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Phytochemical Analysis And Proximate Composition Of Methanol Extract Of Pentadesma Butyracea Leaves

Oyetayo Abayomi Mathew[1], Bayode Mayowa[2]
Department Of Chemical Sciences, Olusegun Agagu University Of Science And Technology, Nigeria
Department Of Plant Biology And Biotechnology, University Of Benin, Nigeria

***Abstract*:**
Background: *Plant-derived Phytochemicals have attracted increasing attention owing to their broad spectrum of application. Phytochemical analysis is very important in identifying new sources of therapeutically and industrially important compounds like Alkaloids, Flavanoids, Phenolic Compounds, Saponins, Steroids, Tannins, Terpenoids, etc. This study investigated the phytochemical constituents and proximate composition of leaf extract of Pentadesma butyracea using methanol as extraction solvent.*
Material and Methods: *The fresh leaves of Pentadesma butyracea were collected from the botanical garden at the University of Benin, in Nigeria. The leaves were washed under running tap water, air-dried, and then homogenized to a fine powder and stored in tight air bottles. The fine powder was used for extraction with methanol. The methanol extracts of the Pentadesma butyracea leaves were used for the phytochemical analysis. The phytochemical tests and proximate composition were conducted using standard procedure.*
Result: *The Phytochemical analysis revealed the presence of glycosides, alkaloids, saponins, flavonoids, phenols, and terpenoids, while tannins and steroids were absent. Proximate analysis indicated high levels of ash (39.51%) and fat (35.05%), with moderate moisture content (11.61%) and low crude fiber (5.68%), nitrogen (1.12%), and crude protein (7.03%). These findings support the traditional use of Pentadesma butyracea in herbal medicine and suggest its potential as a source of bioactive compounds.*
Conclusion: *This study confirms the presence of important phytochemicals and significant levels of Fat and Ash in Pentadesma butyracea leaves.*
Key Words: *Pentadesma butyracea, phytochemicals, proximate analysis, medicinal plants, cold maceration, traditional medicine.*

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I. Introduction

Medicinal plants are the richest bioresource of drugs in the traditional system of medicine and are also responsible for the different colors, flavors, and smells of the plant. They also function as medicaments. These medicinal values of plants lie in some chemically active substances that produce a definite physiological action on the human body (Karunyadevi *et al*., 2009)[1]. There are thousands of species of medicinal plants used globally for the cure of different infections. These plants are used as antimicrobial agents and several works have been carried out by scientists to find out its scientific basis (Omotayo, 1998)[2]. Since ancient times, people have been exploring nature particularly plants in search of new drugs. This has resulted in the use of a large number of medicinal plants with curative properties to treat various diseases.

Pentadesma butyracea is a dense forest species with a distribution area reaching from Sierra Leone to Cameroun; it is a tree, with a height of about 20m, which was found in the north of Benin in forest galleries and along the waterway.

P. butyracea butter was used in traditional medicine as massage oil, in skin and hair care, and the manufacture of soap for its softening, lubricating, and healing qualities. It was used to retard the aging of the skin in patented cosmetic preparation.

Despite its widespread ethnomedicinal use, limited scientific information exists on the phytochemical constituents and nutritional profile. This study aims to fill that gap by conducting a phytochemical analysis and proximate composition of methanol extract from the leaf of *P. butyracea*.

II. Material And Methods

Plant Collection and Preparation

Fresh leaves of *Pentadesma butyracea* were collected from the botanical garden of the University of Benin, Nigeria. The plant was authenticated by the university's taxonomy unit. The leaves were washed, air-dried, powdered, and stored in airtight container.

Extraction

Cold maceration was used in the extraction. Three kilograms (3kg) of dried powder was soaked in 1.5 L of methanol for 72 hours with periodic agitation. The extract was filtered and concentrated using a rotary evaporator at 65°C.

Apparatus

Test tube, beaker, rotary evaporator, dish, burner, dropper, crucible, weighing balance, filter paper, water bath, measuring cylinder, muslin cloth, muffle furnace, oven, and desiccators.

Reagents

Methanol, Distilled water, H_2SO_4, NaOH, HCl, Mayer's reagent. All reagents were of analytical grade.

Phytochemical Analysis Procedure

Phytochemical analysis was conducted using standard procedures (Trease and Evans (1989)[3], Sofowora (1993)[4], Ushie *et al.,*2016)[5] and Santhi *et al.,*2016)[6]. Tests included:

Tests for Alkaloids

Extract was dissolved in dilute hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

Mayer's Test

Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Tests for Flavonoids

Alkaline Test

Extract was treated with few drops of dilute sodium hydroxide solution. Formation of a yellow color, which turned colorless on addition of dilute acid, indicated the presence of flavonoids.

