



PHYTOCHEMICAL SCREENING AND ANTIOXIDANT VITAMINS OF ACACIA NILOTICA POD

^{1a*}ABDULLAHI TANIMU BINDAWA, ^{1b}SAFIYANU ADAMU LAME, ^{1c}SHAMSIYA MUHAMMAD UMAR and ^{1d}AISHATU SALEH KILAWA

¹Department of Science Laboratory Technology, Federal Polytechnic, Kaltungo, Gombe state, Nigeria.

Corresponding Author: tanimuabdullahibindawa@gmail.com

ABSTRACT

Acacia nilotica is also known as Gum Arabic tree, Babul, Egyptian thorn, or Prickly Acacia is multipurpose nitrogen fixing legume tree. The aim of this work was to determine the phytochemical constituent and antioxidants vitamins of *Acacia nilotica* pod. The *Acacia nilotica* were obtained from Dange Shuni and the pod was also obtained by removing the seeds. They were properly screened and identified by Botanist in the herbarium of botany unit, biological science, Usmanu Danfodiyo University, Sokoto. 5g of the sample with 100ml of distilled water was soaked overnight and filtered to obtain the filtrate (extract). The samples were analyzed using spectrophotometer. Phytochemical analysis showed the presence of Alkaloids, Flavonoids, Saponins, Tannins, Cardiac glycosides, Steroids, Glycosides, Balsam and Volatile oils. However, anthraquinones were not detected. The quantitative result showed Alkaloids to be (15.9 ± 0.31), Flavonoids (3.8 ± 1.22), Tannins (0.1077 ± 0.00058), Steroids (0.073 ± 0.00038) and Glycosides (5.77 ± 0.015). The antioxidant vitamins analyzed showed vitamin C concentration to be (2.3018 ± 0.0017), vitamin E (329.9 ± 0.20) and vitamin A equivalent (84.72 ± 0.39). This shows that *A. nilotica* pod is rich in Phytochemicals and antioxidant vitamins.

Keywords: Phytochemical, *Acacia nilotica*, Vitamins and Antioxidant

INTRODUCTION

People have used various plants and their derivatives for medical purposes, including the treatment of infectious diseases. The use of plants as sources for novel antimicrobial as well as antioxidant agents offers advantages. Plants are readily accessible and inexpensive, extracts or compounds from plant sources often demonstrate high-level activity against bacteria, and they rarely have severe side effects (Gorlenko *et al.*, 2020). Most pharmacological activities of medicinal plants are traced to their secondary metabolites. Secondary Metabolites are smaller molecules when compared to the constituents of primary metabolites like proteins, carbohydrates, and lipids (Iarayanan *et al.*, 2019). Furthermore, an herbal product from medicinal plants is

produced through a number of processes such as extraction, fractionation, purification, and concentration of plant materials (Upadhyay, B., et al 2010). According to recent studies conducted by the World Health Organisation (WHO), about 80% of the world's population relies on traditional medicine (Daniel, 2017).

Acacia species are multipurpose trees distributed worldwide which comprise nearly 1200 species of the Fabaceae family belonging to the tribe Acaciaceae. Approximately 152 chemical components have been discovered from the *Acacia* species during the last seven decades (Madjid, O. A., et al 2020). The main factors for their possible biomedical values are the isolated key compounds (flavonoids, terpenoids, and phytosterols, phenolic acids, hydrocarbons,

fatty acids, and other substances) accumulated primarily on leaves, stem barks, and pods. Protein and mineral content are abundant in numerous species, especially in the leaves, twigs, and pods (Madjid *et al.*, 2020). Acacia species have been extensively investigated because of their widespread ecological amplitude, dietary, and beneficial properties (Jevitha *et al.*, 2021). Acacia nilotica especially and other Acacia species are used in local traditional medicine by people as remedy for various health problems like cancers of (ear, eye or testicles) and indurations of liver and spleen, condylomas and excess flesh. It is also used for colds, congestion, coughs, diarrhea, dysentery, fever, gallbladder, hemorrhage, hemorrhoids, leucorrhea, ophthalmia, sclerosis, smallpox and tuberculosis (Rao *et al.*, 2019). The aim of this research work was to determine the phytochemical constituents and antioxidants vitamins of *Acacia nilotica* pod.

