Original Article

The Qualitative and Quantitative Analysis of Fruit of *Acacia nilotica* plant

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ABSTRACT

The objective of this study is to enlighten readers about the phytochemicals found in *Acacia nilotica*trees. The *Acacia nilotica* species, often called "Babul" or "Kikar," is widely distributed in tropical and subtropical regions. *Acacia nilotica* is a significant, multifunctional medicinal plant that has been used to cure a variety of diseases. It is a member of the Fabaceae family. The goal of this work is to identify the phytochemicals in the hydroalcoholic*Acacia nilotica* extract. A thorough investigation has shown that the phytochemicals of *Acacia nilotica's*hydroalcoholic extract were assessed for phenolic, flavonoid alkaloid, saponin, carbohydrate, protein, and triterpenoid compounds. The extract of *Acacia nilotica*contains alkaloids in the amount of 8 %, flavonoids in 6 %, and saponin in 2.24 %.

Keywords: Alkaloid, Acacia nilotica, Phytochemicals, and Quantitative analysis.

India is abundant in medicinal plants, both wild and cultivated, creating a significant natural and economic health that must be conserved and expanded for the growth of the economy, the riches of the country, and the well-being of its citizens. There is a long history of traditional medicine helping people all around the world. Natural remedies having therapeutic qualities have been used for as long as human civilization itself. For a very long time, the principal sources of medications were minerals, plants, and animals [1]. A common misconception is that natural products are less hazardous than pure chemicals. Manytimes, it is discovered a plant or a plant extract contains some medicinal properties that are not present in the plant's isolated, pure constituents [2-4].

Vachellianilotica is the common name for *Acacia nilotica*[5, 6]. From Egypt, through the Maghreb and Sahel, south to Mozambique and KwaZulu-Natal, South Africa, and east via the Arabian Peninsula to the Indian Subcontinent subcontinent and Burma, the *Acacia nilotica*or*Vachellianilotica* is a native species. Outside of its native region, it has extensively naturalized, especially in Australia and Zanzibar. Livestock spreads it.

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MATERIAL AND METHODS

Collection of plant material

The local areas of Methwade, Sangola, and Solapur in Maharashtra were used to pick the fresh fruits of (*Acacia nilotica*) (India). The fruit's pieces were then dried for 20 days in the shade. It was ground into a fine powder using these dried fruits. Fruit powder was sieved using a sieve with a 212 mm opening, and before extraction, the powder was kept at room temperature in a polythene bag to preserve it from moisture.

Authentication of Sample

The plant was identified and authenticated by botanist Dr.Tembhurnikar Sir, SangolaMahavidyalaya,Sangola.

Pharmacognostic evaluation

The moisture content, total ash value, acid insoluble ash

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value, and water-soluble ash value are proximate data that are included in the pharmacognostic evaluation of the fruit of the (plant name).

1. **Morphological Characters :**The color, flavor, and shape of the roots are among the morphological characteristics of the fruit of (plant name). This information is listed in table no.

2. Proximate Analysis

A. Moisture content

A Petri dish that had already been weighed was filled with 5 gm of drug powder, which was then baked for five hours at 105^{0}C. After cooling in the desiccator, the Petri plate was weighed. The method was continued until after 30 minutes of heating and cooling, and the weight difference was then recorded.

B. Ash Value Determination [7]

a. **Total Ash Value :**Porcelain dish that is flat, thin, and ignited. 2 g of air-dried powder should be measured into a porcelain dish. Support dish on a triangle of pipe-clay set on the ring of a retort stand. A dish should be supported over a burner to be heated. Heat until vapors virtually stop being released, then lower the dish and increase the heat until all of the carbon has been burned off. then allow drying in desiccators. Regarding the dried sample of the unrefined drug, weigh the ash and calculate the amount of total ash [7].

b. **Acid insoluble Ash Value :**Follow the instructions in the procedure to guarantee of total ash value estimation of a drug to the letter. Additionally, use 25 ml of oil. hydrochloric acid to wash the ash from the dish used for total ash value estimation in a 100 ml container. Wash the accumulation twice with warm water after running it through an "ashless" channel paper. Light the fire in the cauldron, then remove the weight. Put the channel paper and accumulation in a cauldron and heat it steadily until the development of fumes stops, then heat it even more steadily until all the carbon has been analyzed. In a desiccator, cool. Calculate the acid insoluble ash value using the residue's weight and an air-dried powder as a reference [7].

