



Assessment of the phytochemical analysis of *Acacia nilotica* plant

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Abstract

Acacia nilotica is an important multipurpose medicinal plant. It was used for the treatment of various diseases and is widely distributed throughout the tropical and sub-tropical regions. The different parts of *Acacia nilotica* like roots, leaves, bark, gum, flowers and pods were recognized as a component of traditional medicine in different countries. This study is aimed at the assessment of the phytochemical profile, alkaloids, saponins, tannins and flavonoids of leaves and stem bark extracts of *Acacia nilotica*. The stem bark and leaves of the plant were collected, dried for three weeks, and ground into powder using mortar and pestle. Soxhlet extractor was used to extract the phytochemicals with petroleum ether and methanol as solvents. The extracts were tested for alkaloids, flavonoids, saponins and tannins. Then, the flavonoids were separated by lead acetate method and the isolated ones were subjected to pH determination and solubility profile test. The thin layer chromatography of the various fractions was done using suitable solvent media and the R_f values were calculated. The results show that alkaloids were found in all the extracts, while saponins, tannins and flavonoids were found in the methanol extracts but not in the petroleum ether extracts of both the leaves and the stem bark. The isolated ones were all found to dissolve in water, but not in organic solvents like acetone and methanol. Various R_f values ranging from 0.05 – 0.93 were obtained. This implies that the extracts contain both polar and non-polar secondary metabolites. The pH values obtained show that the extracts are slightly acidic to slightly alkaline. Phytochemical analysis of *Acacia nilotica* plant extracts indicated the presence of tannins, saponins, flavonoids, and alkaloids which contain chemical constituents of pharmacological importance. This will definitely provide a cheap source of these compounds which are used in pharmaceutical, perfumery and many other industries.

Keywords: *Acacia nilotica*, phytochemicals, thin layer chromatography, plant extract

Introduction

Plants have provided agents which serve as starting products for the synthesis of some useful drugs, for instance steroidal drugs like corticosteroids, the sex hormones and oral contraceptives. Early synthesis used cholesterol or bile acids but modern methods favour plant based-steroids like diosgenin (from yam) or stigmasterol (from soyabean oil) because they are abundant.

Acacia nilotica, commonly known as gum Arabic tree or babul is one species of the genus, *Acacia* which belongs to the *Fabaceae* (Family with many therapeutic properties). *Acacia nilotica* is a standard sized tree and is generally grown in tropical and subtropical areas, like in Africa and Asia. Other names of *Acacia nilotica* are “Kikar” “*Acacia arabica*” “Babul” and “Babool” (Barbosa *et al.*, 2014) [4]. *Acacia nilotica* is a plant with an umbrella formed crown with low branches, which are often scattered. It grows to a height of 3 to 5 meters. *Acacia nilotica* is an imperative plant which has numerous medicinal applications across its various parts, including its barks, leaves, pods, seeds, and gum, which are used as therapies for diarrhea, fever, coughs, skin diseases, and hemorrhoids (Attah, *et al.*, 2016).

The anti-inflammatory, antioxidant, anti-microbial, and anti-diabetic properties of *Acacia nilotica* are attributed to the presence of bioactive compounds like tannins and flavonoids. Also *Acacia nilotica* plays important role in oral hygiene, as a diuretic and for addressing respiratory and digestive issues due to the presence of phytochemicals like tannins, flavonoids, and gallic acid (Maldinit *et al.* 2011) [16].