MATERIALS AND METHODS

Sample Collection

The *Acacia nilotica* were obtained from Dange Shuni and the pod was also obtained by removing the seeds. They were properly screened and identified by Botanist in the herbarium of botany unit, biological science Department, Usmanu Danfodiyo University, Sokoto.

Preparation of Aqueous Extract and Phytochemical Screening

For qualitative screening, 5g of the sample with 100ml of distilled water was soaked overnight and filtered to obtain the filtrate (extract).

Test for Alkaloids

Two-milliliter (2ml) of extract was stirred with 2ml of 10% aqueous hydrochloric acid. A few drops of Wagner's reagent were added

to the extract. A reddish-brown precipitate was formed. (Harborne, 1973).

Test for Tannins

In 2-3ml of extract, 5% of FeCl_3 was added drop by drop. A dark green coloured complex was formed indicating the presence of condensed tannins. (Harborne, 1998; Trease and Evans, 1973).

Test for Saponin

Two milliliter (2ml) of the extract and 2ml of distilled water were mixed and shaken thoroughly, the whole tube was filled with froth that lasts for several minutes indicating the presence of saponin. (Harborne, 1998; Wall *et al.*, 1954).

Test for Glycosides

One milliliter of 50% H_2SO_4 was added to 2ml of the extract in a test-tube. The mixture was heated in boiling water for 15minutes. It was then cooled and neutralized with 10% NaOH, 2ml of Fehling's solution was added and the mixture was boiled again. A brick-red precipitate was not observed indicating the absence of glycosides. (Harborne, 1973).

Test for Flavonoid

Three milliliter of aliquot of the filtrate and 1ml of 10% NaOH were mixed. A yellow color that was developed indicates the presence of flavonoid. (El-Olemylet *et al.*, 1994; Harborne, 1998).

Test for Cardiac Glycosides (KELLER-KILLANI'S TEST)

To 2ml of the extract, 2ml of 3.5% FeCl_3 was added and allowed to stand for one minute. 2ml of conc. H_2SO_4 was then carefully poured down the wall of the tube so as to form a lower layer. A reddish-brown ring formed at the interface indicating the presence of cardiac glycosides.

Test for Steroids

To 2ml of extract, 2ml of chloroform was added and 2ml of sulphuric acid was carefully added to form a lower layer. A reddish-brown color formed at the interface indicating the presence of steroidal ring. (Harborne, 1973).

Test for Saponin Glycosides

The extract (2.5ml) was mixed with 2.5ml of Fehling's solution A and B; a bluish green precipitate was formed showing the presence of saponin glycosides. (El-Olemy *et al.*, 1994).

Test for Volatile oils

1 milliliter (1ml) of the fraction was mixed with dilute HCl. A white precipitate was formed indicating the presence of volatile oils. (Evans, 1980).

Test for Anthraquinone

Five milliliter (5ml) of extract was placed in a test-tube and 5ml of benzene was added and

shaken. The mixture was then treated with 5ml of 10% ammonia. The mixed was observed. (Harbone, 1973).

Determination of Alkaloids

About 5g of powdered plant sample was extracted with 100ml of methanol: water (1:1 v: v) mixture and solvent evaporated. The resultant residue was mixed with 20ml of 0.0025M H₂SO₄ and partitioned with ether to removed unwanted materials. The aqueous fraction was basified with strong NH₃ solution and then extracted with excess chloroform to obtain the alkaloids fraction or separated by filtration. The chloroform extraction was repeated several times and the extract was concentrated to dryness. The alkaloid was weighed and the percentage was calculated with reference to initial weight of powder (Trease and Evans, 1978).

$$\% \text{ Alkaloid} = \frac{\text{weight of alkaloid residue}}{\text{weight of sample}} \times 100$$

Total Tannins

0.1g of the powdered sample was put in to a 100cm³ conical flask and 50cm³ of distilled water was added. The flask gently heated to boiling for 1 hour and filtered while hot.

