Evaluation of Proximate, Phytochemical and Pharmacological Profile of Cassia torra Native to Zuru Town, Kebbi State Nigeria
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ABSTRACT
Cassia tora, is commonly found in Nigeria, it’s annual shrub which grows in a wild land. The plant and its different parts are used for their nutritional and medicinal purposes. In the present study, an attempt was made to investigate nutritional contents, phytochemical analysis and medicinal evaluation of Cassia tora. The crude powder extracts of the leaves of the plants were taken for the study. The phytochemical screening was done for the selected plants. Phytochemistry of the plant reveals that Cassia tora contains anthraquinones, protein, carbohydrates, saponins, glycosides, fixed oils and fats. Several chemical contents such as naphthopyrone glycosides, flavonoids, glycosides, anthraquinone, etc. have been separated from this plant. The plants has been found to have hepatoprotective, antigenotoxic, antipsoriatic, antinociceptive, immunostimulatory, anticancerous, antifeedant, larvicidal, antiproliferative, hypolipidemic, antimutagenic activity. It also has a property of mild laxative and diuretics so can be used in constipation, dyspepsia and flatulence. Its decoction is used for healing and washing of wounds.
INTRODUCTION
Herbal medicine also known as botanical medicine or phytomedicine-refers to using plants seeds, flowers, roots for medicinal purpose. Traditional medicine similarly known as native or folk medicine is a prehistoric and cultured bound method of healing, diagnosis, prevention, enhancement or management of physical and mental sickness that advanced over generation within numerous society before the epoch of contemporary medicine (Abraham et al., 2009; WHO, 2000). It is widely used to maintain health even though it is limited by lack of precise diagnosis, hygiene, standardization and ethics in dosage and sometimes exaggerated claims which cannot be proven scientifically (Anupama 2017; Akshay and Manish, 2021). Herbalism has a long traditional use outside of conventional medicine. It is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease (Chaurasia et al., 2011). The medicinal action of plants is unique to a particular plant species, consistent with the concept that the combination of secondary metabolites in a particular plant is taxonomically distinct for three medicinal plants and their description and uses respectively (Akshay and Manish, 2021). It is thus clear that, plants contain mixture of several phytochemical components known as secondary metabolites that may act independently, additively, or in collaboration to recuperate health conditions. This significant treasure of structurally diverse potential bioactive organic molecules and higher plants as potential source of new drugs is still highly unexploited (Smita and Patil 2010; Kinghorn 1992; Hostettmann et al., 1996; Elujoba 1999; Shok 1999). There has recently been a resurgence of curiosity in herbal remedies, as the usage of plant's stem bark, florals, seeds, roots, leaves, or berries for medicinal resolves has a long custom of use outside orthodox remedy and is fetching more mainstream as advances in investigation and quality control, along with innovations in clinical research, shows the value of herbal medication in curing and inhibiting ailments (UMMC 2016).

LITERATURE REVIEW
Cassia tora is a legume that is an annual herbaceous herb in the genus senna. The plant is a shrub of up to 30-90 cm high that is sometimes separated in the monotypic genus diallobus (Chidume et al, 2002). It consists of pinnate leaves that grows wild in Africa, Asia, North, Central, and South America and Oceania, and is considered a predominantly serious weed in many places growing on well-drained fertile soil ( Nature Serve, 2007; Smita & Patil, 2010). It is said to have an antiasthenic and purgative effect, as well valuable for the eyes. It is used in the treatment of ringworm, psoriasis, leprosy, rejuvenate vision, ulcers and skin diseases. As a traditional remedy, the kernels are frequently roasted, then boiled in water to make tea (Dirar, 1984). The new leaves of Cassia tora are sometimes used as vegetable in Africa and other part of the world and the plant is cultivated in houses for this purpose in several countries including Cameroon, Senegal, Nigeria, Ghana and Ethiopia. Here in the present study Cassia torra plant was taken for phytochemical screening and plant extracts was taken and
evaluated, the phytochemical constituents were studied by qualitative analysis for performing various chemical tests.

METHODOLOGY

Collection and Identification of Plant Material

The fresh leaves of Cassia torra were collected in the months of August (2023) from the surrounding farmlands of Zuru town. The plant was authenticated at the herbarium by experts of Department of Plant Science, Usmanu Danfodiyo University Sokoto a voucher specimen (UDUS/2023/PS/01) was retained.

Preparation of Plant Extracts

The fresh leaves were washed under running tap water to clear the dust or any other surface impurities. The leaves were dried for two weeks in a well-ventilated place under shade, with intermittent mixing. The dried leaves were grinded to coarse powder using a mechanical blender. The leaf powder (150g) was extracted with ethanol in a Soxhlet apparatus for 24 h. The solvent was recovered in vacuo to yield a residue (35 g) referred to as Cassia tora ethanolic leave extract.

