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Phytochemical Screening and Elemental Analysis of Gum Arabic (*Acacia senegal*)

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Abstract The study was carried out is to assess the chemical composition of *Acacia senegal.* The phytochemical and elemental analysis of the leaf extract was also carried out according to standard methods. The results of phytochemical analysis showed that the extract contained carbohydrates, tannins, cardiac glycosides, flavonoids, terpenoids, saponins and alkaloids. The elemental analysis of the plant leaf had concentration levels of the elements (mg/l) manganese (1.56), lead (0.002), zinc (0.57), potassium (60), calcium (56.0), magnesium (24.0), copper (0.22), cadmium (0.05), nickel (0.16) and iron (0.04). The presence of bioactive compounds, low concentrations of mineral elements present in the leaf of *Acacia senegal* may justify the traditional use of the plant for the treatment of diseases and also the plants may be safe for use as herbal medicine with less concern for toxic effects of heavy metals.

Keywords Phytochemical, Screening, *Acacia senegal*, Elements

Introduction

Gum Arabic (*Acacia senegal*) is a leguminous tree, drought-tolerant which is widely distributed in both African and Asian countries and found naturally or in plantation. Morphological, is a pale white to orange–brown solid, which breaks with a glassy fracture. The Phytochemical constituents of *Acacia senegal* include: Carbohydrate, Alkaloids, Tannins, Saponins, Flavonoids, Terpenoids and Cardiac glycosides [1].

The use of *Acacia senegal* to obtain superior quality in many products has become so accepted in certain foods. It is an essential element in many industries pharmaceutical, medicine, cosmetics, in local medicinal and other industries. Human dietary intake studies have indicated a reduction in blood cholesterol levels when 25grams/day are ingested in solution. *Acacia senegal* stabilizes emulsions increases the viscosity, adds smooth feel to the skin and forms a protective coating used as an adhesive and constricting for facial masks and face powder [2]. Microencapsulating is a process where droplets of liquids, solids, or gases (core) are coated by thin film (coatings) e.g. gum arabic, which protects the core until it is needed [1]. The coating on a core is semi-permeable and protects the core from severe conditions and controls substances flavoring into the core. The major use for encapsulation in food industry is for liquid flavors. Encapsulation has been able to mask bitter tastes of compounds, reduce volatility and flammability of liquids, control release of materials, provide protection to compounds, and reduce toxicity, separate reactive

materials and to make liquids behave like solid. Micro encapsulation by spray drying is the most economical and flexible way for the food ingredient to retain the needed properties in the final food products. Gum arabic is listed in British Pharmacopoeia (1993), as an effective suspending acid and has been employed to suspend insoluble drugs and to prevent the precipitation of heavy metals from solution through the formation of colloidal suspensions [2]. In food products gum arabic is used as a functional ingredient, which means that the typical function of gum arabic, are emulsifier, flavoring stabilizer and retards sugar crystallization [3]. In addition, gum arabic is acceptable dietary intake due to its non-toxic, odorless, colorless, tasteless, so it does not affect the flavor, color or odor of the food to which it is added. The aim of this study is to assess the chemical composition of *Acacia senegal.*

Materials and Methods

Materials

All the chemicals and reagents used in this study were of analar grade and were used as such without further purification. The reagents such as ethyl acetate (Sigma-Aldrich), methanol and chloroform (JHD-China, and Merck Chemical) were purchased from Cardinal Chemicals and Scientific Equipments, Zaria, Kaduna, Nigeria. Equipment and apparatus used include analytical weighing balance (Model JA103p), electric oven (Gallenkamp Model OV-160), Spectrometer (Perkin Elmer Analyst 2000), Flame photometers (Jenway, model PFP7), Kjeldahl digestion chamber, Muffle furnace (Lenton, Model 4423, England), Desiccators, Mortar and Piston (glass) and Hot plate.

Methods

Collection and Identification of Plant Materials

The leaf of *Acacia senegal* was identified based on botanical features described by Chothani and Vaghasiva [4]. These were collected from the University of Maiduguri campus and further authenticated by a Botanist in the Department of Biological Sciences, Faculty of Science, University of Maiduguri.

Extraction of *Acacia senegal* Leaves

Freshly harvested *Acacia senegal* leaves were thoroughly rinsed in distilled water, air dried under shade at room temperature in the laboratory and ground into fine powder using mortar and pestle as described by Tiwari *et al.* [5]. Standard polar solvents (water, ethanol, and methanol) weight ratio of 10:1 (v/w) was used for the extraction as described by Tiwari *et al.* [5]. A 100 g each of powdered leaves material was extracted in 1000 mls of distilled water, methanol and ethanol using a Soxhlet extractor as described in earlier studies by Tiwari *et al.* [5], to obtain the aqueous, methanolic and ethanolic extracts respectively. The crude extracts were concentrated on an aluminum tray using hot air oven at 40-50 ^{o}C as described by Biu *et al*. [6], to remove the solvent, leaving behind the crude extract. The dried extracts were weighed and stored at room temperature (27 ^{o}C) in sealed glass bottles.

