Preliminary phytochemical screening and proximate analysis of *Indigofera tinctoria* L. (Uhe) Pod.

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**Abstract**

The present study was conducted to evaluate the phytochemicals and proximate analysis of *Indigofera tinctoria* pod. The proximate analysis revealed the presence of moisture (68.68%), crude fiber (14.83%), carbohydrate (12.17%), protein (2.63%), ash content (1.01%) and lipid content (0.68%). In the phytochemical analysis using ethanolic extracts (Hot and Cold) and aqueous extracts, the following bioactive chemicals were observed; Alkaloids, and Flavonoids were present, while Tannins, Saponins and Cardiac glycosides which are present in most medicinal plants were absent. The results of this study showed that *Indigofera tinctoria* pod contained reasonable amount of nutrients and few phytochemical constituents which suggests the application of the pod as supplementary sources of antimicrobial agent and essential nutrient to man and livestock.

**Key-words:** Preliminary, Phytochemical, Proximate Analysis, *Indigofera Tinctoria* L.
also providing energy to the body, which helps in the body mechanisms.

Fig. 1. Image of Indigofera tinctoria plant.

*Indigofera tinctoria* L. (also called true indigo, known as uli or uri by Igbo’s, elu or alu by Yoruba’s and Baba by Hausa’s), a native of Malaysian Archipelago. It is a deciduous spreading tropical shrub or sub shrub, that may be annual, biennial, or perennial, depending on climatic conditions and belongs to the family of pea known as fabacea that typically grows to 2-3 inch tall (Fig. 1). As suggested by the common name, this shrub was the original source of the blue dye known as indigo. It has been naturalized to tropical and temperate Asia, as well as parts of Africa, it has been in cultivation worldwide for many centuries. Today most dye is synthetic, but natural dye from *Indigofera tinctoria* is still available, marketed as natural coloring (Randall, 2012). The plant is widely grown as a soil-improving groundcover.

Fig. 2. Image of Indigofera tinctoria pod (fresh and dry).

*Indigofera* tinctoria pod are oval shaped and elongated, 4-angled or flattened and often curved with many seeds (Salehi-Surmaghi et al., 1992). Dye is obtained from the processing of the plant pod using different extraction techniques such as soxhlet (i.e. hot solvent-ethanol) method, cold Ethanol Extraction method and Aqueous method. By using any desire solvent either organic or water. *I. tinctoria* pod has been used traditionally in drawing different designs on the body and also in treating measles. While the leaves have many traditional and commercial uses, the most common being as a natural dye for textile industry for achieving numerous decoration techniques, manure for promoting hair growth, in folk medicines for the treatment of numerous diseases such as, epilepsy, nervous disorders, asthma, bronchitis, fever, complaints of stomach, liver, kidney and spleen, as a rabies prophylactic, and as an ointment for skin diseases, wounds, sores, ulcers and hemorrhoids, antidote for snake bites and to treat insect and scorpion stings (Salehi-Surmaghi et al., 1992). The family *Indigofera* has been known to be dye stain producers, as well as being a herbal remedy to several diseases. Not much has being known about its nutritional compositions. Thus, the knowledge of this study will reveal its efficacy as a food supplement and its therapeutic properties. Hence the study was undertaken to evaluate the phytochemical composition and proximate profile of *I. tinctoria* pods.

**Material and methods**

This study was carried out at Umuduru Ekwe, Isu local government Imo state, with the latitude 5° 71’N and 5° 15’E. It lies in the Tropical rainforest region of south Eastern Nigeria. They are two distinct season in the Area namely: the dry season (November – March) and the rainy season (April – October). Its average annual temperature is 31°C, Humidity (%) 66 and annual rainfall of 1806mm. The forest vegetation here is characterized by variety of plants including plants with medicinal value like *I. tinctoria*. This plant grows and survive under the describe climate conditions.