According to Ali (2011) [1], in traditional medicine, *Acacia nilotica* is widely used because it has anti-microbial, anti-plasmodia, and antioxidant activities and used for treatment of human immune-deficiency virus (HIV), hepatitis C virus and cancer. The bark and pods have been reported to have inhibitory effect against hepatitis virus (Hussaini *et al.*, 2000) [6]. It is useful for treatment of venereal diseases, nausea, burns and wounds, and stomach-ache. It is also beneficial to prevent of premature ejaculation, relieve irritation in acute gonorrhoeal and leucorrhoea, urine genital disorders such as pelvic prolapse (Farzana *et al.*, 2014) [19]. Boiled leaf extracts are used for curing chest pain or pneumonia, powerlessness and chest illness, fever, malaria, headache, coughs, painful joints, backache, stomach ulcers are among many other medicinal uses of this plant. It has been demonstrated that different parts of the *Acacia nilotica* have tannis, stearic acid, ascorbic acid, carotene, protein, fibre, calcium, magnesium and selenium (Lukman *et al.*, 2014) [15].

The plant phytochemical and their products are generally non toxic and contain many medicinal benefits. Antioxidant activities of plants are commonly correlated with their polyphenolic compounds content (Singh and Kumari, 2015) [27].

It has been found that *Acacia nilotica* has effect on central nervous system activities due to potent acetylcholinesterase inhibitory activities Shittu (2010) [25]. Studies have confirmed anti-diabetic activities. However, pods and tender leaves are considered very beneficial in folk medicine to treat diabetes mellitus (Gilani *et al.*, 1999) [5].

It has been reported that the leaf extract of *Acacia nilotica* had significant chemopreventive and anti-mutagenic activity than the other parts (Kalaivani and Mathew, 2010) [7].

The Chemo preventive and anti-mutagenic activity of the leaf extract of *Acacia nilotica* was the most significant, followed by the flower extract and then by gum (Meena *et al.*, 2006) [18].

The extract of *Acacia nilotica* is found to stimulate the synthesis and release of prolactin in the female rate and may be give a better result for lactating women (Lompo *et al.*, 2004)

Acacia nilotica pods have reported inhibited HIV-1 induced cythopathogenicity (Kauret *et al.*, 2005) [8].

Acacia nilotica extracts was performed for qualitative analysis of leaves, pods and bark extract of plant. The obtained result suggested the presence of phenolic compounds in all the tested extracts. (Sawada., 2012) [23].

The first extraction using petroleum ether removes oily and waxy substances. While the second extraction using methanol ensures the removal of highly polar substances like the flavonoids, chlorophylls, alkaloids, *etc.* Such observation has been recorded (Koolen *et al.*, 2013) [11].

Statement of the Problem

Acacia nilotica is a medicinal plant widely used in traditional systems of medicine in Africa and Asia for treating inflammations, infections, diarrhoea and skin diseases. The anti-inflammatory, antioxidant, anti-microbial, and anti diabetic properties of *Acacia nilotica* are attributed to the presence of bioactive compounds like saponins, alkaloids, tannins, flavonoids and phenolic compounds. In spite of these ethnomedicinal applications and pharmacological activities, the phytochemical profile of *Acacia nilotica* remains inconsistently characterized across different geographical regions, plant parts, and extraction methods. Again, there are limited comparative data that correlate the variations in the reports of the earlier studies. In this research, phytochemical screening of *Acacia nilotica* was carried out with the view to further substantiate the earlier claims by various researchers on its potential use in traditional medicine.

Objectives of this Study

The main objective of this study is to assess the phytochemical analysis of *Acacia nilotica* plant. Specifically, the research will seek to:

1. prepare extracts from the stem bark and leaves of *Acacia nilotica* plant;
2. carry out preliminary screening of secondary metabolites, *viz*: alkaloids, saponins, flavonoids and tannins on the stem bark and leaves of the plant;
3. carry out various tests on secondary metabolites, *viz*: alkaloids, saponins, flavonoids and tannins;
4. assess the selective precipitation of flavonoids using lead acetate in the methanol extracts and carry out thin layer chromatography for further characterization of the extracts;
5. determine the pH and solubility profile of the extracts in polar and non-polar solvents.

Materials and Methods

The sample collection and preparation

The plant *Acacia nilotica* stem bark and leaves were obtained within the Usman Dan Fodiyo University, Sokoto

State, Nigeria. The sample was authenticated by Botanists in the Department of Botany of the Faculty of Sciences, Usman Dan Fodiyo University, Sokoto. The stem bark and leaves were dried under the sun for three weeks. After drying, both were ground independently, using mortar and pestle into powdery form to increase the surface area hauling the oil particles; after which isolation of essential oils, via extraction was carried out.