c. **Water Soluble Ash Value :**Follow the procedures outlined in the methodology to ensure an accurate assessment of a medication's total ash value. More thoroughly clean the dish used to measure the total ash in the 100 ml measuring device by adding 25 ml of water to it. Filter using "ashless" filter paper, and then wash the accumulation twice with hot water. Cool weight, start a fire and light the cauldron. Put the filter paper and residue in a saucepan; gently heat until the development of fumes stops, then more vigorously until all the carbon has been released. In desiccators, cool. Calculate the acid-insoluble ash using the residue's weight and air-dried powder [7].

Figure no. 1: Acid insoluble ash value and water soluble ash value

I. Determination of extractive Value

The Ayurvedic Pharmacopoeia of India's approach was used to calculate the percentage of extractive value that is soluble in alcohol and the percentage of extractive value that is soluble in water for powdered drugs [8].

Principle

The evaluation of a medicine's quality and purity using its water and alcohol soluble extractive value is employed when the constituents of the drug cannot be easily assessed by other methods.

- **Alcohol Soluble Extractive Value**

Procedure

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of alcohol of specified strength in a closed flask for 24 hours, shaking frequently during 6 hours and allowing to stand for 18 hours. Filter rapidly, taking precaution against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish and dry at 1050 C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air dried drug [8]

Calculation

Alcohol soluble extractive value % = (B – A) *4*100/w

Where,

A–weightofthe emptydish(gm)
B–theweightofdish+residue (gm)
W-theweight ofplantmaterialtaken(gm)

- **Water soluble extractive value**

Procedure

In a glass stoppered conical flask, 5 gm of the air dried, coarsely powdered medication was macerated for 24 hours with 100 ml of chloroform water (95 ml of distilled water and 5 ml chloroform), shaking the mixture constantly for the first 6 hours, and then allowing it to stand for 18 hours. quickly filtered while taking care to prevent solvent loss. 25 mL of the filtrate was evaporated to dryness in a shallow dish or Petri plate with a flat bottom made of tar. To dry the residue, 2 mL of alcohol was applied. The content was shaken and then dried once more in a water bath. The Petri plates were dried in the hot air oven at 105˚ C for 1 hour and cooled in the desiccator for 30 minutes and weighed. Up till a constant weight was attained, the process was repeated. In relation to the air-dried medication, the percentage of extractive value that is water soluble was calculated [8].

Calculation

Water solubleextractivevalue%=(B–A) *4*100/W

Where,

A–weightoftheemptydish(gm)
B–theweightofdish+ residue (gm)
W– theweightofplantmaterialtaken(gm)

II. Preparationofextracts

Using a mixer grinder, the fruit portion of *Acacia nilotica* was ground. The produced extract was tested for phytochemical content.

Procedure

In a soxhlet system, 45 g of drug powder was extracted using a hydroalcoholic (i.e., water:ethanol = 50:50) solvent. Powdered material was poured into the Soxhlet device after which the solvent system was added. Start heating at 55˚C once one solvent cycle is finished. Continue heating the solvent until it becomes colourless, after which extract was collected and allowed to air dry [8].

Figure 2:Diagramorphotographofextractionmethodbysoxhletextractor

QualitativePhytochemicalTests

A) Detection ofAlkaloid

1. **The Wagner's Test:** An extract that was exposed to a small amount of Wagner's reagent (Iodine in Potassium Iodide). Alkaloids are present because pink earthy colored precipitate forms.
2. **Mayer's Test:** extract that has been exposed to a small amount of Mayer's reagent (Potassium mercuric iodide). Alkaloids are present when a precipitate with a cream tint forms.
3. **Dragenoff's test** involves treating an extract with a few drops of the Dragenoff reagent (solution of Potassium bismuth iodide). Alkaloids are present when a reddish brown precipitate forms.