**Sample Collection and Identification**

Fresh pod and leaves of *Indigofera tinctoria* was collected from Umuduru Ekwe Isu local government Area of Imo state. And were identified by a Botanist, Dr. C. M. Duru of Biology Department, Federal University of Technology, Owerri, Imo State, Nigeria.

**Sample Preparation/Processing of Sample of Extracts**
The freshly collected pods of *Indigofera tinctoria* were shade dry for 1 1/2 month, and finally oven dry at 40\(^\circ\)C in Biology laboratory. The dried pod was weighed with an electronic weighing balance, so as to obtain a constant weight before pulverizing. Then the pod was pulverized to a very fine powder using mortar and pestle. The homogenized powder was stored in an airtight container for further use.

### Extraction

**Squeeze / Fresh extract:** The freshly collected pod was pulverized using clean mortar and pestle, then strained into a container and a drop of formalin was added to the fresh extract, the container was corked very well before storage.

**Dry extract:** These was achieved by the following methods;

#### Soxhlet Method (Hot Ethanol Extraction)

**Fig. 3.** Image of set-up of Soxhlet Extraction process.

**Procedures for soxhlet extraction:** 20 g of finely pulverized pod of *Indigofera tinctoria* was weighed using an electric weighing balance, and then poured into a thimble, also, 100 ml of absolute ethanol was measured using calibrated measuring cylinder and then poured into a conical flask of 500 ml. The apparatus for soxhlet was set-up as shown above and then allow to run for some hours, so as to obtain the extract from the sample, the ethanol was evaporated from the extract using water bath at 40\(^\circ\)C and finally stored in a sample collection container.

#### Cold Ethanol Extraction: 20 g of finely pulverized pod of *Indigofera tinctoria* weighing, poured into an empty Bama bottle, 100 ml of absolute ethanol was also added to the contents in the bama bottle after measuring, and then allowed to stand for 24 hours without disturbance. After 24 hours, I then decant into another clean container so as to obtain the extract. The extract was heat in a water bath at 40\(^\circ\)C so as to evaporate the solvent and then stored in a sample collection container until when needed.

#### Aqueous method: 20 g of finely pulverized pod of *Indigofera tinctoria* weighed, poured into an empty Bama bottle, 100 ml of distilled water was also added to the contents in the bama bottle after measuring, and then allowed to stand for 24 hours without disturbance. After 24 hours, I then decant into another clean container so as to obtain the extract. The extract was heat in a water bath at 40\(^\circ\)C so as to evaporate the solvent and then stored in a sample collection container until when needed.

### Preliminary Qualitative Phytochemical Screening

Preliminary qualitative phytochemical screening was done with method of Sanjappa (1995) and Daffre (2008). The pod of *I. tinctoria* extracts (i.e. soxhlet extract using ethanol, cold ethanolic, and aqueous extract) were all analyzed for the presence/absence of saponins, flavonoids, alkaloids, tannins, and cardiac glycosides.

**Saponins:** The presence of saponins was determined by Frothing test. 2ml of the pod extract was vigorously shaken with 2ml distilled water and was allowed to stand for 10 minutes and classified for saponin content as follows: No froth indicate absence of saponins and stable froth more than 1.5 cm indicated the presence of saponins (Effiong, 2009).

**Flavonoids:** 2ml of the extract was dissolved in dilute NaOH. A yellow solution that turns colourless on addition of con. HCl indicates the presence of flavonoids.

**Alkaloids:** 2ml of the pod extract was acidified with 1% HCl (i.e. 1ml of con. HCl in 99ml of distilled water) and was treated with few drops of Wagner reagents in a test tube. A reddish brown precipitates indicates the presence of alkaloids (Firn, 2010).

**Tannins:** 2ml of the extract was treated with about 10mls of distilled water and then few drops of 1% ferric chloride (FeCl\(_3\)) solution were added. The
occurrence of blue-black, green or blue greenish precipitate indicates the presence of tannins (Harborne, 2010).