Qualitative Phytochemical Analysis

A small portion of the aqueous, ethanolic, methanolic extracts were tested for the presence of secondary metabolites such as simple sugars, carbohydrates, soluble starch, tannins, phlabotannins, cardiac glycosides, glycosides, terpenoids, saponins, flavonoids and alkaloids, using the methods described by Brain and Turner, [7]; Vishnoi, [8]; Harborne, [9]; Evans, [10] and Sofowora, [11].

Test for carbohydrates: A 4g of the aqueous, ethanol and methanol extracts were boiled in 50mls of distilled warm water on a hot plate for 3 minutes. The mixtures were filtered using Whatman No.1 filter paper while hot and the filtrate was allowed to cool and used for the following tests:

General test- (Molisch's Test): Few drops of Molisch's reagent were added to 2mls of each extract obtained, followed by the addition of 1 ml of concentrated tetraoxosulphate (VI) acids by the side of the test tube, so that it forms a layer beneath the aqueous layer. The mixtures were then allowed to stand for 2 minutes and then diluted with 5 mls distilled water. Formation of a red or dull violet colour at the interphase of the two layers indicated the presence of carbohydrates [10].

Barfoed's Test (General Test for Monosaccharides): one **(**1) ml of each extract were mixed with 1ml of Barfoed's reagent in a test tube and then heated on a water bath for 2 minutes. A red precipitate of cuprous oxide indicated the presence of monosaccharides [10].

Fehling's Test: Standard Test for free Reducing Sugars: 2mls of already prepared extracts were heated with 5 ml of equal volume of Fehling's solution A and B. The formation of a red precipitate of cuprous oxide indicated the presence of a reduced sugar [10].

Test for Combined Sugar: About 0.2 g of each extract was hydrolyzed by boiling with 5mls of dilute hydrochloric acid (HCI) and the resultant solution was neutralized with sodium hydroxide (NaOH) solution. A few drops of Fehling's solution was added to it and heated on a water bath for 2 minutes. Formation of a reddish-brown precipitate of cuprous oxide indicated the presence of combined reducing sugar [10].

Selivanoff's Test (Standard Test for Ketoses): Few crystals of resorcinol and 2 mls of concentrated hydrochloric acid (HCI) were added to a 2 mls of each extract already prepared and boiled for 5 minutes. A reddish coloration indicated the presence of ketose [10].

Test for Pentose: About one (1) ml of hydrochloric acids (HCI) and a little quantity of phloroglucinol was added to 2 ml of the already prepared solution of the extracts in a test tube. The mixtures were then heated over a low flame. Appearance of a red color indicated the presence of pentose [8].

Test for Soluble Starch: Two (2) mls solution of already prepared extracts were boiled with 1ml of 5 % potassium hydroxide (KOH), cooled and acidified with tetraoxosulphate (VI) acid (H_2SO_4). A yellow coloration indicated the presence of soluble starch [8].

Test for Anthraquinone: (Borntrigger's Test): About0.5 g of aqueous, ethanolic and methanolic extract was shaken with 10 ml of benzene and then filtered. 5ml of 10 % ammonia solution were added to the filtrate, and the mixtures were shaken. The appearance of a pink, red or violet color in the ammoniacal (lower) phase indicated the presence of free anthraquinones [10].

Test for Combined Anthraquinones (Borntrigger's Test): About0.5 g of aqueous, ethanolic and methanolic extract were shaken with 10 ml aqueous sulphuric acid (H_2SO_4) and then filtered while hot, the filtrate was shaken with 5 ml of benzene; the benzene layer separates and half its own volume of 10 % ammonia solution was added, appearance of a pink, red or violet color in the ammoniacal (lower) phase indicated the presence of combined anthraquinones [10].

Test for Alkaloids: Half gram (0.5 g) of crude extracts were dissolved in 10 mls of aqueous, ethanol, methanol and filtered followed by addition of 2 mls filtrate to 1% HCl. The mixtures were then boiled and filtered, then treated with few drops of Meyer's reagent. A positive test was indicated by appearance of a creamy or brownish precipitate [10].

Test for Flavonoids: Pew's test described by Evans [10] was used to test for flavonoids. 5.0 g of each extract were boiled in 2mls of 5 % aqueous, ethanol and methanol to which magnesium ribbon was added. The mixtures were then warmed followed by the addition of 5 drops of concentrated HCl. The formation of an orange or red color indicated the presence of flavonoids.