B) Detection ofGlycosides[7]

1. **Legal's Test:** Add a mixture of sodium nitroprusside and sodium hydroxide to the extract along with 1 ml of pyridine. The development of pink to red shading is evidence that glycosides are present.
2. **Borntrager's Test**: Add dilute H2SO4 to 3 ml of extricate. Filter and bubbles. Add an equivalent volume of chloroform to the cold filtrate. Shake firmly. Distinguish the organic soluble, and add ammonia. The ammonia layer changes from pink to red.
3. **Libermann's Test**: Perform Libermann's Test by combining 3 ml of an extract with 3 ml of acetic anhydride. both hot and cold few drops of concentrated

H_2SO_4. A blue hue is visible.

C) DetectionofFlavonoids

1. **Alkaline Test:** A very small amount of sodium hydroxide solution was used to treat the extract. The presence of flavonoids is indicated by the development of an outstanding yellow coloring that becomes dull with the expansion of diluted acid.
2. **Lead acetate Test**: Extract was given a light lead acetate treatment, consisting of a few drops. The yellow shading solution quickly revealed the presence of flavonoids [9-11].

D) DetectionOfCarbohydrates [7]

1. **Molash's Test:** Add a few drops of alpha-naphthol solution to 2-3 ml of extract in an alcohol shake, then add concentrated H_2SO_4 from the test tube's side. At the point where the two liquids come together, a violate ring forms.
2. **Barfoed's Test**: To perform the Barfoed Test, combine equal volumes of the test solution and Barfoed's reagent (extract). In a bubbling water bath, warm the blend for one to two minutes before cooling. We get a red PowerPoint.
3. **Fehling's Test**: Perform Fehling's Test by combining 1 ml of each Fehling's A and B solution, then adding a bubble for one instant. Add an equal volume of the test extract mixture.

Place the test tube in a pan of bubbling water for five to ten minutes. A yellow, black, and red PowerPoint presentation is initially viewed.

E) DetectionofSaponins

Foam Test: Shake the water-based drug extract ferociously to check for foam. We saw persistent foam [7].

F) Diterpene detection

Copper acetate Test: Extract was given a brief treatment with copper acetate solution. Diterpenes were present, as evidenced by the formation of a green hue.

G) Triterpene detection

Salkowski's Test: Extracts were dissolved in chloroform, and the resulting solution was then shaken and left to stand after being added a few drops of concentrated sulfuric acid. The presence of triterpenes was suggested by the color's appearance of golden yellow [6].

H) Resin Detection

1. **Acetone-water Test**: Filtered acetone was used to dissolve the extract. The acetone solution was shaken after a small amount of water was added. The presence of resins was suggested by the appearance of turbidity.

I) Phenol detection

1. **Ferric chloride Test**: A few drops of ferric chloride solution were added to the extracts. Phenols were present as evidenced by the creation of blurry black color [9-11].

J) Protein Detection

1. **Xanthoproteic Test:** A few drops of a concentrated nitric acid solution were added to the extracts. Proteins were present, as shown by the formation of a yellow color.
2. **Ninhydrin Test:** A 0.25 percent ninhydrin reagent was added to the extract and boiled for a short period. Amino acids were present, as indicated by the formation of blue color.

Quantitative Determination

Alkaloid Determination

Materials:
10%aceticacid,Ethanol,ConcentratedAmmoniumhydroxide

Procedure

According to Harborne's methods, 1.25 grams of root powder were placed in a 250 ml beaker together with 100 ml of 10% acetic acid in ethanol, and the mixture was let to stand for 4 hours. The extract was next concentrated in a water bath to reduce it to one-fourth of its initial volume, and then 10-15 drops of concentrated ammonium hydroxide were added drop by drop, causing a precipitate to form immediately after filtering. The mixture is sedimented after three hours, the supernatant is discarded, the precipitate is washed with 10 ml of 0.1 M ammonium hydroxide, and the filtrate is then run through Gem filter paper. The extract was baked to dry it out.