Instrument and apparatus

Electrical weighing balance, beakers, filter paper, Hot plate, measuring cylinder, separating funnel, reflux condenser, and water bath.

Preparation of plant extracts

A given mass, 100 g of the powdered leaves were extracted with petroleum ether (60-80°C) for 2 hours, using a Soxhlet extractor. The extract was put in a 250 cm³ volumetric flask and labeled, LA₁. Again, the powdered sample was extracted with methanol for about one hour. The extract was allowed to cool and poured into a 250 cm³ standard volumetric flask and labeled, LA₂. Then, a 100 g of the ground stem were extracted with petroleum ether for 2 hours using Soxhlet extractor. The extract was then transferred into a 250 cm³ standard volumetric flask and labeled, ST₁. The stem was further extracted with methanol for about one hour. The extract was allowed to cool and then transferred into a 250 cm³ volumetric flask and labeled, ST₂. The plant samples were designated as: -

LA₁ as the petroleum ether extract of the leaves

LA₂ as the methanolic extract of the leaves

ST₁ as the petroleum ether extract of the stem

ST₂ as the methanolic extract of the stem

Experimental

Preliminary phytochemical screening of secondary metabolites

Saponin: A given mass, 0.5 g extract was mixed with 5 cm³ of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion. (Qius *et al.*, 2014) [20]

Flavonoids: A given mass, 0.5 g extract was shaken with 1 cm³ of dilute ammonia solution. A yellow observation indicates the presence of Flavonoids. (Sawada *et al.*, 2012) [23]

Tannins: A given mass, 0.5 g extract was boiled in 20 cm³ of water in a test tube and then filtered. Few drops of 0.1% ferric chloride (FeCl₃) was added and observed for brownish green or blue black colour.

Alkaloids: A given mass, 0.5 g extract was dissolved in 1.5 cm³ of 2% HCl and treated with two drops of Mayer's reagent. Turbidity and formation of creamy white precipitate was regarded as evidence for the presence of free alkaloids in the extract (Roy, 2017) [21].

Test for Flavonoids and Tannins

The same mass, 2.0 g each of the extracts LA₁, ST₁, LA₂, and ST₂ were treated with ferric chloride (FeCl₃) solution. The colour change was observed. The presence of bluish green or blue-black green which changes to reddish brown if in excess indicates the presence of flavonoids or tannins.

Test for Alkaloids

A given mass, 2.0 g of the extracts LA₁, ST₁, LA₂, and ST₂, were each treated with Wagner's reagent, Hager's reagent, Dragendorff's reagent and Mayer's reagent separately. The intensity of precipitation was observed in each case. Precipitation or turbidity indicates the presence of alkaloids.

Test for Saponins

A given mass, 2.0 g of the extracts LA₁, ST₁, LA₂, and ST₂ were each shaken with water in a test tube and the frothing observed in each case. Frothing persisting on warming was taken as an evidence for the presence of saponins.

Test for Flavonoids

A mass of 2.0 g of the extracts LA₁, ST₁, LA₂, and ST₂ were each treated with aluminium chloride (AlCl₃) solution and the colour change was observed. Test using aluminium chloride gives a yellow colour (flavones, flavonols, flavonoids, chalcones and aurones).

