potential sources of useful food and drugs. Studies are on going to ascertain the nutritive and vitamin potential of *Acacia* species in order to know comprehensively their food and medicinal values, so as to be fully exploited for enhancement of life of mankind.

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