International Journal of Engineering Inventions
e-ISSN: 2278-7461, p-ISSN: 2319-6491
Volume 15, Issue 1 [January 2026] PP: 06-10

Proximate and Phytochemical Screening of Indigofera conferta Linn.

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Abstract
This study investigated the proximate and phytochemical composition of Indigofera conferta. leaves to evaluate their potential nutritional and medicinal properties. Standard analytical techniques were employed to determine the presence and concentration of bioactive compounds such as alkaloids, tannins, saponins, flavonoids, steroids, and glycosides, as well as proximate components including ash, moisture, lipid, crude protein, fiber, and carbohydrate. The results showed that carbohydrates (72.59 ± 0.11 g%) and lipids (21.18 ± 0.01 g%) were the most abundant nutrients, indicating significant energy potential. Phytochemical analysis revealed the presence of flavonoids (++), saponins (+), tannins (++), glycosides (++), and steroids (++). These findings suggest that Indigofera conferta is a rich source of essential phytochemicals and nutrients, supporting its traditional use in herbal medicine and indicating potential for pharmaceutical and nutraceutical applications.
Keyword: *Indigofera conferta, Phytochemicals, Proximate, Medicinal Properties, Nutrients*

Date of Submission: 25-12-2025 Date of acceptance: 06-01-2026

I. Introduction

Medicinal plants are vital in traditional and modern healthcare systems because of their therapeutic compounds that serve as precursors for drug development (Sofowora, 1993). The genus Indigofera, belonging to the Fabaceae family, comprises species widely distributed across tropical regions and known for various pharmacological properties including antimicrobial, anti-inflammatory, and antioxidant activities (Aiyegoro & Okoh, 2010). Indigofera conferta Linn. is traditionally employed in the management of fever, inflammation, and infections. However, limited scientific data exist regarding its phytochemical and nutritional profiles. Phytochemicals such as alkaloids, flavonoids, saponins, tannins, and glycosides are secondary metabolites that play essential roles in plant defense and human health, contributing to antioxidant, antimicrobial, and anti-inflammatory effects (Trease & Evans, 1978). Furthermore, proximate analysis provides insight into the nutritional composition of plants, which is crucial in assessing their dietary significance and potential industrial applications (Pearson, 1976). Hence, this study aims to evaluate the proximate and phytochemical constituents of Indigofera conferta leaves to support its ethnopharmacological use and provide a scientific basis for its nutritional and medicinal value.

II. Materials and Method

Sample Collection

Fresh leaves of *Indigofera conferta* were collected from Zaria Local Government Area, Kaduna State, Nigeria. The leaves were thoroughly washed, air-dried at ambient temperature for seven days, and pulverized into a fine powder using a mechanical grinder. The powdered samples were stored in airtight containers for subsequent analysis.

Proximate Composition Analysis

Proximate analysis was determined using methods of the Association of Official Analytical Chemists (AOAC (2003). Determining the percentage moisture content involve loss of weight due to drying the samples in an oven at (50-60^{o}C) for 5 hours, by relating the weight of an empty bottle at initial stage, weight of empty bottle plus the sample.

The weight of empty bottle plus the sample minus initial weight of empty bottle all over weight of empty bottle plus sample (2g) times one hundred after drying. The ash content was determined by heating the sample above the boiling point of water at a temperature of 550-570oC in order to burnt all the organic matter until the appearance of white ash. The content was obtained by relating the weight of crucible plus the sample weight (2g) minus weight of an empty crucible all over the weight of crucible after burning the organic matter

minus initial weight of the crucible time's one hundred. The crude fiber and lipid content were determined by soxhlet extraction. In a case two gram (2g) of the samples were placed in an empty thimble and then extracted at lower temperature (40-60oC) using petroleum ether, 25% sulphuric acid and sodium hydroxide respectively. The two parameters weight were obtained by relating the weight of the thimble plus the initial weight of the thimble all over the weight of the thimble, sample and cotton wool minus the weight of the thimble after extraction times one hundred. Crude protein content was also measured by subjecting sample (2g) to digestion using concentrated sulphuric acid and Kjedahl catalyst tablet followed by distillation in 10% boric acid, ammonium hydroxide and which is finally back titrating the aliquot using 0.1N hydrochloric acid. The content of carbohydrate was estimated by subtracting out the sum of ash, crude fiber, crude protein and crude fat from one hundred.