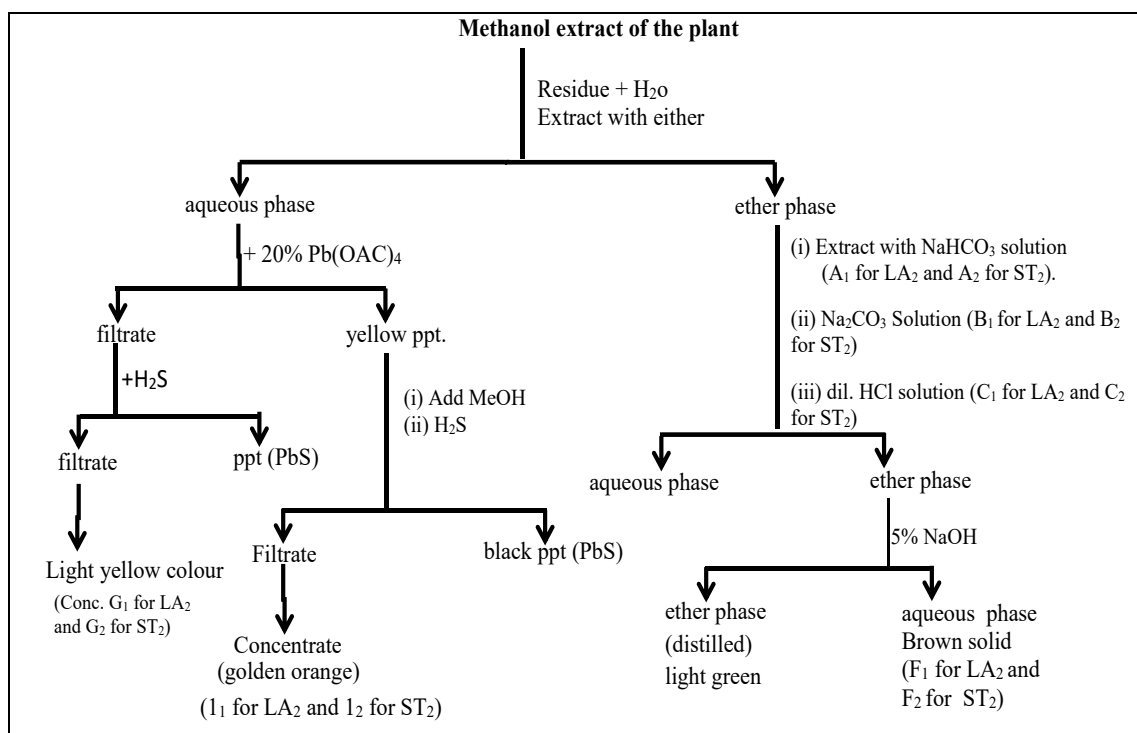
Selective precipitation of flavonoids using lead acetate

The methanol extracts of both the leaves and stems, that is, LA₂ and ST₂ respectively were each dissolved in water and extracted with diethylether. The diethylether phase was

sequentially extracted with NaHCO₃, Na₂CO₃, HCl and NaOH. The resulting aqueous extracts and the final ether extracts were concentrated. The NaHCO₃, Na₂CO₃ and NaOH extracts were each treated with HCl; while the HCl extract was extracted with NaOH solution. For LA₂ and ST₂ the products obtained after extraction with NaHCO₃ were A₁ and A₂ respectively; with Na₂CO₃, the products obtained were B₁ and B₂; with HCl, the products were C₁ and C₂ and with NaOH, the products obtained were F₁ and F₂ respectively. Each of the products was then purified by washing with acetone, chloroform, ether and methanol. TLC and solubility tests in methanol and water were carried on the products.

For the other aqueous phase, 20% lead acetate was added and the resulting precipitate filtered. A yellow precipitate was formed and dissolved in methanol and decomposed by passing H₂S gas to remove lead as PbS, black precipitate on filtering. H₂S was also passed through the filtrate and the precipitated PbS filtered. Each of the solution was concentrated; the concentrates were labeled, G₁, G₂, I₁ and I₂. The procedure is as shown on Scheme 1.

Methanol extract of the plant



Scheme 1: Selective precipitation of flavonoids using lead acetate

Samples A₁, A₂, B₁, B₂, C₁, C₂, F₁, F₂, I₁, I₂, G₁, and G₂, are fractions obtained on selective precipitation of the extracts with lead acetate as shown on Scheme 1.