**Cardiac glycosides:** Keller-kiliani test was performed to assess the presence of cardiac glycosides. 2ml of the extract was treated 2ml of glacial acetic acid containing 1 drop of ferric chloride solution. To this solution a few drops of concentrated sulphuric acid (H\_2 SO\_4) was added. A brown ring formation at the interphase indicates the presence of deoxy sugar characteristics of Cardiac glycosides (Kapoor et al., 1969).

**Proximate Analysis**

The grounded samples (fresh) were subjected to proximate analysis for the determination of the major nutritional component of the plant pod. This method partitioned nutrients in the plant pod into six components: Moisture (dry matter), ash, crude protein, crude fat (ether extract), crude fibre and carbohydrates as described by (Kumar et al., 2009).

**Moisture determination**

The method used is the indirect distillation method employing drying oven. Porcelain\ or silica crucible\ dishes are used, weigh 5g-10g of the sample into a pre-weighed, pre-dried and cooled crucible or dish and dry in the oven at 70\^-80\^\circ\mathrm{C} for 2hrs and at 100-110°\mathrm{C} [usually 105\^\circ\mathrm{C}] until constant weight is obtained .cool the crucible and its content of dried sample in a dessicator before weighing.

\[
\% \text{ moisture} = \frac{W_2 - W_1}{W_1} \times 100
\]

Where  
\(W_1 = \) initial weight of empty crucible  
\(W_2 = \) weight of crucible and the sample before drying  
\(W_3 = \) final weight of crucible and sample after drying.

**Determination of Ash Content**

Crucible are thoroughly washed, cleaned and placed in a hot air- circulation oven for 24hrs and cooled to room temperature in a desiccator. The empty crucible are then transferred to the muffle furnace to burn out all the organic matter and also to stabilize the hotness of the crucible at room temperature range of 550.6, before cooling to room temperature in a desiccators. Weigh accurately 2-5g of finely ground sample. The pre-weighed crucible are transfer into a pre-heated furnace and ash for 3-5 hours at 600\^\circ\mathrm{C}. the crucible and its content is cooled in a desiccators and re-weigh.

The percentage ash content is calculated as:

\[
\% \text{ Ash} = \frac{W_3 - W_1}{W_2} \times 100
\]

Where, \(w_1 = \) weight of empty crucible  
\(W_2 = \) weight of the crucible and sample before ashing  
\(W_3 = \) weight of the crucible and ash  
Organic matter \% = 100 – ash content \%

**Determination of Fibre Content**

The sample [2g] was weighed accurately in a fibre flask and 100ml of 0.25N H\_2 SO\_4 was used to dissolve the mixture and was heated for one hour with the heating mantle. The hot mixture was filtered through a sieve cloth. The filtrate was thrown off and the residue was return to the fibre flask to which 100ml of 0.3IN NaOH was added and heated under reflux for another one hour. The mixture was filtered through a fibre sieve cloth and 10ml of acetone was added to dissolve any organic constituent. The residue was washed with about 5ml host water twice in the sieve cloth before it was finally transferred into the crucible. The crucible and the residue was oven dried at 105\^\circ\mathrm{C} over night to drive off moisture. The oven dried crucible containing the residue was cooled in a desiccators and later weighed to obtain the weight \(w_1\). The crucible with weight \([w]\) was transferred to the muffle furnace for ashing at 550\^\circ\mathrm{C} for 4hours. The crucible containing white or grey ash [free of carbonaceous material] was cooled in the desiccators and weighed to obtain \(w_2\). The difference \(w_1 - w_2\) gives the weight of fibre (Kumar et al., 2009). The percentage fibre was obtained by the formula:

\[
\% \text{ fibre} = \frac{w_3 - w_2}{W_1} \times 100
\]