The filtrate was collected in a 50cm³ volumetric flask. The residue was washed several times and the combine solution made to the volume with distilled water. To 0, 1, 2, 3, 4, and 5 cm³ of the standard tannic acid and 10cm³ of the sample solution in a 50cm³ volumetric flask, 2.5cm³ Folin-Denis reagent and 10cm³ Na₂CO₃ solutions were added and made to volume with distilled water. The flask was allowed to stand for 20minutes after which the optical density was measured at 760nm. The calibration curve was plotted

from which the concentration of tannic acid in the sample was determined. (Trease and Evans, 1978).

Total Flavonoid

5g of the sample was hydrolyzed by boiling in 100ml of hydrochloric acid solution for 30 minutes. The hydrolysate was filtered to recover the extract(filtered). The filtrate was treated with ethylacetate drop wise until in excess. The precipitated flavonoid was recovered by filtration using a weighed filter paper after drying in the oven at 100°C for 30 minutes; it was cooled in a dessicator and reweighed. The difference in weighed gave the weighed flavonoid which was expressed as a percentage of the weighed of the sample analyzed. (Harborne, J.B., et al 1982).

$$\% \text{ Flavonoid} = \frac{\text{weight of filter paper + sample} - \text{weight of empty filter paper}}{\text{weight of sample}} \times 100$$

Total Steroids

About 0.1 g of the powdered sample was put in a 100 ml volumetric flask and 10 ml of 100% methanol was added then the mixture

was allowed to stand for 1 hour after which it was filtered using Whatman's filter paper No.1 in a 100 ml beaker. The sample and blank were prepared as shown in Table 1.

Table 1: Preparation of sample and blank for steroid determination

Reagents	Sample	Blank
Methanolic extract	1 ml	-
0.5% FeCl ₃ containing H ₂ SO ₄	2 ml	2 ml
Potassium Hexacyanoferrite	0.5 ml	0.5 ml
Distilled water	-	1 ml

The absorbance of both the sample and blank were taken at 780 nm using a spectrophotometer and the concentration of steroids in the sample was calculated using a standard steroid curve. Tyler (1994).

solution was centrifugated and the whole of the separated supernatant was collected with a pipette which was used for spectrophotometry measurements. The standard sample was prepared as above (using 1ml of the standard solution instead of the analysed liquid) without centrifugation. The absorbance of sample (Ax) and of standard (As) was measured at 700nm. (Rotkowskiet *al.*, 1998).

Determination of Antioxidant Vitamins

Vitamin C

1ml of the analysed liquid was measured into the centrifugal test-tube and 1ml of the Phosphotungsate Reagent was added, the solution was mixed thoroughly and left in a room temperature for 30 minutes. The

The concentration (Cx) of Vitamin C (um) in the analysed liquid was calculated using the formula:

$$\text{Concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standart}$$

Vitamin E

0.5ml of the analysed fluid was measured into the test-tube I and 0.5ml of anhydrous ethanol was added, the solution was shaken vigorously for 1 minutes. 3ml of xylene was added and shaken vigorously for another 1 minutes the test-tube (solution) was centrifugated for ten minutes. Simultaneously, 0.25ml solution of betaphenanthroline was measured into a separate test-tube labeled as test-tube II. 1.5ml of the extract upper layer was collected and transferred into the test-tube II, the content was mixed. 0.25ml FeCl₃ solution was added to the test-tube II, mixed

and of H₃PO₄ solution was added and the content (solution) was mixed again. This way, the test sample was obtained for spectrophotometry. The standard sample was prepared (0.5ml of the standard solution, instead of the analyse liquid) using Trolox-prepared as the test sample. The standard sample was not centrifugated. (Rotkowskiet *al.*, 2005).