Thin layer chromatography (TLC)

All the four extracts; ST₁, ST₂, LA₁ and LA₂ were subjected to thin layer chromatography. Three solvent media were used, including; benzene: diethylether (3:1 v/v), butanol: acetic acid: water (4:1:5 v/v/v), and chloroform: benzene (2:5 v/v).

Each of the samples was spotted on a silica gel aluminium plate. The plate was then immersed in the solvent and the "development" started instantly. The various separated components were observed and the retention factor (R_f) values were calculated. The R_f values for each spot was calculated thus: $R_f = \frac{DCP}{DSF}$

where: DCP = distance travelled by the component; DSF = distance travelled by the solvent front

Solubility Test

A given mass, 0.5 g of F₁, F₂, G₁, G₂, I₁ and I₂ was each placed in water and shaken. Some amount of each were also placed in methanol and shaken.

pH Values

The pH values of the samples F₁, F₂, G₁, G₂, I₁ and I₂ were determined using a pH meter.

Results and Discussion

Results

Flavonoids and Tannins

The results obtained from the test for flavonoids and tannins done using Ferric chloride as the reagent are as shown on Table 1.

Table 1: Results obtained from the test for flavonoids and tannins

Plant Extract	Colour
LA ₁	Yellowish green
ST ₁	Orange
LA ₂	Bluish-black green
ST ₂	Bluish green

Alkaloids

The results obtained from the test done using Dragendorff's, Mayer's, Wagner's and Hager's reagents were used to detect alkaloids are shown on Table 2.

Table 2: Results obtained from the tests on turbidity with alkaloidal reagents

Extract	Dragendorff's	Mayer	Wagner's	Hager's (picric acid)
LA ₁	+	-	+	-
ST ₁	+	-	+	+
LA ₂	++	+	+++	+
ST ₂	++	+	+	+

where: +++ = turbidity; ++ = slight turbidity; + = very slight turbidity; - = no turbidity

Saponins

The results obtained from frothing tests done to detect the presence of saponins are shown on Table 3.

Table 3: Results from frothing test for saponins

Extract	After shaking and warming with water
LA ₁	No frothing
ST ₁	Frothing
LA ₂	No frothing
ST ₂	Frothing

Thin layer chromatography (TLC)

The results obtained from the TLC on all the four extracts, using the different solvent mixtures are shown on Tables 4 and 5; while the results obtained from the TLC on all the extractants from the diethylether phase sequentially extracted with NaHCO₃, Na₂CO₃, HCl and NaOH are shown on Tables 6 – 11.

Table 4: TLC of the extracts using the solvent mixture; chloroform: benzene (2:5 v/v)

Extract	DSF	DCP	R _f value	Colour
LA ₁	54	37	0.69	Pale-green
	54	50	0.93	Deep-green
	54	23	0.43	Light-yellow
ST ₁	56	12	0.21	Brown
	56	43	0.77	Green
	56	55	0.98	Red
LA ₂	60	30	0.50	Brown
	60	35	0.58	Red
	60	38	0.63	Pale-green
	60	43	0.72	Green
	60	48	0.80	Yellow
ST ₂	67	6	0.10	Green
	67	35	0.52	Pale-green

Table 5: TLC on the extracts using the solvents' benzene: diethylether (3:1 v/v)

Extract	DSF	DCP	R _f value	Colour
LA ₁	68	9	0.13	Brown
	68	48	0.71	Yellow
	68	60	0.88	Pale-green
	68	65	0.96	Green
ST ₁	55	43	0.78	Pale-Yellow
	55	41	0.75	Brown
	55	52	0.95	Red
LA ₂	75	4	0.05	Pale-green
	75	6	0.08	Brown
	75	60	0.80	Red
	75	69	0.92	Green
ST ₂	53	4	0.08	Faint-blue
	53	43	0.81	Pale-red

Table 6: TLC on A₁ and A₂ after NaHCO₃ extraction using the solvents, butanol: acetic acid: water (B:A:W, 4:1:5 v/v/v)