Test for Saponins: The extracts were subjected to the frothing test to identify saponins. A 0.5 g of crude extracts were dissolved in water and shaken thoroughly in a test tube. The appearance of frothing indicated the presence of saponins [10].

Test for Tannins: Five gram (5.0 g) of crude extracts were added to 10 mls of distilled water, shaken thoroughly and filtered through a sieve, followed by the addition of 2 mls of the filtrate to an equal volume of 10 % ferric chloride. The formation of a blue-black precipitate indicated the presence of tannins [10].

Test for Terpenes: This was determined using Lieberman-Buchard's test as described by Evans [10]. 5.0 g of each extract were boiled in ethanol filtered and the filtrate dried. It was then dissolved in 2 mls of chloroform and 1ml of acetic acid anhydride added. Concentrated sulphuric acid was then added gently until solution formed two layers. The formation of a blue top layer and a red lower layer indicated the presence of terpenes.

Elemental Analysis

Ashing and Digestion procedures: About2-10g of pulverized air-dried leaves of *Acacia senegal* (gum Arabic) was packed into a porcelain crucible and placed in a muffle furnance maintained at 450 ± 20 ^{o}C (50 ^{o}C/h). After 24hrs, the crucible was removed from the furnace and cooled to room temperature. The ash was then dissolved in 1 N hydrochloric acid and the final solution was diluted in 0.2 % nitric acid (HNO_3), to dehydrate silica and completely digest organic substances (Szkoda and Zmudzki, 2005). An Atomic Absorption Spectrophotometer (Perkin-Elmer 4110 ZL) equipped with a graphite furnace and AS-72 autosampler (GF AAS), at National Research Institutes for Chemical Technology (NARICT), Zaria was used for the determination of metal elements in *Acacia senegal* as described by Szkoda and Zmudzki [11].

Results and Discussion

Results of the phytochemical screening of the aqueous, ethanolic and methanolic leaf extracts of *Acacia senegal* revealed the presence of chemical compounds such as carbohydrates, tannins, cardiac glyosides, terpenoids, saponins, alkaloids and flavonoids (Table 1). Results of the elemental analysis of leaf extract of *Acacia senegal* shows the presence of elements such as manganese, lead, zinc, potassium, calcium, magnesium, copper, cadmium, nickel and iron (Table 2). When compared with standard permissible limits by WHO, lead and nickel were high, manganese, zinc, potassium, calcium, magnesium, copper and iron were low while cadmium was normal (Table 2).

The phytochemical screening of the leaf of *Acacia senegal* revealed the presence of chemical compounds such as carbohydrates, tannins, cardiac glycosides, terpenoids, saponins glycosides, alkaloids, and flavonoids. Tannin is a general descriptive name for a group of polymeric phenolic subAstances capable of tanning leather or precipitating gelatin from solution, a property known as astringency [12]. They are found in almost every plant part: bark, wood, leaves, fruits, and roots [14]. They have been found to have antidiarrhoeal effect and these substances may precipitate proteins of the enterocytes, reduce peristaltic movement and intestinal secretion [14]. Tannins also can be toxic to filamentous fungi, yeasts, and bacteria. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity [15]. At least two studies have shown tannins to be inhibitory to viral reverse transcriptases [12]. Cardiac glycosides which tend to possess synergism or additive activity as it has been found before [16], and also used for the treatment of heart related problems [17]. Terpenoids are synthesized from acetate units, and as such they share their origins with fatty acids. Terpenenes or terpenoids are active against bacteria, fungi, protozoa and viruses [18-24]. In 1977, it was reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria [25]. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds. Accordingly, Mendoza *et al* [22] found that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduced their antimicrobial activity. Food scientists have found the terpenoids present in essential oils of plants to be useful in the control of *Listeria monocytogenes* [26]. Oil of basil, a commercially available herb, was found to be as effective as 125 ppm chlorine in disinfecting lettuce leaves [27]. Saponins have been reported to posses insecticidal activity [28], antitumorigenic effect [29], molluscicidal effect and spermicidal activity [30], anxiolytic activity [31] and Anti-bacterial activity [32]. Alkaloids have been found to have microbiocidal effects against *Giardia* and *Entamoeba* species [21], the major antidiarrhoeal effect is probably due to their effects on transit time in the small intestine. While many alkaloids are poisonous, some are used medicinally as analgesics (pain relievers) or anaesthetics, particularly morphine and codeine, and for other uses. Flavonoids have also been found to possess some activity and their activity is probably due to their ability to complex with extra cellular and soluble proteins and also complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes [33] probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes [33]. Catechins, is the most reduced form of the C3 unit in flavonoid compounds and has been found to inhibit *Vibrio cholera in vitro* [34], *Streptococcus mutans* [33, 35-36], *Shigella* [37], and other bacteria [35]. The catechins inactivated cholera toxin in *Vibrio* [34] and inhibited isolated bacterial glucosyltransferases in *S. mutans* [38],