Calculation

The percentageofAlkaloids isexpressedmathematically

$$\text{Percentalkaloid} = \frac{\text{Weightofalkaloid}}{\text{Weightofsample}} \times 100$$

Flavonoiddetermination

Materials: 80%aqueous Methanol,Ethanol

Procedure

The method described by Ejikeme et alwas used to determine the presence of flavonoids. It involved adding 50 ml of 80 percent aqueous methanol to 2.5 g of root powder (the sample) in a 250 ml beaker. The mixture was then covered and left to stand for 24 hours at room temperature. The residue was then extracted three times with the same amount of ethanol after the supernatant was discarded. The entire Root sample solution was filtered using Whatman filter paper number 42. The filtrate from the Rost sample was then put in a crucible and dried over a water bath. The crucible's contents were then cooled in a desiccator and underweight.

Calculation

The percentageofflavonoidswascalculatedby

Weight of flavonoid
Percent flavonoid=--------------------------------------- X 100
Weightofsample

Saponindetection

Materials

20 %aqueousethanol,Diethylether,n-butanol,5%sodiumchloride

Procedure

The method described by Ejikeme et al. was used to complete the quantitative determination of saponin. 5 grams of Root powder sample was placed in a 250 ml conical cup with 100 ml of 20 percent fluid ethanol. The mixture was heated to a temperature of 55 ° C over a water bath for four hours while being constantly mixed. Following filtration, the residue was once again extracted using 100 ml of 20% watery ethanol. This filtrate was continuously mixed for 4 hours while being heated to a constant 55 ° C. On a water bath, the combined concentrate was reduced to 40 ml. 20 ml of diethyl ether was added to this concentrate in a 250 ml separating funnel and vigorously mixed. The ether layer was removed and the aqueous layer recovered from it. This was extracted twice using 10 ml of 5 percent sodium chloride and 60 ml of n-butanol. After that, the sodium chloride layer was removed, and the residual solution was added to the crucible that had already been preheated in the oven to a consistent weight. To bring the weight of this solution to a constant, heat it first in a water bath.

Calculation

Thepercentagesaponincontentwascalculatedas

Weight of saponin
PercentSaponin=--X 100
Weightofsample

RESULTS

Pharmacognosticevaluation

1. Morphologicalcharacters

The macroscopic analysis of *Acacia nilotica* fruits' color, scent, taste, size, and form is considered a morphological characteristic. In table No. 1, this is noted.

Table no. 1:Morphologicalcharacteristicsof fruitsof *Acacia nilotica*

Morphologicalfeatures	Observations
Color	Greyishyellow
Odorandtaste	Pungent,bitterandastringent
Shape	Podlike structure

2. Proximateanalysis

Moisture content, total ash value, acid insoluble ash value, water soluble extractive value, and alcohol soluble extractive value are among the parameters used in the proximate analysis. Their results are presented graphically in graph No. 1 and recorded in Table No. 2

Table no. 2:proximatevaluesofFruitsof*Acacia nilotica*

Parameters	Determinedvalue(%)
Moisturecontent	6.66%
Totalashvalue	7.35%
Watersolubleashvalue	2.1%
Acid insolubleashvalue	5.5%
Watersolubleextractivevalue	9.5%
Alcoholsolubleextractivevalue	10.32%