Vitamin A

Three test-tubes were labeled as test, standard and blanks 1.0ml of sample was pipette into the test-tube labeled as test, 1.0ml ethanol was

pipette into all test-tube respectively, 1.0ml standard working reagent was pipette into the test-tube labeled as standard, 3.0ml Pet ether was pipette into all test-tubes respectively and 1.0ml of distilled water was added into all test-tubes respectively. The test-tubes were shaken thoroughly and centrifuged at 3000rpm for 5mins.

RESULTS

The results of phytochemical screening (both qualitative and quantitative) and antioxidants vitamin of *Acacia nilotica* pod were presented in the Table 2, 3 and 4 respectively.

Table 2: Phytochemical composition of *Acacia nilotica* pod

Phytochemicals	Observations
Flavonoids	+
Tannins	+
Saponins	+
Glycosides	+
Alkaloids	+
Cardiac glycosides	+
Steroids	+
Saponin glycosides	+
Balsam	+
Anthraquinones	-
Volatile oils	+

KEY: (+) = Detected or Present, (-) = Not detected

The above table of phytochemical qualitative composition of *Acacia nilotica* pod showed that phytochemical constituents; flavonoids, tannin, saponin, alkaloids, cardiac glycosides, steroids, saponin glycosides, volatile oils, balsams and glycoside are present but anthraquinone was not detected.

The above table of phytochemical quantitative composition of *Acacia nilotica* pod showed that the pod contain higher Alkaloids constituents which is found to be 15.9 ± 0.31 compared to the glycosides, flavonoids, tannins and stereoids which finds to be 5.77 ± 0.015 , 3.8 ± 1.22 , 0.1077 ± 0.00058 and 0.073 ± 0.00038 respectively.

Table 3: Quantitative phytochemical composition of *Acacia nilotica* pod.

Phytochemicals	Concentration (g%)
Alkaloids	15.9 ± 0.31
Glycosides	5.77 ± 0.015
Flavanoids	3.8 ± 1.22
Tannins	0.1077 ± 0.00058
Steroids	0.073 ± 0.00038

All the values were expressed as Mean \pm Standard deviation for three replicates.

Table 4: Antioxidant vitamins of *Acacia nilotica* pod

Antioxidants vitamins	Concentration (mg/dl)
Vitamin A Equivalent	84.72 ± 0.39
C	2.3018 ± 0.0017
E	329.9 ± 0.20

All the values were expressed as Mean \pm Standard deviation for three replicates.

The above table of antioxidants vitamins of *Acacia nilotica* pod showed that the pod contain higher vitamin E constituents which is found to be 329.9 ± 0.20 compared to the vitamin A equivalent and vitamin C which finds to be 84.72 ± 0.39 and 2.3018 ± 0.0017 respectively.

DISCUSSION

Herbal plants were originally researched due to their broad medicinal values since the classical era especially *Acacia* species are utilized to cure a range of illnesses due to their high antioxidant activity. Phytochemicals are bioactive, non-nutrient and naturally occurring compounds in plants (Cheikyoussef *et al.*, 2015). Several phytochemical compounds detected in *Acacia nilotica* pod (Table 1 and 2) are known to have health benefits, physiological activities and medicinal value. Alkanoids, cardiac glycosides, Steroids, saponins, glycosides, balsam, volatile oils, flavonoids, and tannins were tested positive while anthraquinone was tested negative. These results are similar to the previous studies done on other species by

(Abdel-Farid *et al.*, 2014) and (Atiku *et al.*, 2016) who found that *Acacia nilotica* and *Acacia seyal* have saponins, phenolics and flavonoids and *Acacia seiberiana* possess flavonoids, tannins, steroids, alkaloids and saponins. (Hedimbi *et al.*, 2012) also reported on the presence of saponins, flavonoids and polyphenols in *P. leubnitziae*. Alkaloids have contributed to the majority of the poisons, neurotoxins and traditional psychedelics and social drugs e.g. nicotine, caffeine, methamphetamine (ephedrine), cocaine, and opiates consumed by humans (Adamu *et al.*, 2013). Tannins may be employed medicinally in effective protection of the kidneys, antiinflammatory, antidiarrheal, haemostatic and antihemorrhoidal compounds.