Phytochemical Screening

Preliminary phytochemical screening was performed to identify the presence of major bioactive compounds using standard methods with minor modifications (Shaikh & Patil, 2020)

Quantitative Determination of Phytochemicals

Determination of Alkaloid

Alkaloids was determined using method as reported by (Trease and Evans, 1978).

Principle

As with most alkaloids, salts are soluble in water, while the alkaloids free bases are soluble in organic solvent. This fact is made use of in extraction of the free alkaloids with organic solvent.

Procedure

Five grams of powdered plant sample was extracted with 100 ml of methanol water (1:1 v:v) mixture and solvent evaporated. The resultant residue would mix with 20 ml of 0.0025M H_2SO_4 and partitioned with ether to removed unwanted materials. The aqueous fraction was basified with strong NH_3 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 would be concentrated to dryness. The alkaloid would be weighed and the percentage was calculated with reference to initial weight of powder.

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

Where A = Absorbance of the colour at 495 nm

Determination of Flavonoids

The precipitation method was used (Bohm and Kocipai, 1994).

Excess iodine was determined by titration, rendering acidic with sodium thiosulphate standard solution

Procedure

a weighed sample five (5) gram of water lily was hydrolyzed by boiling in 100 mls of hydrochloric acid solution for about 35 minutes. The hydrolysate was filtered to recover the extract (filtrate), the filtrate was treated with ethyl acetate drop wise twice until in the precipitated flavonoid would be recovered by filtration using a weighed filter paper after drying in the oven 100^C for 30 minutes, it was cool in a desiccator and weighed. The difference in weighed gave the weighed of flavonoid which was expressed as a percentage of the weighed of sample analyzed.

$$\% \text{ flavonoid} = \frac{W2-W1}{5g} \times 100$$

Where:

$5g = weig$eight sample

W_1 =weight of filter paper

W_2 =weight of filter paper $+sample$sample

Determination of Tannins

Tannins was determined by the method of (Trease and Evans, 1978).

Principle

The method is based on quantitative consumption of tannins and pseudo tannins to iodine in alkaline medium, a character which is attributed to their phenolic nature. True tannins, in contrast to pseudo tannins can be removed from the extract by precipitation with gelatin. This can permit the determination of each group of constituents alone.

Procedure
Powdered sample (0.1) was put into a 100 cm3 conical flask and 50 cm' volumetric flask. The residue would be washed several times and the combined solution made up with distilled water to 0, 1, 2, 3, 4 and 5 cm3 of the standard tannic acid and 10 cm^3 of the sample solution in a 50 cm3 volumetric flask, 2.5 cm3 Folin-Denis reagent and 10 cm^3 of Na_2CO_3 solution was added and made to volume with distilled water. The flask would be allowed to stand for 20 minutes after which optical density would be measure at 760 nm. The calibration curve was plotted from which the concentration of tannic acid in the sample was extrapolated.

Determination of Saponins
Saponins was determine using method of (El-Olemyl et al., 1994)
Principle
Saponins are soluble in water or boiling dilute alcohol and are precipitated on the addition of acetone.
Procedure
From powdered plant extract, five (5) grams was place in a 250 ml flask containing 30ml of 50% alcohol. The mixture would be boiled under reflux for 30minute and would immediately filter while hot through a coarse filter paper.
Two grams (2g) of charcoal was added; the content was boiled and filtered while hot. The extract was cool (some saponins may be separated) and an equal volume of acetone was added to complete the precipitation of saponins. The separated saponins was collected by decantation and dissolved in the least amount of boiling 95% alcohol and filtered while hot to remove any insoluble matter.
The filtrate was allowed to cool at room temperature thereby resulting in the precipitation of saponins. The separated saponins would be collected by decantation and suspended in about 2 ml of alcohol and filtered. The filter paper was immediately transferred to a desiccator containing anhydrous calcium chloride and the saponins would be left to dry. The was weighed with reference of extract used.

$$\%\text{saponin} = \frac{W_2 - W_1}{5g} \times 100$$

Where
$5g = weig$eight sample
W_1 =weight of filter paper
W_2 =weight of filter paper $+ sample$sample

Determination of Glycosides
Glycosides were determine using spectrometric method (El-Olemyl et al,1994)