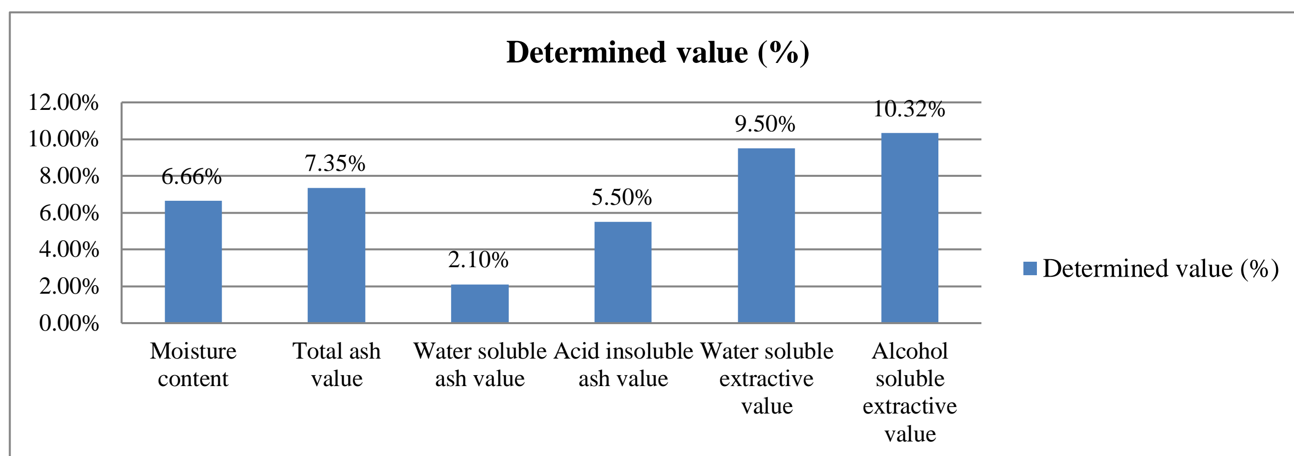


Figure no. 3:proximatevaluesoffruitpowderof*Acacia nilotica*

PhytochemicalEvaluation

The detection of the presence or absence of phytochemicals in plant and herbal medicine extracts is known as phytochemical evaluation.

1. Qualitative Chemical Test

The results of the qualitative phytochemical tests for the fruit extracts of *Acacia nilotica* are shown in Table No..3.

Table no. 3 : Qualitative chemical test

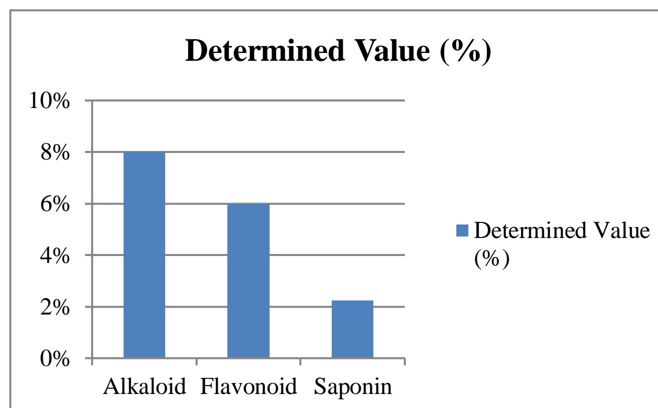
Chemicalconstituents	Chemicaltest	Extract
Alkaloid	Wagner'sTest	+
	Mayer'sTest	+
	Dragenoff'sTest	+
Glycosides	Legal'sTest	+
	Borntrager's Test	
	Libermann'sTest	+
Flavonoid	AlkalineTest	+
	LeadAcetateTest	+
Carbohydrates	Molish'sTest	+
	Barfoed'sTest	+
	Fehling'sTest	+
Saponin	FoamTest	+
Diterpenes	CopperacetateTest	-
Triterpenes	Salkowski'sTest	+
Resins	Acetone-watertest	+
Phenol	FerricchlorideTest	+
Proteins	XanthoproteicTest	-
	NinhydrinTest	-

(- for Absent, + for Present)

QuantitativeDetermination

Tableno.4:Resultsofthequantitativeanalysisof*Acacia nilotica*

NameofthePhytochemicalconstituent	Percentage of phytochemical constituentpresent
Alkaloids	8%
Flavonoids	6%
Saponins	2.24%



FigureNo.4:Quantitativeanalysisof*Acacianilotica*FruitExtract.

DISCUSSION

Collectionofplant material

The local areas of Methwade, Sangola, and Solapur in Maharashtra are where the fresh *Acacia nilotica* fruits were gathered (India). The fruit's pieces were then dried

for 20 days in the shade. To create a fine powder, these dried fruit pieces were ground. Fruit powder was sieved using a 212 mm sieve before being placed in a thin polythene bag to keep out moisture. Fruits were shade-dried, coarsely ground, and stored in hermetically sealed containers for later use.Using a Soxhlet apparatus, the fruits of *Acacia nilotica* were extracted in a hydro-alcoholic solvent. The macroscopic analysis of the color, scent, taste, size, and shape of the fruits of *Acacia nilotica* is considered a morphological characteristic.Moisture content, total ash value, acid insoluble ash value, water-soluble extractive value, and alcohol soluble extractive value are among the parameters used in the proximate analysis. *Acacia nilotica* fruit powder underwent proximate examination, including measurements of moisture content, total ash value, acid insoluble ash value, water soluble extractive value, and alcohol soluble extractive value.