Tannins are used in the treatment of wounds emanating from varicose ulcers and hemorrhoids. It is also used to stop bleeding during circumcision (Njoku and Akumufula, 2007). Saponins prevent the excessive intestinal absorption of cholesterol and reduce the risk of cardiovascular diseases such as hypertension (Akinpelu and Onakoya, 2006). These compounds are well known to have curative activity against several human problems such as diuretic, choleric, spasmodic, chronic eczema, diarrhea, dysentery, skin diseases and menstrual disorders (Brinkhaus *et al.*, 2005).

The result of antioxidant vitamins also shows higher concentration of vitamin E followed by vitamin A equivalent and then vitamin C. Vitamin C was recognized as the bioactive molecule that was missing in the diet of sailors, causing scurvy (Baron 2009). Vitamin C is known to take part in many physiological processes, and has been proposed to have a beneficial or therapeutic role in immune responses, cardiovascular disease and cancer (Dervelle, and Baron 2008). Vitamin E is a group of eight antioxidant lipophilic molecules, four of which are tocopherols and

four of which are tocotrienols. It is mostly found in green vegetables, grains, nuts and various vegetable oils, as well as in eggs and milk. Although it is commonly known today for its antioxidant properties, the first biological role attributed to vitamin E was its necessity for fetal survival (Evans *et al.*, 1922). Today vitamin E is known to possess many biological properties, including antioxidant activity and the ability to modulate protein function and gene expression.

Vitamin A and β -carotene have important roles in protection against numerous infections including mastitis. Potential pathogens are regularly present in the teat orifice, and under suitable circumstances can invade and initiate clinical mastitis. Any unhealthy state of the epithelium would increase susceptibility of a mammary gland to invasion by pathogens. There are reports of improved mammary health in dairy cows supplemented with β -carotene and vitamin A during the dry and lactating periods (Chew and Johnston, 1985).

CONCLUSION

Acacia nilotica pod is rich in Phytochemicals and antioxidant vitamins, and it has been in use since ancient times to treat a wide range of diseases in traditional system medicine. Oxidative processes are vital for normal cellular function, and have a pivotal role in various physiological systems, including normal vascular physiology. When given in pharmacological doses, which are much higher than doses that can be attained by dietary intake, antioxidants may attenuate both deleterious and beneficial oxidative processes. This may be the reason why clinical trials that use pharmacological doses of antioxidants do not show a beneficial effect on disease progression when given indiscriminately to all individuals regardless of their baseline level of oxidative stress.

