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Determination of Medicinal Potentials, Nutritional Value and Mineral Content of Acacia Tree

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Abstract: *The medicinal importance of the bark of acacia tree was analyzed to determine their proximate, phytochemical and antinutritional contents using standard analytical procedures and hence its medicinal potentials were determined. The phytochemical analysis revealed the presence of phytochemicals such as tannins, saponin, alkaloid, cardiac glycoside, steroid, balsam and glycoside, but flavonoid, saponin glycoside, volatile oil and anthraquinone was not present. The percentage proximate values for moisture content, ash, crude fat, crude protein, crude fibre, nitrogen and carbohydrate content in the bark of acacia tree were determined. The results were: moisture-38.8%, ash-19.05%, lipid-41.4%, nitrogen-3.4%, fibre-7.0%, carbohydrate-65.97%, and crude protein-2.98%. The antinutritional analysis showed the presence of oxalate (0.65ml) and phytate (7.7ml).*

Keywords: *Acacia Tree. Medicine, Mineral, Potentials, & Nutrition*

INTRODUCTION

Medicinal chemistry also known as pharmaceutical chemistry can be explained as a discipline at the intersection of chemistry, especially synthetic organic chemistry and pharmacology and other various biological speciaities where they are involved with chemical synthesis, design and development for market pharmaceutical agent or bid-active molecules (drugs), compound used as medicines are most often organic compounds which are often divided into broad classes of small organic molecules (e.g. atovastin, fluticasone) and "biologics" (erythropoietin insulin glargine), the latter which are most of medicinal preparations of protein (natural and recombinant antibodies, hormonrs e.t.c) (Hughes et al, 2011)

Inorganic and organomettalic compounds are used as drugd (e.g., lithium and platinum base agents such as lithium carbonate and cis-platin as well as sallium), in particular medicinal chemistry is the most practice-focusing on small organic chemistry aspect of natural product and computational chemistry in close combination with chemical biology, enzymology and structure biology, together

aiming at the discovery and development of new therapeutic agents. Practically speaking, it involves chemical aspects of identification, and then systematic, thorough synthetic alteration of new chemical entities to make them suitable for therapeutic use, it includes synthetic and computational aspect of the study of existing drugs and agents in development in relation to their boioactivities (biological activities and properties), i.e understanding their structure activity relationship (SAR). Pharmaceutical chemistry is focused on quality aspects of medicines and aim to as fitness for purpose of medicinal products (Hughes *et al*, 2011)

At the biological interface, medicinal chemistry combines to form a set of highly interdisciplinary sciences, setting its organic, physical, and computational emphasis alongside arcas such as biochemistry, molecular biology, pharmacology, toxicology and veterinary and human medicine; these, with project management, statistics, and pharmaceutical business practices, systematically oversee altering identified chemical agents such as that after pharmaceutical formulation, they are safe and efficacious, and therefore suitable for use for treatment of disease (Johnson *et al,* 2014).

1.1.1 MEDICINAL CHEMISTRY IN THE PATH OF DRUG DISCOVERY

Discovery is the identification of novel active chemical compounds often called “hits” which are typically found by assay of compounds for a desired biological activity (Hughes *et al*, 2011). Initial hits can be from repurposing existing agents towards new pathologic process, and from observation of biologic effects of new or existing natural products from bacteria, fungi and plants (Crag *et al*, 2013).

In addition, hits also routinely originate from structural observations of small molecules “fragments” bounded to therapeutic target (enzymes, receptors, e.t.c.), where the fragments serve as a starting point to develop more chemically complex form by synthesis. Finally, hits also regularly originate from en-mass testing of chemical compound ahainst biological targets where the compounds may be from novel synthetic chemical libraries known to have particular properties (kinase inhibitory activity diversity or drug-likeness, e.t.c, or from historic chemical compound collections or libraries created through combinatorial chemistry, while a number of approaches towards the identification and development of hits exist, the most successful techniques are based on chemical and biological initiation development in team environments through years of vigorous practice aimed solely at discovering new therapeutic agents (Harvey *et al*, 2008)

2.0 METHODS

2.1 SAMPLE COLLECTION AND TREATMENT

The sample was collected from Northern part of Nigeria within Sokoto metropolitan specifically in Umaru Ali Shinkafi Polytechnic, Sokoto State, Nigeria. The bark of acacia tree was removed and it was sun dried for five (5) days, it was grinded using mortar and pestle. The sample after collection was kept in an ice-frozen container and immediately transported to the laboratory and kept in the refrigerator at 4^{o}C until it was ready for use.