Procedure
One gram (1g) of the extract was extracted in 10ml of 70% alcohol and mixture was filtered. From the filtrate, eight (8 ml) of the mixture would be added to 8ml of 12.5% lead acetate (to precipitate resins, tannin and pigments). The mixture was shake well, completed to volume (100 ml) with distilled water and filtered. The filtrate (50 ml) was pipetted into another 100ml volumetric flask and 8ml of 4.7% disodium hydrogen phosphate (Na2HPO4) solution (to precipitate excess lead) would be added. The mixture was made up to the volume with distilled water and mixed. The mixture was filtered twice through a Whatman No. filter paper. Baljet reagent (10 ml) would be added to 10 ml of purified filtrate. A blank sample of 10 ml of distilled water was also added to 10 ml Baljet reagent. The two would be allowed to stand for one hour (time maximum for colour development). The intensity of the colour wase read at 495 nm using spectrophotometer against a blank (20 ml distilled water). The colour was stable for several hours.
The percentage of total glycosides was calculated digitoxins by simply using the E^{1cm} 1% of given digitoxins (= 170)
The percentage $of glycosides = \frac{A \times 100}{17} \times g\%$
Where A = Absorbance of the colour at 495 nm

III. Results

Table 1: Proximate Composition of *Indigofera conferta* Leaves Extract

Parameters	Concentration (g%)
Ash Content	2.71±0.0173
Moisture Content	1.62±0.010
Lipid	21.18±0.01
Fibre	1.36±0.010
Crude Protein	1.89±0.121

Carbohydrate	72.59±0.110

The values are expressed as Mean± Standard deviation

Table 2: Phytochemical Screening of *Indigofera conferta* Leaves Extract

Parameters	Results
Flavanoids	++
Saponins	+
Tannins	++
Glycosides	++
Saponin Glycosides	++
Cardiac Glycosides	++
Balsam	++
Anthraquinone	+

Table 3: Phytochemical Composition of *Indigofera conferta* Leaves Extract

Parameters	**Concentration**
Flavanoids (g%)	8.89±0.0316
Alkaloids (g%)	1.19±0.0212
Saponin (g%)	0.336 ±0.0206
Tannins (mg/dl)	27.59±0.394
Steroids (Mmol/L)	40.55±0.135

The values are expressed as Mean± Standard deviation

IV. Discussion

The fat content concentration is within the range as reported by Borges et al. (2008) and Imam et al., (2013), which facilitate the fat-soluble vitamins absorption., thus; consumption of the plant in significant quantities would therefore not constitute a risk factor to some pathogenic stages such as diabetic mellitus, obesity and coronary heart diseases due the presence of fibre content in the plant which plays a significant role in a health problems associated with heart disorder, bowel cancer and appendicitis (Pyke, 1979). The nutrient information and antioxidant properties would enhance efforts to promote wide use of plants because of their nutritional benefits and medicinal properties (Wasagu et al., 2013).

The qualitative phytochemical screening revealed the presence of key bioactive compounds such as flavonoids, saponins, tannins, glycosides, and steroids, suggesting that Indigofera conferta leaves possess significant pharmacological potential. Quantitative analysis also indicated high levels of flavonoids (8.89 ± 0.0316 g%) and steroids (40.55 ± 0.135 mmol/L), which contribute to antioxidant and anti-inflammatory properties, it is also reported by Lata and dubey (2010), the medicinal benefits of a plant might be attributed to the quality of the bioactive constituents. As such, presence of these bioactive constituents such as terpenoids, anthraquinones, steroids, saponins, tannins, alkaloids, flavonoids and phenolics in N. lotus stem extract might be responsible for its potential use as a drug against pathogenic microbes. According to Omulokoli *et al*., (1997), Newman et al., (2000) and Zakaryan et al., (2017) alkaloid, tannins, saponins and flavonoids possesses antimicrobial activities. However, Saxena et al., (2013) reported that terpenoids and phenolics possess antimicrobial activity.

V. Conclusion

The findings study reveals that *Indigofera conferta* leaves are a valuable source of bioactive compounds and nutrients. The presence of phytochemicals supports its ethnomedicinal applications and highlights its potential for use in pharmaceutical formulations. The high carbohydrate and lipid contents also suggest that the plant may serve as a supplementary food source. Further research is recommended to isolate and characterize the active constituents responsible for the observed properties.

Acknowledgement

This work was financially supported by Tertiary Education Trust Fund of Nigeria (TETFUND) under institutional based reserach with batch No.: 2023/Vol 11 BATCH 9.

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