**Determination of Crude Fat / Liquid**

Using the (Kumar et al., 2009) method, weigh 2-5g of fine ground dry sample into a thimble and place into a soxhlet apparatus containing a glass wool.
Attach a dried pre-weighed 500ml round bottom flask [containing few crystals anto bumping chips] to the base of the extractor and clamp to a retart stand. Pour about 300ml of petroleum either into the barrel containing the thimble and place the assembled unit in an electrothermal heater with the top of the extractor connected to a reflux condenser. Turn on the source of water supply connected to the reflux condenser and the source of heater to enable the solvent in the flask to boil and extract the lipids in the sample for about 3-6 hours on completion, remove the thimble and redial either by distillation. Place the flask and the collected lipids in an oven at 70°C for a few minutes, completely remove all the either residue and cool in a desiccator before re-weighing. The process of cooling should continued until an accurate weight is obtained. The percentage lipid is calculated thus:

\[
\text{Crude Lipid [\%]} = \frac{\text{weight of lipid} \ [w_1] \times 100}{\text{Weight of sample} \ [l]}
\]

Where \( w_1 = \text{weight of flask and content after extraction} - \text{weight of flask before extraction}. \)

**Determination of Crude Protein Content**

Kheldahl method was used for the determination of crude protein. Exactly 8g of the sample and 3g of copper sulphate catalyst and 25mls of concentrated sulphuric acid was heated over a burnsen flame in a fume cupboard to expel any poisonous gas. It was then heated with shaking at intervals for 1 hour until the mixture became clear. 400ml of distilled water was added followed by the addition of 50ml of 2% boric acid with 1ml methyl red indicator, 75ml of 50% NaOH was added to make the solvent alkaline. The ammonia was distilled into the boric acid solution. 250mls of the distillate was collected after washing the walls of the receiver and the condenser. The distillate was titrated with 0.1N sulphuric acid. The percentage protein was calculated from the percentage nitrogen content of the sample as follows:

\[
\text{% Nitrogen} = \frac{\text{ml acid} \cdot N \text{ acid} \times 1.4}{\text{Weight of sample}}
\]

Therefore, \( \text{Crude protein} = \text{% Nitrogen} \times 6.25 \)

**Determination of Total Available Carbohydrates [by difference]**

The total carbohydrate content of the sample was estimated as the Nitrogen free extract (NFE). The arithmetic different methods involve adding the total percentage value of crude volume.

\[
\text{Total CHO} = 100 - [\% \text{ fibre} + \% \text{ protein} + \% \text{ Moisture} + \% \text{ ash} + \% \text{ fats}]
\]

**Results**

**Table 1. Qualitative result of phytochemical contents of Indigofera tinctoria pod extracts.**

| Phytochemical screened | Type of Extract/Indication |
|------------------------|---------------------------|
|                        | Hot Ethanolic (Soxhlet)   |
|                        | Cold Ethanolic            |
|                        | Aqueous                   |
| Alkaloids              | +                         | +                         |
| Flavonoids             | +                         | +                         |
| Saponins               | –                         | –                         |
| Tannins                | –                         | –                         |
| Cardiac glycosides     | –                         | –                         |

From the table above, there was presence of Alkaloids and Flavonoids in all the extracts of *I. tinctoria* pod and absence of Saponins, Tannins and Cardiac glycosides in all the extracts of *I. tinctoria* Pod.

**Table 2. Result of proximate analysis Of Indigofera tinctoria Pod.**

| Parameter Checked    | Percentage Composition |
|----------------------|------------------------|
| Ash Content          | 1.01                   |
| Moisture Content     | 68.68                  |
| Crude Fibre Content  | 14.83                  |
| Crude Protein        | 2.63                   |
| Lipid Content        | 0.68                   |
| Carbohydrate         | 12.17                  |

From Table 2 above, *Indigofera tinctoria* pod has high moisture content of 68.68\%, crude fiber content 14.83\%, carbohydrate 12.17\%, crude protein 2.63\%, ash content 1.01\% and lipid content 0.68\%.