REFERENCES

- Abdel-Farid, B. I., Sheded, M. G., & Mohamed, A. E. (2014). Metabolomic profiling and antioxidant activity of some Acacia species. *Saudi Journal of Biological Sciences*, 21, 400-408.
- Adamu, H.M., Ushie, O.A., D. S. Lawal, D.S., Oga, I.A. (2013). Phytochemical Screening of Fruit of Azanza garckeana and Root of Acacia macrothyrsa. *International Journal of Traditional and Natural Medicines*, 3(1): 19-25.
- Akinpelu, D.A and Onakoya, T.M (2006). Antimicrobial activities of medical plants used in folklore remedies in south-western Africa. *Journal Biotechnol*; 1078-1081.
- Atiku, A., Oladipo, O. O., Forcados, E. G., Usman, S. A., Mancha, D. M. (2016). Anti-nutritional and phytochemical profile of some plants grazed upon by ruminants in North Central Nigeria during the dry season (January to April). *International Journal of Livestock Production*, 7(4), 19-23. doi: 10.5897/IJLP2015.0268.
- Baron, J.H. (2009). Sailors' scurvy before and after James Lind--a reassessment. *Nutr. Rev.* 2009, 67, 315-332.
- Brinkhaus, B., Hentschel, C. and Scand J. (2005). Herbal medicine with curcuma and fumitory in the treatment of irritable bowel syndrome: a randomized, placebo controlled, doubleblind clinical trial. *Gastroentrol.*, 40:936-943.
- Cheikyoussef, A., Sammers, W. R., & Kahaka, G. (2015). Qualitative and quantitative analysis of phytochemical compounds in Namibian *Myrothamnus flabellifolius*. *International Science Technology Journal of Namibia*, 5, 71-83.
- Chew, B.P. and L.A. Johnston (1985). Effects of supplemental vitamin A and β carotene on mastitis in dairy cows. *Journal Dairy Sci.* 68(suppl. 1):19.
- Daniel Ndongo (2017). Antibacterial, Antioxidant and Phytochemical investigation of Acacia Arenaria, Aloe Esculenta, and Pechuel-loeschea Leubnitziae. MSc thesis, Biochemistry Department, University of Namibia.
- Dervelle, F.; Baron, B. (2008). Vitamin C: is supplementation necessary for optimal health? *Journal Alternative Compl. Med.*, 14, 1291-1298.
- Evans, H.M.; Bishop, K.S. (1922). On the existence of a hitherto unrecognized dietary factor essential for reproduction. *Science* 1922, 56, 650-651.
- Gorlenko CL, Kiselev HY, Budanova EV, Zamyatnin AA, Ikryannikova LN (2020) Plant secondary metabolites in the battle of drugs and drug-resistant bacteria: new heroes or worse clones of antibiotics? *Antibiotics*.
- Hedimbi, M., Kaputjaza, D. K., Hans, R. H., Mumbengegwi, D. R., and Bock, R. (2012).
- Phytochemical analysis and antimicrobial screening crude extracts from Pechuel-Loeschea leubnitziae (Kuntze) O. (Asteraceae). *Journal of Research in Microbes*, 1, 039-043.
- Larayetana, R., Ololade Z. S., Ogunmola, O. O, Ladokun, A. (2019) Phytochemical constituents, antioxidant, cytotoxicity, antimicrobial, antitrypanosomal, and antimalarial potentials of the crude extracts of *Callistemon citrinus*. *Evidence-Based Complementary and Alternative Medicine* 2019:e5410923.
- Madjid, O. A., Sanni A., Lagnika, L. (2020). Chemical diversity and pharmacological properties of genus acacia. *Asian Journal of Applied*



- Sciences* 13:40–59.
- Njoku, P. C., and Akumufula, M. I. (2007). Phytochemical and nutrient evaluation of *Spondias mombi* Leave. *Pakistani Journal of Nutrition.*, 6: 613-615.
- Upadhyay, B., Singh, P. T., & Kumar, A. (2010). Ethno-medicinal, phytochemical and antimicrobial studies of *Euphorbia tirucalli* L. *Journal of Phytology*, 2(4), 65-77.
- Rao, T. N., Riyazuddin, P. Babji, N. Ahmad, R. A. Khan, I. Hassan, (2019). Green synthesis and structural classification of *Acacia nilotica* mediated-silver doped titanium oxide (Ag/TiO₂) spherical nanoparticles: Assessment of its antimicrobial and anticancer activity. *Saudi Journal Biol Sci*, vol. 26, pp. 1385-1391.
- Trease GE, Evans WC: Textbook of Pharmacognosy. 1978, BalliereTindall: London, 12 Harborne JB: Phytochemical Methods - A Guide to Modern Techniques of plant analysis. 1998, Chapman and Hall: London