Test for Glycosides

Glycoside Test

0.5 mL of the extract was dissolved in 1 mL of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

Phenol Tests

Ferric Chloride Test

10 mL of the extract was treated with few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

Test for Saponins

Foam Test

1 mL of the extract was shaken with 5 mL of distilled water. Formation of stable persistent foam indicated the presence of saponins.

Test for Tannins

1 mL of the extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. Formation of a dark green color indicated the presence of tannins.

Test for Terpenoids

Salkowski s Test

0.5 mL of the extract was mixed with 2 mL of chloroform, and 3mL of concentrated H_2SO_4 was carefully added to form a layer. An appearance of a reddish brown color interface indicated the presence of terpenoids.

Tests for Steroids

Liebermann-Burchard Test

2 mL of acetic anhydride was added to 0.5 mL of the extract in a test tube, followed by the addition of 2 mL of sulfuric acid. A color change from violet to blue or green indicated the presence of steroids.

Proximate Analysis Procedure

Moisture, ash, crude fat, crude fiber, and nitrogen content were determined using AOAC (2005) methods. Protein content was calculated by multiplying the nitrogen content by a conversion factor of 6.25.

Determination of Moisture Content

An empty dish and lid were dried in the oven at 105ºC for 30minutes and transferred to the desiccator to cool. The empty dish and lid were weighed. 3g of sample was weighed to the dish and it was spread to the uniformity. The dish with the sample was placed in the oven. It was dried for 3-5 hrs at 105ºC. After drying with a constant weight, the dish was transferred with a partially covered lid to the desiccator to cool.

The dish and its dried sample were reweighed.
Calculation

$$\text{Moisture (\%)} = \frac{(W1 - W2) \times 100}{W1}$$

W1 = weight (g) of sample before drying
W2 = weight (g) of sample after drying.

Determination of Protein Content

Sample (0.5 – 1.0 g) was placed in a digestion flask. 5g of Kjeldahl catalyst and 200ml of conc. H_2SO_4 was added. A tube containing the above chemical except for the sample as blank was prepared. The flask was placed in an inclined position and heated gently until frothing ceased and it was boiled briskly until the solution clears. It was cooled and 60ml of distilled water was cautiously added.

Immediately the flask was connected to the digestion bulb on the condenser and with the tip of the condenser immersed in a standard acid and 5-7 drops of mix indicator in the receiver. Rotated flask to mix content thoroughly; then heated until all NH_3 was distilled. The receiver was removed, the tip of the condenser was washed and titrated excess standard acid distilled with standard NaOH solution.

Calculation

$$\text{Protein (\%)} = \frac{(A\text{-}B) \times N \times 14.007 \times 6.25}{W}$$

A = volume (ml) of 0.2N HCl used sample titration
B = volume (ml) of 0.2N HCl used in blank titration
N = normality of HCl
W = weight (g) of sample
14.007 = atomic weight of nitrogen
6.25 = the protein conversation factor for fish and its by-products

Determination of Ash Content

The crucible and lid were placed in the furnace at 550ºC overnight to ensure that impurities on the surface of the crucible are burned off and the crucible was cooled in the desiccator (30 min). The crucible and lid were weighed to 3 decimal places. About 5g of the sample was weighed into the crucible. Heated over low Bunsen flame with the lid half covered. When fumes were no longer produced, the crucible and lid were placed in the furnace and heated at 550ºC overnight. During heating, the lid was not covered. After completing the heating, to prevent loss of fluffy ash the lid was cooled down in the desiccators. The ash with crucible and lid was weighed when the sample turned gray.

Calculation

$$\text{Ash (\%)} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}}$$

Determination of Fat Content

The bottle and lid were placed in the incubator at 105ºC overnight to ensure that the weight of the bottle is stable. About 3-5 g of sample was weighed to filter paper and was wrapped. The sample was put into extraction thimble and transferred into soxhlet. 250ml of petroleum ether was filled into the bottle and placed on the heating mantle. Soxhlet apparatus was connected and the water was turned on to cool them and then the heating mantle was switched on. The sample was heated for about 6-8hrs. The solvent was evaporated by using the vacuum condenser. The bottle was incubated at 80-90ºC until the solvent was completely evaporated and the bottle was completely dried.

After drying, the bottle with a partially covered lid was transferred to the desiccator to cool. The bottle and its dried content were reweighed.

Calculation

$$\text{Fat (\%)} = \frac{\text{Weight of fat} \times 100}{\text{Weight of sample}}$$

Nitrogen Determination

Digestion

0.5 g of the sample to the nearest 0.001g was weighed and transferred to the Kjeldahl flask of the digestion apparatus. 15g of a mix of potassium sulphate and catalyst (copper (II) sulphate pentahydrate), 12 ml of sulphuric acid was added and mixed.