**Discussion**

The proximate analysis of *Indigofera tinctoria* pod revealed that 14.83\% of fibre and 12.17\% carbohydrate the fibre content together with the cellulose contents of *Indigofera tinctoria* pod provides substrate for cellulose action. Also the
moisture content revealed 68.68% showing that this pod has high water storage capacity. The crude fibre contents of *Indigofera tinctoria* pod (14.83%) were high and compared favourably with those of *Lasianthera africana* (15.3 – 18.1% dry mass) (Lockeett et al., 2000) and *Heinsia crinata* (13 – 15% dry mass) (Oladé, 2005). Although intake of dietary fibre can lower the serum cholesterol level, risk of coronary heart disease, hypertension, diabetes and colon and breast cancer, the major problem associated with nutrition of vegetables by human is the high fibre content which can cause intestinal irritation and lower bioavailability (Salehi-Surmaghi et al., 1992). Ash contents (an index of mineral contents in biological mass), were relatively lower in *Indigofera tinctoria* pod (1.01%) than in fluted pumpkin (9.68%), bitter leaf, (15.86%) and *Moringa oleifera* (15.09%) leaves [20]. Hence, this pod could however not be a good source of mineral elements. The crude protein content in *Indigofera tinctoria* pod (2.63%) is low when compared to 24% in *Amaranthus vividis* (Sena et al., 1998), 20.72% in *Moringa oleifera* (Segelman et al., 1969), 21.0% in *Lasianthera africana* and 15.0% in *Heinsia crinata* (Sena et al., 1998). Phytochemical screenings of the *Indigofera tinctoria* pod revealed the presence of Alkaloids and Flavonoids in all the three different extracts with the absence of the most known plant bioactive components like Saponins, Tannins, Cardiac glycosides which have been involved in the treatment of many ailments. Although some researchers have found them in the leaves (Who, 2002). The question then arises, does it healing remedy ascribed to the pod extract in treating measles and chicken pox (all viral disease) is conferred to the presence of this two phytochemicals (alkaloids flavonoids) or are there other micro-bioactive compounds present in this pod that has helped the traditional medicine men to achieve this feet;causing the age long use of *Indigofera tinctoria* pod extract in the treatment of disease ?.

More investigation is needed on the phytochemical analysis of this pod, so as to properly ascertain the presence of the bioactive chemical or confirm the sole existence of these two already identified.

**Conclusion**

Based on my findings, the result of this research work, it showed that *Indigofera tinctoria* pod contained appreciable amounts of nutrients such as carbohydrate, protein and fibres which are nutritional requirements of both humans and livestock which suggest that the pod could be useful as feeding supplement to improve health and growth performance in humans and livestocks. The pharmacological effect of the phytochemical constituents such as alkaloids, and flavonoids of the plant pod can explain the rationale for the use of this plant pod in the treatment of infections in traditional medicine. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. Therefore, the outcome of this project work suggest that the selected plant pod could probably be a veritable and cheaper substitute for conventional drugs since the plant is easily obtainable and the extract can easily be made through a simple process.

**Recommendations**

Regarding both proximate and phytochemical compositions obtained as a result of plant pod analysed, it is suggested that *Indigofera tinctoria* pod is a promising native plant drug, whereas should be considered most depth pharmacological studies. It is recommended that further studies has to be carried out to isolate, characterize and elucidate the structure of the bioactive compounds from the plant for industrial drug formation.

Recommendation for further studies for proximate and phytochemical components of this plant pod to be characterized and indexed for a better identification, production potential for use as synthetic antibiotics and nutrient value.

It is also recommend that:

1. The most active extracts of this plant pod should be subjected to isolation and structural elucidation to know the actual compounds of therapeutically value.
2. The potentially useful phytochemical structures present in this plant pod should be synthesized chemically.

Toxicological evaluation of the pod should be carried out.

**Conflict of interest:** All authors declare no conflict of interest.

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