2.1.1 Extraction of the Plant Material

Soxhlet method of extraction was employed. In this case, 40 grams of sample was weighed and placed in the upper chamber in a thimble. 400ml of methanol was used as solvent. The flask was heated

and the vapour was condensed to extract the sample in the thimble. The process was run for 6 hours; the sample was evaporated to dryness using steam evaporator. The dried extracts were weighed and kept in well labeled sterile sample bottles (Oajele's *et al,* 2008)

40g of Sodium hydroxide pilled was weighted and dissolved in 100cm^3 with distilled water in a 1000Cm^3

2.2 .1 MOISTURE CONTENT DETERMINATION

Principle: the Principal is based on heating of the sample to eliminate all the water content in the sample this can be achieved by placing the sample in an oven at about 105^{o}c for 24 hours.

Procedure: Three crucibles were washed, dried in an oven lat 105$_o$C), cooled in a desiccator and weighed W1.2g samples was weighed into each crucible (W2) and heated in an oven at 105 ^{o}C for 24 hours. The crucible were removed from the oven, cooked were then returned into the oven and weighted after 3 hours interval until a constants value is obtained. The percentage moisture the sample was calculated using equation 2.1.

$$\% \text{ moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.1.)$$

2.2.2. DETERMINATION OF ASH CONTENT

Ash content was determined using AOAC method three crucibles were washed, dried in an oven at 80oc for 30 minutes, cooled in desiccators and weighed as W1 2g of each sample was placed in each crucible and weighted as W2. the crucibles where then heated in a multiple furnace at 600oc for 2 hours the crucible where then cooked in a desiccators and weighed as W3. the samples percentage ash content was calculated using equation 2.2.

$$\% \text{ Ash} = \frac{W3 - W1}{W2 - W1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.2)$$

W1 = weighed of empty dish + crucible

W2 = Weight of crucible + sample before digestion
W3 = weighted of crucible + sample after digestion

2.21 DETERMINATION OF CRUDE PROTEIN

Principle: The principle of the method is based on the transformation of protein nitrogen and other compounds other than nitrate and nitrate of ammonium sulphate by concentrated tetra sulphate (vi) acid.

Organic Nitrogen + cone H_2S0_4 reduction $(NH_4)_2\ S0_4$

Organic matter oxidation Co_2

The subsequent addition of an excess amount of NaOH in closed system will neutralized the acid and releases ammonia which is distilled into boric acid indicator solution

$(NH_4)_2\ S0_4 + {}_2NaOH \dashrightarrow NH_3 + NH_2\ S)_4 + {}_2H_20$

$NH_3 + H_3BO_3$ ----------→ $NH_4 H_2 BO_3$

The solution was then back titrated against 00/N HCl to end point 9

Procedure: the procedure involves three steps i.e. Digestion, distillation and titration

Digestion: 2kg sample (in triplicate) were placed into Kjeldahl digestion flask, tablet of Kjeldahl Catalyst, 20cm^3 of Con.H_2S0_4 were added to each flask and then stirred to soak the sample, the flasks were then heated gently in an electric heater in fume chamber until the solution become clear; the clear solutions were looked and diluted to 100cm^3 mark of the volumetric flask with distilled water.

DISTILLATION: - the distillation apparatus was washed by passion through for about 5 minute – 2OO cm^3 OF 20% boric acid were pipette into a 100cm^3 conical flask and an indicator (methyl pimple) added the chemical flask was then placed at the receiving end of the condenser in such a way that it touches the liquid. 10 cm^2 of the diluted digested solution and 20cms of the 40% NaOH were added into the Kjeldahl digestion flask and then close the inlet. Steam was passed through the digested time (to heat the sample) point the quantity of the ammonia distillate and the boric acid-indicator become about thrice (50ml) original volume.

Titration: the distillate was titrated against 0.01N HCl to end point. The samples under protein was calculated from the tire value using equation 2.3

$$Cp\ (5) = \frac{(a-b) \times 0.01m\ Hcl \times 14 \times C \times 6.25}{d \times e} \times 100 \quad .....(2.3)$$

a. tire value for the digested sample
b. titre value for the blank
c. volume to which the digest was made up
d. Volume of alig not used in distillation
e. Weighted of dried sample
f.

2.2.1 DETERMINATION OF CRUDE LIPID

Procedures: The soxhlet method was adapted for these determinations 2g of the sample were weighted into porous thimble, to which it is mouth was plugged with cotton. The thimble was placed in an extraction Chamber, which was suspended above receiving flask (previously weighted) containing petroleum either (BP. 40-60 ^oC) and below a condenser. The flask was heated on a heavy mantle and the oil extracted, extraction lasted for eight hours after which the thimble was removed from the soxhlet and the apparatus reassembled and heated over waster bath to recovery the either. The flask containing the dry cloth and placed in an oven at 100oc for 30 minutes. After the heating, the flask was cooled in a desiccators and weighted; the percentage crude lipid content was calculated using the equation 2.4.