Phytochemical Screening

- Test for Tannins
  The test for tannins was performed by boiling small quantity of the extract with 20 cm³ distilled water in a water bath for 5 minutes and was filtered while hot. It was followed by adding little drops (2-3) of 10% ferric chloride reagents to small size of cool filtrate (Trease & Evans, 1996).

- Test for Cardiac Glycosides
  The legal and Killer-Kiliani test were adopted for cardiac glycosides test. The crude leave extract (0.5 g) was added to 2 cm³ acetic acid followed by adding concentrated H₂SO₄ down the test tube (Trease & Evans, 1996).

- Test for Alkaloids
  The ethanolic leave extract (0.5 g) was mixed with 5 cm³ of 1% aqueous hydrochloric acid on a water bath, the filtered extract was divided into three small portions and 2 drops of Dragendroff’s, Mayer’s reagent and Wagner’s reagent were respectively added (Soforowa, 1993; Trease & Evans, 1996).

- Test for Saponins
  Frothing test of the crude extract (2 g) was adopted for the identification of saponins (Silva et al., 1998).

- Test for Reducing Sugar
  The detection of reducing sugar was employed by adding Fehling's solution to small portion of the extract and boiled for 5 minutes (Silva et al., 1998).

- Test for Anthraquinones
  The crude extract (2g) was also examined for free and combined anthraquinones. Identification of free anthraquinones was carried out by mixing the crude extract (1g) with 10 cm³ of chloroform for 5 min with continuous shaken and then filtered. It was then followed by the addition of 10% ammonia
solution to equal volume of the filtrate. For combined anthraquinones, powdered crude extract (1 g) was boiled for 5 minutes with 2 cm$^3$ of 10% hydrochloric acid. The boiled mixture was filtered in a test tube, cooled and partitioned against equivalent quantity of chloroform. The upper layer was pipetted off and transferred into a test tube followed by the addition of half its volume of 10% ammonia solution and shaken gently (Ajayi et al., 2011).

- **Test for Flavonoids**
  Flavonoid was determined using Shinoda test. A minute quantity of the extract was suspended in methanol. Some bits of magnesium chips were enhanced followed by 5 droplets of concentrated hydrochloric acid (Silva et al., 1998).

- **Test for Terpenoids/Steroids**
  Terpenoids/Steroids were determined by Lieberman burchard test and Salkowski test respectively. To the former, a small portion of crude extract was suspended in chloroform. Equivalent volume of acetic anhydride was added, and concentrated H$_2$SO$_4$ was added down the test tube. And to the latter, small quantity of the crude extract was mixed with 2 cm$^3$ of chloroform. The layer was treated with 3 drops of concentrated H$_2$SO$_4$ (Silva et al., 1998; Ajayi et al., 2011).

- **Test for Phlobatannins**
  The test of phlobatannins was carried out by suspending small quantity of the extract in distilled water, the mixture was then shaken in a test tube and filtered. 1% aqueous hydrochloric acid was added to the aqueous solution, and was then boiled with the help of Hot plate stirrer (Ajayi et al., 2011).

- **Test for Fixed oils and Fats**
  Fixed oils and fats were identified by pressing small amount of the extract between two filter paper to observe the presence of an oily stain (Trease & Evans, 1996).

- **Test for Proteins**
  The test of protein was carried out by treating the extract with 1 cm$^3$ of 10% sodium hydroxide solution and heating, followed by droplet of 0.7% copper sulphate solution (Trease & Evans, 1996).

**Gas Chromatography Mass Spectrometry Analysis and Compound Identification.**

Gas Chromatography Mass Spectrometry analysis was performed on Perkin Elmer, Claurs 680 GC coupled with PE SQ-8C mass analyzer using Elite-5MS, 30 M X 0.25 mm, 0.25 µm capillary column. Carrier gas, helium; temperature programming, initial 400C, hold time 5.0 min, ramp rate 120C/min to 2600C and finally held isothermally for 5 min. The injector temperature was 2500C and carrier flow was at 1 cm$^3$/min, Programmable Split-Splitless Injector (PSSI) in split mode (1:50) with injector volume 1 µl using autosampler. The ion source
temperature was set at 1800°C, transfer line temperature was 2000°C, and the ionization of the sample components was performed in electron ionization mode at an ionization voltage of 70eV. Mass range was used from m/z 50 to 550 amu. The compounds were identified by comparison of their mass spectra with those of the internal reference mass spectra library (NIST/Wiley) using standard methods (Joulain and Konig 1998).