Sample	DSF	DCP	R _f value	Colour
A ₁	66	8	0.12	Brownish
	66	20	0.30	Pale-Yellow
	66	23	0.35	Green
	66	32	0.48	Yellowish
A ₂	62	4	0.06	Pale-brown
	62	41	0.66	Green

Table 7: TLC on B₁ and B₂ after Na₂CO₃ extraction using the solvent (B:A:W, 4:1:5 v/v/v)

Sample	DSF	DCP	R _f Value	Colour
B ₁	50	14	0.28	Pale-brown
	50	18	0.36	Dark-brown
	50	22	0.44	Green
	50	40	0.80	Pale-yellow
B ₂	59	3	0.05	Blue

Table 8: TCL on C₁ and C₂ after HCl extraction using the solvents (B:A:W, 4:1:5 v/v/v)

Sample	DSF	DCP	R _f Value	Colour
C ₁	58	4	0.07	Faint-blue
	58	44	0.76	Brown
	58	52	0.90	Yellow
C ₂	53	8	0.15	Blue
	53	40	0.75	Green

Table 9: TLC on F₁ and F₂ after NaOH extraction using the solvents B:A:W, 4:1:5 v/v/v

Sample	DSF	DCP	R _f Value	Colour
F ₁	56	18	0.32	Green
	56	31	0.55	Faint-green
	56	41	0.72	Faint-yellow
	56	52	0.93	Brown
F ₂	50	32	0.64	Brown
	50	46	0.92	Green

Table 10: TLC on G₁ and G₂ after precipitation with Pb (OAC)₄ using the solvents (B:A:W 4:1:5 v/v/v)

Sample	DSF	DCP	R _f Value	Colour
G ₁	55	43	0.78	Pale-yellow
	55	50	0.91	Green
G ₂	53	16	0.30	Pale-brown
	53	45	0.85	Green

Table 11: TLC on I₁ and I₂ after precipitation with Pb(OAC)₄ using the solvents (B:A:W 4:1:5 v/v)

Sample	DSF	DCP	R _f Value	Colour
I ₁	56	32	0.57	Yellow
	56	45	0.80	Brown
I ₂	55	2	0.04	Bluish
	55	25	0.45	Yellowish
	55	44	0.79	Brown

Solubility

The solubility tests with various solvents on the samples F₁, F₂, G₁, G₂, I₁ and I₂ were conducted. They were all found to dissolve in water, but not in organic solvents like acetone and methanol.

pH Value

The results of the pH values of the samples are as shown on Table 12.

Table 12: pH value of the samples

Sample	pH value	Colour
F ₁	7	Brown
F ₂	9	Light-yellow
G ₁	9	Light-green
G ₂	6	Dirty-white
I ₁	6	White
I ₂	7	White

Summary of the findings

The overall results of the tests done on LA₁, LA₂, ST₁ and ST₂ are summarized in the table 13. (Note + denotes presence and - denotes absence).

Table 13: Summary of the Results

Extract	Flavonoids and Tannins	Alkaloids	Saponins
LA ₁	-	+	-
LA ₂	+	++	+
ST ₁	-	+	-
ST ₂	+	++	+

Discussion of the findings

As can be seen from the summary of the overall results (Table 13), alkaloids were identified in all the extracts, but flavonoids and tannins were found only in the methanol extract of the leaves and stem of *Acacia* (LA₂ and ST₂), not in the ether extracts. This could be simply because they are highly polar compounds. This could be due to their wide range of solubility; though being bases, they are mostly extracted by the alcohol as can be seen from the ++ in LA₂ and ST₂.

This is in agreement with the findings of Kumar and Panday (2020) [13] who observed the presence of flavonoids and tannins in alcohol extract of plant parts.