The Kjeldahl flask was heated moderately at first, swirling from time to time until the mass had carbonized and the foam had disappeared; then heated more intensively until the liquid was boiling steadily. When the solution becomes clear and light green it was boiled for another two hours, then left to be cooled. It was distilled into boric acid and titrated.

Titration

The content of the collecting flask with the sulphuric acid standard volumetric solution using a burette was titrated and the amount of titrant used was recorded.

When colorimetric end-point detection was applied, the end-point was reached when the color of the solution changes from green to red. The burette reading was estimated to be the nearest 0, 01 ml.

Blank test

To confirm that the reagents are free from nitrogen, a blank test was carried out (digestion, distillation, and titration) using only reagents (no sample is added).

Calculation of results

Nitrogen content in the sample:

$$\text{MN (g/kg)} = \frac{(Va - Vb) \times CHCl \times MN \times 100}{Mvz}$$

Va = volume of standard HCl solution when titrating sample (l)
Vb = volume of standard HCl solution when titrating blank (l)
CHCl= concentration of HCl (mol/l)
MN = nitrogen molar mass (g/mol)
Mvz = weight of sample (g)

Crude Fibre Determination

3g of the prepared sample to the nearest 10 mg was weighed and placed in a glass beaker, 200ml of sulphuric acid (1.25 %) was added and the baker was covered with the condenser. The liquid was brought to a boil within 5 ± 2 minutes and it was boiled vigorously for exactly 30 minutes. Opened the tap to the discharge pipe and, under vacuum, filter the suspension of sample in 1.25 % sulphuric acid through the fritted glass crucible (containing cca 1.5 cm of sea sand) and washed the residue with three consecutive 30 ml portions of boiling water, ensuring that the residue was filtered dry after each washing. Put the sample from fritted glass crucible back to the glass beaker (with the minimal proportion of the sea sand) and poured 200 ml of potassium hydroxide solution (1.25 %) into the beaker and covered the baker with the condenser. Bringing the liquid to a boiling point within 5 ± 2 minutes and boiled vigorously for exactly 30 minutes. Filtered and repeated the washing procedure used for the sulphuric acid step. After the final washing and liquid aspiration, the residue in the crucible was washed with acetone and water ensuring that the residue was filtered dry after each washing. The crucible was dried to constant weight in the oven at 103^{o}C. After drying, it was cooled in the desiccator and weighed rapidly. The crucible was placed in a muffle furnace and ash at 520^{o}C to 550^{o}C overnight. After heating, it was cooled first in the furnace and then in the desiccator before weighing.

Calculation

$$\text{CF(g/kg)} = \frac{Ma - Mb \times 100}{Mvz}$$

Ma = weight of crucible and fibre after drying (g)

Mb = weight of crucible and fibre after ashing (g)
Mvz = weight of the sample (g)

III. Result

Table no 1: Phytochemical analysis of methanol extract of *Pentadesma butyracea* leaves

Name of Test	Test Applied/Reagent Used	Methanol Extract
Alkanoids	Mayer's Reagent	+
Flavonoids	Alkaline Reagent Test	+
Glycosides	Extract + H_2O+ NaOH	+
Phenol	Ferric Chloride Test	+
Saponins	Foam Test	+
Steriods	Extract + Acetic Anhydride + H_2so_4	-
Tannins	Ferric Chloride Test	-
Terpenoids	Salkowski's Test	+