$$\%\ \text{crude lipid} = \frac{\text{weight oil extracted}}{\text{Weight of sample}} \times 100 \quad .....(2.4)$$

2.2.2 DETERMINATION OF CRUDE FIBRE

procedure: 2g of each powdered sample were transferred into 1 lire compactly spotless beaker, and 175Cm^3 boiled water and 2.5cm^3 of 10% H2S04 were then added, the contents were boiled for 30

minute the residues was raised with hot distilled water the raised residue was then sharpened back into the spotless beaker and 175 Cm^3 of boiled

2.2.5 DETERMINATION OF AVAILABLE CARBOHYDRATE

Procedure: This involves obtaining the available carbohydrate content by calculation having estimated all order factions by proximate analysis. I.e. by sums heating other parameters analyzed from100% dug matters as shown in equation 2.6

Available carbohydrate = 100 – (% Ash + % crude fire + % Lipid + % crude protein ……. ……..2-5

2.2.3 DETERMINATION OF MINERAL CONTENTS

The mineral contents of the three (3) samples were determined using the standard methods by Evans *et al* (1965), Bray *et al* (1945) and Jackson M.L. (1958).

2.3 PHYTOCHEMICAL TESTS

Materials For Phytochemical Analysis

Test-tube, conical flask, spatula, weighing balance, shaker machine

Reagents Used

10% NaOH Sodium hydroxide

5% Ferric Chloride Solution

5ml of Fehling's solution

2ml of 10% aquences hydrochloric acid

Wagner's reagent, H_2SO_4, Chlproform, ethanol. Alcoholic ferric chloride solution

5ml of 10% Ammonia solution, dilute HCl.

2.3.1 Test for Flavonoid

3ml aliquot of the filtrated and 1ml of the 10% NaOH sodium hydroxide, a yellow colour was developed; this indicated the possible appearance of flavonoid compounds.

2.3.2 Test for Tannins

Ferric chloride solution 5% ferric chloride solution was added drop by drop, 2-3mls of the extract and the colour produced was noted. Condensed tannins gave a dark green colour, hydrolysable tannins gave blue-black colour.

2.3.3 Test for Saponin

5ml of the extract was placed in a test tube + 5ml of water and it was shaken strongly. The whole test tube was filled fronth that lasted for several minutes.

2.3.4 Test for Glycosides

2.5ml of 50% H_2SO_4 was added to 5ml of the extract in a test tube. The mixture was heated in boiling water for 15 minutes. It was cooled and neutralized with 10% NaOH, 5ml of Fehling's solution was added and the mixture was boiled. A brick-red precipitate was observed which indicated the presence of glycosides.

2.3.5 Test for Alkaloids

About 2ml of each extract was stirred with 2ml of 10% aqueous hydrochloric acid. 1ml was treated with a few drops of Wagners reagent and second 1ml portion was treated similarly with Mayers reagent. Turbidity or precipitation with either of these reagents was taken as

preliminary evidence for the presence of Alkaloids.

2.3.6 Test for Cardiac Glycosides (Keller-killiani's test)

To one herb extract, 2ml of 3.5% ferric chloride solution was added and allowed to stand for one minute. 2mls of Conc. H_2SO_4 was carefully poured down the wall of the tube so as to form a lower layer. A reddish brown ring at the interface indicated the presence of cardiac glycoside.

2.3.7 Test for Steroids (Salwoski)

This was carried out according to the method of Harbone 1973. 2ml of the extract was dissolved in 2ml of chloroform. 2ml of sulphuric acid was carefully added to form lower layer. A reddish-brown colour at the interface indicated the presence of a steroidal ring.

2.3.8 Test for Saponin Glycosides

To 2.5ml of the extract was added 2.5ml of Fehling's solution A and B, a bluish green precipitate showed the presence pf saponin glycosides.

2.3.9 Test for Basalms

9.5ml of the extract was mixed with equal volume of 90% ethanol, 2 drops of alcoholic ferric chloride solution was added to the mixture. A dark green colour indicated the presence of basalms.

2.3.10 Test for Anthraquinones

2ml of each plant extract was shaken with 10ml benzene, and 5ml of 10% ammonia solution was added. The mixture was shaken and the presence of a pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of anthraquinones.

2.3.11. Test for Volatile oils

1ml of the fraction was mixed with dilute Hcl. A white precipitate was not formed, this indicated the absence of volatile oils.

2.4 DETERMINATION OF ANTINUTRITIONAL FACTOR

2.4.1 Determination of Oxalate

Oxalate was determined using the method of Day and Underwood (1986)

Principle

Oxalate was precipitated as calcium oxalate, the concentration was determined by titration with sodium permanganate which gave a faint pink end point.