possibly due to complexing activities. This latter activity was borne out in *in vivo* tests of conventional rats. When the rats were fed a diet containing 0.1% tea catechins, fissure caries (caused by *S. mutans*) was reduced by 40% [39]. *In vitro* and *in vivo* experiments have shown that flavonoids are able to inhibit the intestinal secretory response induced by prostaglandin E2 [40]. This is close to the findings of Mudi and Salisu [41], who reported that Acacia senegal stem bark extracts contain steroids, tannins etc. The results also showed the presence of metal elements such as manganese, lead, zinc, potassium, calcium, magnesium, copper, cadmium, nickel and iron. These results are similar to the finding of Islam *et al*. [42], Anderson, *et al* [43] and Osman, *et al* [44] who reported that the major elements present in *Acacia senegal* were Ca, Mg, Na, K, Fe, and Cu. However, the metal elements Mn, Pb, Zn, Cd, Ni which were detected in this research work were not detected by Islam, *et al* [42], Anderson, *et al* [43] and Osman, *et al* [44]. *Acacia senegal* is a complex mixture of polysaccharides, protein and prabinoglacto protein specie. It has been shown to be highly heterogeneous and is found in nature as mixed calcium, magnesium, potassium and sodium salts of a polysaccharic acid (*arabic* acid). However, other heavy elements such as Zn, Al, Cd, Cu, Cr, Pb, and Co may also be present but in very small quantities (Islam, *et al* [42], Anderson, *et al* [43] and Osman, *et al* [44]). However, it is evident that the elemental composition is the main reason which contributes to different colors and appearance of different nodules of the same variety or even within different regions in the same nodule. Moreover, Fe and Cu are transition metals which form colored complexes, therefore they can be considered to be the main reason for coloration. Most elements are soil-dependent; therefore their amounts are expected to increase or decrease within different regions in the same nodule [32].

Table 1: Qualitative Phytochemical Screening of the Aqueous, Ethanol, and Methanol Extract of the Leaf of *Acacia senegal*

Phytochemical Constituents		**Inferences**		
		Aqueous	**Ethanol**	**Methanol**
Carbohydrates Test	Molisch's	+	+	+
	Barfoed's	-	-	-
	Free reducing sugar (Fehling's)	-	-	+
	Combined reducing sugars	-	+	+
	Ketoses	-	-	-
	Pentose	+	-	+
	Soluble starch	-	-	-
Tannins	Ferric chloride	-	-	-
	Lead	-	+	+
Phlabotannins		-	-	-
Glycosides	Free anthraquinone	-	-	-
	Combined anthraquinone	-	-	-
Cardiac glycosides	Solkowski's test	+	+	+
	Liebermann-Buchard test	+	+	+
	Terpenoids	+	+	+
Saponins	Frothing	+	+	+
Flavonoids	Shinoda's	-	-	+
	Ferric chloride test	-	-	-
	Lead acetate	+	-	+
	Sodium hydoxide	+	+	-
Alkaloids	Dragendroff's reagent	+	+	+
	Mayers reagent	+	+	+

Key: **+** = Present, **-** = Absent

Table 2: Elemental Analysis of *Acacia senegal* Leaf

Metal Element	Symbol	Concentration (mg/l)	WHO Limit (mg/kg)	Inference
[b]Manganese	Mn	1.5628	2	Low
[*]Lead	Pb	0.002	0.01	High
[b]Zinc	Zn	0.5671	3	Low
[a]Potassium	K	60	3500	Low
[a]Calcium	Ca	56	1000	Low
[a]Magnesium	Mg	24	400	Low
[b]Copper	Cu	0.2163	2	Low
[*]Cadmium	Cd	0.0532	0.05	Normal
[b]Nickel	Ni	0.1562	0.05	High
[a]Iron	Fe	0.041	0.1	low

Keys: *Heavy metals, [a]Macro elements, [b]Micro elements: Source of Permissible Limits (WHO, 1998).

Conclusion

This study implicated the presence of phytochemicals of high therapeutic values. Therefore, the widely reported bioactivities of the *Acacia Senegal* may be attributed to the presence of these bioactive constituents, which may explain its diverse traditional uses for the treatment and management of various ailments. Further research of these active ingredients in the different parts of will help to authenticate the diverse claims of traditional healers on the use of this plant in the cure of several diseases. The low concentrations of mineral elements with the exception of lead and nickel, in the leaf of *Acacia senegal* may indicates the plant do not bioaccumulate these elements and may be safe for use as herbal medicine with less concern for the toxicity effect.

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