Saponins were also identified in the plant extracts, indicated by (+) as found in the alcoholic extracts, LA₂ and ST₂ on the Table 13. This is because saponins are polar, due to their glycosidic attachments. They are also surface active agents with soap-like properties, which explain why their aqueous alcoholic extracts foam when shaken. This is in line with the observation of Wang and Zhang (2020) [29] who identified saponins in plant extracts.

Various R_f values ranging from 0.05 – 0.93 were obtained. This implies that the extracts contain both polar and non-polar secondary metabolites. The values obtain are in line

with the literature. A desirable R_f value lies between 0.3 and 0.7.

The R_f values is important in chromatography because it allows us to identify the component of a mixture. By comparing R_f values of a component in a mixture to the R_f values of known compounds, we can determine the identity of the component. This is because; Low R_f values < 0.3 show that the compound is polar and sticks strongly to the polar silica gel stationary phase, therefore does not move far with the non-polar solvent.

R_f values of 0.4 – 0.6 show intermediate polarity; while R_f values of 0.7-1.0 depicts the compound as non-polar, implying that it has low affinity for silica and moves almost as fast as the solvent.

There are similarities and differences in R_f values obtained. This could be as a result of the stationary phase used, the polarity of the solvent, sample size, vessel saturation, the temperature and the concentration of the components in the mixture.

This was then followed by series of extractions and precipitations of the flavonoids in the distilled methanol extracts (LA₂ and ST₂) which yielded crystals. The crystals were all found to be soluble in water but not in organic solvent like methanol.

Conclusion

Alkaloids, tannins and saponins were found to be present in *Acacia nilotica*. Flavonoids are re-isolated. They were found to be much and soluble in water. Thus, since the plant has been shown to possess these phytochemicals, they can be isolated and identified using spectroscopic methods. This will definitely provide a cheap source of these substances (alkaloids, flavonoids, tannins and saponins *etc*) which are used in pharmaceutical, perfumery and many other industries.

References

1. Ali LA, Adesokan AA, Salawu OA, Akanji MA, Tijani AY. Anti-plasmodial activity of aqueous root extract of *Acacia nilotica*. African Journal of Biochemistry Research,2011;5(7):214-219.
2. Aniszewski T. Elsevier, 2017. doi:10.1016/B978-0-12-811453-0.00001-7.
3. Attah CO, Ibrahim S, Lamido DM, Abdulkadir R, Salisu AR, Ahmad I, *et al*. Preliminary phytochemical and antibacterial screening of *Acanthospermum hispidium*. International Journal for Research in Applied and Natural Science,2011;2(1):101-108.
4. Barbosa ER, van Langevelde F, Tomlinson KW, Carvalheiro LG, Kirkman K, de Bie S, *et al*. Tree species from different functional groups respond differently to environmental changes during establishment. Oecologia,2014;174:1345-1357.
5. Gilani AH, Shaheen F, Zaman M, Janbaz KH, Shah BH, Akhtar MS. Studies on antihypertensive and antispasmodic activities of methanol extract of *Acacia nilotica* pods. Phytotherapy Research,1999;13:665-669.
6. Hussein G, Miyashiro H, Nakamura N, Hattori M, Kakiuchi N. Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. Phytotherapy Research,2000;14(7):510-516.
7. Kalaivani T, Mathew L. Free radical scavenging activity from leaves of *Acacia nilotica* (L.) Wil. Ex