Procedure

One gram (1g) of the sample was added to 75ml of 15% H_2SO_4. The solution was carefully stirred intermittently with a magnetic stirrer for 1 hour and filtered using Whatman No. 1 filter paper. The filtrate (25ml) was then collected and titrated against o.1N $KMnO_4$ solution till a faint pink colour appeared that persisted for 30 seconds. $1cm^3$ of 0.1N $KMnO_4$ = 0.0045g of oxalic acid.

2.4.2 Determination of Phytate

The phytate of each sample was determined using the procedure described by Lucas and Markaka (1975).

Procedure

This entails the weighing of 2g of each sample into 250ml conical flask. Two percent (2%) Conc. Hcl was used to soak the sample in the conical flask for 3 hours. Then content was filtered through a double layer filter paper. Each sample filtrate (50ml) was placed in a 250ml beaker and 10ml of distilled water was added. Ten (10ml) of 0.3% ammonium thiocyanate solution was added to each sample solution as indicator and titrated with standard iron chloride solution which contained 0.00195g iron/ml and the end point was signified b brownish yellow colouration that persisted for 5 minutes. (The percentage phytic acid was calculated). Phytin-Phosphorus $1cm^3$ Fe = 1.19mg. Phytin-pjosphorus was determined and phytate content was calculated by multiplying value of phytin-phosphorus by 3.55.

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 PROXIMATE ANALYSIS

Proximate Composition Value in Percentage

S/NO.	Tests	Percentage
1	Moisture	33.8
2	Fibre	7.0

3	Lipid	5.0
4	Nitrogen	0.48
5	Crude protein	2.98
6	Carbohydrate	65.97
7	Ash	19.5

MINERALS

Na (Mg/kg)	K(Mg/kg)	Ca(Mg/kg)	Mg(Mg/kg)	P(Mg/kg)
142.5	8100	1.65	2.10	6.55

3.1.2 ANTINUTRITIONAL ANALYSIS

Oxalate	0.65ml
Phytate	7.7ml

3.1.3 PHYTOCHEMICAL ANALYSIS

S/NO.	TESTS	RESULT
1.	Flavonoids (+++)	N.D
2.	Tannins (+++)	++
3.	Saponins (++)	++
4.	Alkaloids (++)	++
5.	Cardiac glycosides (++)	++
6.	Saponin glycosides (++)	++
7.	Steroids (++)	++
8.	Balsam (+)	++
9.	Volatile oil (+)	N.D

10.	Anthraquinone (+)	N.D
11.	Glycoside	+++

KEY: N.D = Not detected.

\+ = Trace.

++ = Detected.

+++ = Strongly present

3.3 DISCUSSION

The result obtained in the table shows that the extracts of this plants contain ash (19.05) but according to El Behiary *et al* (2009) arrived at 11.81%, but in my research of fiber, I arrivd at 7.00%, but according to T. Smith *et al*, (2005) arrived at 25.11%, but according to A.M. Fade *et al*, (2002), they arrived at 12.2% in crude protein and I arrived at 2.98% while according to El Behairy *et al* (2009), they arrived at 30.90%. Still also in the finding on lipids, my result was 5%, but according to the research carried out by A. Mohammed *et al* (1997) they showed that it contains 4% of lipid.

Under this proximate analysis, I found that there was a lot of difference in my result compared to those result been conducted before, this difference occurred due to the methods been used because I used soxhlet extraction method while other researchers used NIRS (Near Infrared Reactance Spectroscopy).

The result obtained in table 3 showed that the extracts of this plants contains Tannins, steroid, saponin, alkaloid, balsam, glycoside and cardiac glycoside all present but there was absent of flavonoid, anthraquinone, balsam, saponin glycoside, I realized that there was slightly different from each other which I observed that it is due to the method used for the extraction.

It is widely accepted that the functions of secondary metabolites is that they are active principles and they exhibit pharmacological effect. It can therefore be said that one of the metabolites may be responsible for the antibacterial activity.

CONCLUSION

The phytochemical, antinutritional and proximate study using aqueous extract of the bark of *Acacia nilotica* has shown the presence of secondary metabolites or active principle tested, that is; alkaloid, saponin, steroid, balsams and cardiac glycoside with some antinutritional composition of oxalate 0.3ml, phytate 7.7ml and some proximate composition of moisture 38.8%, fibre 7.0%, ash 19.5%, lipid 5%, crude protein 2.98%, nitrogen 3.4%, carbohydrate 65.97% and minerals.

This research is preliminary work of *Acacia nilotica* plant, therefore knowledge of traditional healers and herbalists should not be overlooked or considered inferior to western method of treatment.

RECOMMENDATION

I hereby recommend that the use of plant (*Acacia nilotica*) in the treatment and curing of all sorts of bacterial infection and all sorts of disease should be encouraged, but attempt should be made to establish the facts scientifically.

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