KEY + = *Present* - = *Absent*

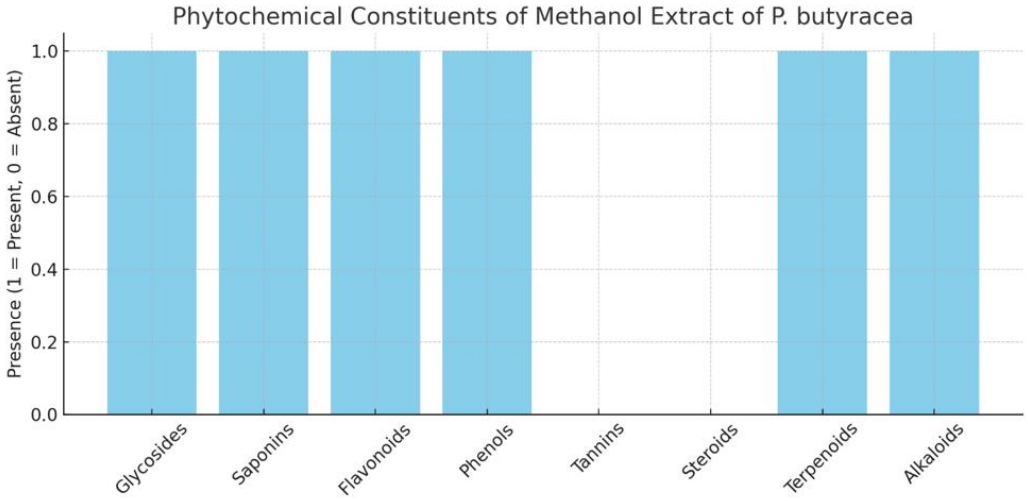
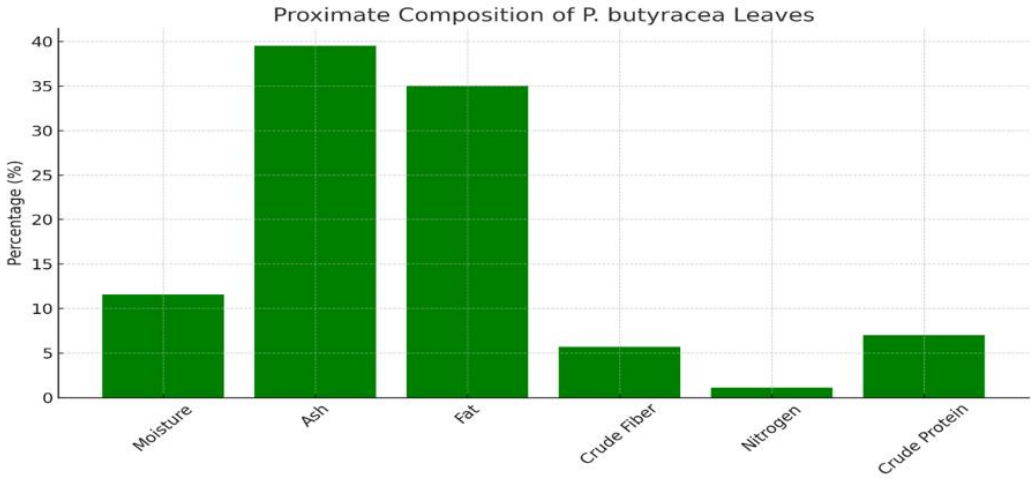


Table no 2: Proximate Composition of *Pentadesma butyracea* leaves (dry sample)

Component	*Pentadesma butyracea*
Moisture%	11.61
Ash%	39.51
Fat%	35.05
Crude Fiber%	5.68
Nitrogen%	1.12
Crude Protein%	7.03



IV. Discussion

The characterization tests showed that glycosides, alkaloids, saponins, flavonoids, phenols, and terpenoids were the groups of compounds present in the methanol extract of *P. butyracea* leaves in the Table 1 above, whereas tannins and steroids were absent. The presence of bioactive constituents has contributed to its medicinal value as well as physiological activity. Flavonoid which was detected has a wide range of pharmacological effects including antioxidant, anti-inflammation, antiplatelet, anti-allergic, cytotoxicity, and reduces the risk for heart disease.

The medical value of the plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of the plant are alkaloids, tannins, flavonoids, and phenolic compounds. It has been estimated that about 25% of all prescribed medicines today are substances derived from plants (Puri, 1999)[7]. Plants have estimated more than 7000 different compounds in use today antibiotics, decongestants, and analysis compounds. The World Health Organization (WHO) estimates that up 80% of the world's people rely on plants for their primary health care Since western pharmaceuticals are other expensive inaccessible or unsuitable and are always accompanied by various side effects (Puri.,1999)[8].

The results of the proximate analysis of methanol extract of *P. butyracea* from the Table 2 above shows Ash content has the highest value while Nitrogen has the least. The Moisture Content accounts for its shelf life after harvest. The moderate Moisture Content provides for an activity of water-soluble enzymes and coenzymes needed for the metabolic activities of the plant. The crude fibre content obtained from this study suggests that P. b*utyracea* is not a potential source of dietary fibre (roughages) because the value is small. A high level of fibre is known as an anti-tumorigenic and hypocholesterolemic agent. The high content of fat indicates the presence of high cholesterol which is very dangerous to human health. The low presence of protein implies it is not a good supplement. Ash content represents the incombustible component remaining after a sample of the furnace oil is completely burned. A high level of ash indicates much contamination of the plant. There is low nitrogen content in the plant which tends to affect the protein content as it is the building block of protein.

V. Conclusion

This study confirms the presence of important phytochemicals and significant levels of Fat and Ash in *P. butyracea* leaves. These findings provide scientific validation for its ethnopharmacological use and highlight its potential for further drug discovery and nutritional studies.

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