This proximate analysis provides information on the sample's quality as well as aids in the detection of adulteration. Ash value can be used to assess the sample's purity. According to this investigation, the maximum extractive values for alcohol and water are both 10.32 percent and 9.5 percent, respectively.The detection of the presence or absence of phytochemicals in plant and herbal medicine extracts is known as phytochemical evaluation. In order to find the optional metabolites, such as alkaloids, glycosides, flavonoids, saponin, diterpenes, triterpenes, resins, phenols, and proteins in fruit concentrate of *Acacia nilotica* using the hydro-alcoholic solvent, various quantitative phytochemical assays are carried out. The hydro-alcoholic extract of the fruit of the *Acacia nilotica* plant contains alkaloids, glycosides, carbohydrates, saponin, triterpenes, resins, and phenol.To determine the quantity (%) of secondary metabolites (phytoconstituents) contained in the plant powder, quantitative analysis is utilized. According to the current quantitative analytical study, the fruit powder of *Acacia nilotica* includes a maximum of 8% alkaloid as the main phytoconstituent, 6% flavonoid, and 2.24 % saponin.

CONCLUSION

The plant *Acacianilotica* is a member of the Fabaceae family. The findings of the organoleptic investigation provide a rationale for the traditional usage of *Acacia nilotica*, a plant with characteristics such as a greyish yellow color, a pungent odor, and a bitter taste. The various proximate analysis parameters are evaluated.Both the acid-soluble extractive value and the acid soluble ash value are higher than the water-soluble extractive value. According to protocol, the fruit extract's phytochemical profile was studied. Preliminary phytochemical analysis of the crude fruit extract of *Acacia nilotica* revealed the presence of several phytoconstituents, including alkaloids, flavonoids, glycosides, saponins, and triterpenes. Conferring to the stated quantitative analysis, the fruit extract of *Acacia nilotica*significantly showed the presence of alkaloids in greater amounts.

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REFERENCES

1. De Pasquale. (1984), “Pharmacology, The oldest modern Science”,JournalofEthnopharmacology,11(1),1-16.
2. Dhanukar SA, Kulkarni RA and Rege NN, (2000). Pharmacology of medicinal plants and natural products**.** IndianJournalofPharmacology**32**,S81-S118.
3. Gregory SK, (1999). Nutritional and botanical interventions to assist the adaptation to stress. Altern Med Rev, **4**,249-265.
4. Patwardhan B (2000).Ayurveda: The Designer Medicine: A Review ofEthnopharmacologyandBioprospectingResearch,IndianDrugs,**37(5),**213–27.
5. Wang C, Schuller Levis GB, Lee EB, Levis WR, Lee DW, Kim BS, Park SY, Park E.PlatycodinDandD3isolatedfrom therootofPlatycodongrandiflorummodulate the production of nitric oxide and secretion of TNF-[alpha] in activated RAW 264.7 cells. Int JImmunopharmacol.2004;4:1039–1049.
6. "Vachellianilotica". Germplasm Resources Information Network (GRIN). Agricultural Res Service (ARS), United S Dept. of Agriculture (USDA). Retrieved 30 Jun 2017.
7. Khandelwal KR. Practical book of pharmacognosy. Pune, India: Niraliprakashan. 2005.
8. The Ayurvedic Pharmacopoeia Of India. 2016; Part-I,Volume-IX,1 stEdn .114.
9. Ajith TA, Janardhanan KK. Indian medicinl mushrooms as a source of antioxidant and antitumor agents JC linBiochemNutr. 2007; 40:157–162.
10. Zander DS, Popper H, Jagirdar J, Haque AK, Cagle PT, Barrios R, editors. Molecular pathology of lung diseases. Springer-Verlag New York; 2008.
11. Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several culinary and medicinal herbs are important sources of dietary antioxidants. J Nutr. 2003;133:1286–1290.

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