**Proximate Analysis**

**Determination of Moisture Content** The moisture content was determined by adopting the method described by Udo and Ogunwele (1986), with slight modification. In this method, powdered leaves 2 g (W0) was placed in a pre-weighed crucible (W1) and oven dried for 3 consecutive hours at 105°C. This was weighed after cooling in a desiccator and continued until there was no change in weight (W2) and moisture content was calculated.

\[
\text{Moisture content (\%) } = \frac{W_1 - W_2}{W_0} \times 100
\]

- \( W_1 = \) mass of crucible with sample
- \( W_2 = \) mass of crucible with dried sample
- \( W_0 = \) mass of sample.

**Determination of Ash Content**

Ash is pondered as the total mineral or inorganic residue of the sample. It was determined by adding dried pulverized leaves 2 g (M1) into a weighed crucible (M0) and incinerated successively for 6 hours in a furnace at 550°C. This was transferred into a desiccator, cooled and weighed (M2) and the differences in weight gives the ash content (James, 1995).

\[
\text{Ash content (\%) } = \frac{M_2 - M_0}{M_1} \times 100
\]

- \( M_1 = \) Mass of sample
- \( M_2 = \) Mass of incinerated sample
- \( M_0 = \) Mass of unfilled crucible
**Determination of Crude Lipid Content**

Approximate lipid content was estimated using Soxhlet method of extraction. Crushed sample 5 g (W0) was placed in weighed filter paper. The content was folded and tied with thread (W1), arranged neatly in thimble flask and extracted using hexane for 6 h. The sample was then dried in an oven and transferred into a desiccator to cool and weighed (W2). The process was done in triplicate and approximate lipid was calculated (Udo & Ogunwele, 1986).

\[
\text{Crude lipid (\%) = } \frac{W_1 - W_2}{W_0} \times 100
\]

W0 = Untreated sample

W1 = Filter paper with sample before extraction

W2 = Extracted sample

**Determination of Crude Fibre Content**

The crude fibre content was analyzed by digesting the pulverized defatted sample 5 g (W0) using mixture of 20% H2SO4 and distilled water in ratio 1:5 for 30 min. The residue of the filtered mixture was collected in a beaker containing a mixture of 10% NaOH and distilled water in ratio 1:10 and then boiled for 30 minutes. The solution was then filtered and the residue was treated with boiled distilled water, 10% HCl and washed two times with ethanol and finally three consecutive times with petroleum ether and then transferred into clean weighed crucible and dried at 105°C, cooled and weigh (W1). Finally, incinerated at 550 oC for 1 hour 30 minutes cooled and weighed (W2). Fraction of crude fibre is evaluated using expression below (Udo & Ogunwele, 1986).

\[
\text{Crude fibre (\%) = } \frac{W_1 - W_2}{W_0} \times 100
\]

W0 = Sample’s mass

W1 = Mass of dried sample

W2 = Mass of incinerated sample

**Determination of Crude Protein**

Percentage crude protein was determine using Miro-Kjeldahl method described by AOAC (1990). The crude sample (2 g) was mixed with Catalyst (8 g) and digested using 20 cm3 of concentrated H2SO4 followed by heating in an inclined position. Heating continued with occasional swirling until the solution turns colourless. This was make up to 100 cm3 with distilled water. The digested sample (10 cm3) was distilled using 10 cm3 solution of 50% NaOH (10 M NaOH)
into conical flask containing 10 cm³ of 4% boric acid, two drops of mixed indicator were added and titrated against 0.1 M HCl. The result is given as % nitrogen,

\[(ml \text{ standard acid} - ml \text{ blank}) \times N \text{ of acid} \times 1.401\]

sample mas

Determination of Nitrogen Free Energy (Digestible Carbohydrate) Nitrogen free extract (NFE) was analyzed by mathematical calculation. It is achieved by deducting the sum of percentages of all the nutrients already determined from 100.

\[\text{NFE} = 100 - (\% \text{ crude protein} + \% \text{ crude lipid} + \% \text{ crude fibre} + \% \text{ ash} + \% \text{ moisture}) \text{ weight by dry basis. Hence, energy was evaluated: } \{\text{ratio of } (CP \times 4) + (CFT \times 9) + (NFE \times 4)\}\]

as employed by Isiong and Idiong (1997).

**Statistical Analysis**

The analysis was carried out in triplicates, results were expressed as the mean ± standard deviation. Values were considered statistically significant at t (P ≤ 0.05).

**RESULTS**

The results of the preliminary phytochemical analysis carried out on ethanolic leave extract of Casia tora were summarized in table 1, while table 2 shows proximate composition of the plants ethanolic extracts.