- Delile, an Indian medicinal tree. Food and Chemical Toxicology,2010;48:298-305.
8. Kaur K, Michael H, Arora S, Harkonen P, Kumar S. *In vitro* bioactivity-guided fractionation and characterization of polyphenolic inhibitory fractions from *Acacia nilotica* (L.) Willd. ex Del. Journal of Ethnopharmacology,2005;99:353-630.
 9. Khan H. Green synthetic methods and biological activities of nitrogen and oxygen containing heterocycles. 2021.
 10. Koca M, Yilmaz V. European Journal of Medicinal Chemistry,2020;207:112749.
 11. Koolen HHM, da Silva FM, Gozzo FC, de Souza AQ, de Souza AD. Antioxidant, antimicrobial activities and characterization of phenolic compounds from *Mauritia flexuosa* L.f. by UPLC-ESI-MS/MS. Food Research International,2013;51(2):467-473.
 12. Kuzina V, Augustin JM. Saponin biosynthesis and regulation in plants. Plant, Cell and Environment,2020;43(1):15-33. doi:10.1111/pce.13663.
 13. Kumar S, Pandey AK. Flavonoids: A review of their classification, biosynthesis, biological activities. Journal of Pharmacy and Pharmacology,2020;72(8):1039-1064.
 14. Lompo-Ouedraogo Z, Heide van der D, Beek van der EM, Swarts HJM, Mattheij JAM, Sawadogo L. Effect of aqueous extract of *Acacia nilotica* ssp. *adansonii* on milk production and prolactin release in the rat. Journal of Endocrinology,2004;182:257-266.
 15. Lukman AA, Mikhail ON, Abdulfatai AA, Musbau AA, Adeniyi YT, Salawu OA. Antipyretic and analgesic activities of aqueous extract of *Acacia nilotica* root. Biokemistri,2014;26(2):55-62.
 16. Maldini M, Montoro P, Hamed AI, Mahalel UA, Stochmal WA, Piacente S. Strong antioxidant phenolics from *Acacia nilotica*: Profiling by ESI-MS and qualitative–quantitative determination by LC–ESI-MS. Journal of Pharmaceutical and Biomedical Analysis,2011;56(2):228-239.
 17. Manske RHF, Holmes HI, editors. The Alkaloids: Chemistry and Physiology. Elsevier, 2014.
 18. Meena PD, Kaushik P, Shukla S, Soni AK, Kumar M, Kumar A. Anticancer and antimutagenic properties of *Acacia nilotica* (Linn.) on 7,12-dimethylbenz(a)anthracene-induced skin papillomagenesis in Swiss albino mice. Asian Pacific Journal of Cancer Prevention,2006;7:627-632.
 19. Farzana MUN, Al Tharique I, Arshiya S. A review of ethnomedicine, phytochemical and pharmacological activities of *Acacia nilotica* (Linn.) Willd. Journal of Pharmacognosy and Phytochemistry,2014;3(1):84-90.
 20. Qius X. Natural alkaloids: Basic aspects, biological roles, and future perspectives. Chinese Journal of Natural Medicines,2014;12(6):401-406.
 21. Roy A. A review on the alkaloids: An important therapeutic compound from plants. IJPB,2017;3(2):1-9.
 22. Santos SC, Waterman PG. Phytochemistry Reviews,2017;16(2):267-285. doi:10.1007/s11101-016-9464-6.
 23. Sawada Y, Nakebayashi R, Suzuki M, Sato M. RIKEN tandem mass spectral database (ReSpect) for phytochemicals: A plant specific MS/MS-based data resource and database. Phytochemistry,2012;82:38-45.
 24. Sharma V, JanMedina B. Saponins: Chemistry, biosynthesis, and potential applications. Phytochemistry Reviews,2016;15(3):535-554.
 25. Shittu GA. *In vitro* antimicrobial and phytochemical activities of *Acacia nilotica* leaf extract. Journal of Medicinal Plants Research,2010;4(12):1232-1234.
 26. Singh R, Singh S. Flavonoids: A review of their structure, classification and biological activities. Journal of Medicinal Plants Research,2020;14(15):247-257.
 27. Singh R, Kumari N. Comparative determination of phytochemicals and antioxidant activity from leaf and fruit of *Sapindus mukorossi* L. Pertn., a valuable medicinal tree. Industrial Crops and Products,2015;23:1-8.
 28. Tanaka Y, Ohmiya A. Seeing the world through anthocyanins. Journal of Experimental Botany,2018;69(11):2715-2726.
 29. Wang Y, Zhang H. Saponins: Structure, bioactivity, and potential applications. Journal of Agricultural and Food Chemistry,2020;68(2):533-545.