### Table 1. Phytochemical Screening of the Cassia Tora Leaves

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Phlabotannins</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Steroids/ Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>Fats and Oils</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: + = Present, - = Absent
Table 2. Proximate Composition of Cassia Tora Leaves

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>10.23±0.52</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>7.54±1.08</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>6.01±0.07</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>15.01±0.63</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>27.34±1.16</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>33.69±2.38</td>
</tr>
</tbody>
</table>

Results are Expressed as Means Standard Deviation of Three Replicates

Figure 1. GC-MS Spectral Chromatogram of Ethanolic Extract of Cassia Torra Leaves
DISCUSSION

The result of the phytochemical screening of Cassia tora leaves (Table 1), shows that the following phytochemicals: alkaloids, anthraquinones, flavonoids, tannins, carbohydrates, steroids/triterpenoids, protein, fats, phlobatannins and glycosides were present. The phytochemical constituents observed are very similar to those reported by group of researchers from India except for saponins and triterpenes that were present and absent respectively (Syafii et al., 201). These phytochemical constituents have been reported to be associated with different pharmacological and nutritional activities. Humans consume a wide range of foods, drugs and dietary supplements that are derived from plants. Plants provide nutrients including carbohydrates, protein, fats, mineral salts, vitamins, dietary fiber and many other compounds known as phytochemicals or secondary metabolites (Hatano et al., 1999). Each human body is built up from food containing these five primary constituents (Micheal, 1997). The high content macronutrients carbohydrate (33.69%) and protein (27.34%) present in this plant are fundamental for proper body performance and the body needed large amount of these nutrients as important part of healthy diet. Carbohydrates provides glucose to the body, which is converted to energy that fuels the central nervous system, it is also important for brain functioning and provide energy for working muscles (Valentine et al, 2017). On the other hand, proteins are macronutrients that make up 15 percent of human’s body weight (Sharma et al. 2005). This essential metabolite brakes down in the body to fuel muscle mass which helps metabolism and improve immune system (Mozaffararian et al., 2011).
The proximate analysis of Cassia tora leaves is given in (Table 2). The results showed that it contains ash (15.01%), crude fibre (7.54%), crude protein (27.34%), carbohydrate (33.69%), moisture (10.23%) and crude fat (6.01%). From the results, carbohydrate has the highest value, while crude fat has the smallest. The ash content of 15.01% shows that the leaves are rich in mineral elements. The value is higher compared to 1.80% in sweet potato and 12% in Tribulus terrestris leaves, but lesser than some leafy vegetables normally consumed in Nigeria such as Talinum triangulare 20% (Akindahunsi & Salawu, 2005). The moisture content of the leaves is low 10.23%, this would hinder the growth of microorganism and storage life would be high (Adeyeye & Ayejuyo, 1994). The moisture content of the leaves is lower than that of Acalypha hupsida 11.02%, Acalypha recemosa 11.91% and Acalypha maginata 10.83% (Iniaghe et al, 2009). The dietary fiber can lower serum cholesterol level, risk of coronary heart diseases, hypertension, constipation, diabetes, colon and breast cancer (Ishida et al., 2000; Sui-ming et al. 1989). The data presented in this study provides scientific evidence that ethanolic leaves extract from Cassia tora may contain nutritional principles that are relevant to the management of malnutrition disorder.

GC-MS with NIST library is one of the widely used techniques for identification of the constituents of volatile matter, long chain and branched chain hydrocarbons, alcohols, acids, esters, etc. Twenty (20) major compounds from the methanolic extract of C. tora leaves were identified using GCMS-NIST library. These major compounds and data regarding their reported pharmacological activities was collected from existing literature. DLAlpha-Tocopherol, Linolenic acid, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Trehalose, Tocopheryl acetate, Phytol and Stearic acid as antioxidative and neuroprotective agents. Anti-inflammatory, antimicrobial, antibacterial, antiviral, antiasthmatic, anticancer, anti-malarial, antituberculosis, antirheumatic and cholesterol-lowering properties of the identified compounds have also been reported in the literature. The present report of identification of bioactive compounds of Cassia tora justifies its use in management of various ailments (Yu et al., 2008; Islam et al., 2018). The presence of neuroprotective and antioxidant molecules justifies the use of this plant for mitigation of Al toxicity in this region. Laminitol has been identified as the major phytocomponent in this study having 25.049% concentration. Ethanol extract of Strychnos ligustrina wood rich in Laminitol has been reported to possess antimalarial activity(Syafii et al., 2016).

CONCLUSIONS AND RECOMMENDATIONS

Analysis of the phytochemical and proximate composition of Cassia tora leaves revealed that, the leaves possesses adequate contents of carbohydrate and proteins, with appreciable number of phytochemicals like anthraquinones, flavonoids, Phlabotannins, and Steroids. The high content of carbohydrate and proteins can provide important nutritional supplements in food and support their use as an important source of energy and body builders respectively. The plant has also being widely used in ayurvedic as well as chinese medicine. Pharmacological profile reveals the plant is rich in anthraquinone glycosides and their derivatives. The plants also possesses antioxidiant and neuroprotective activity. Other pharmacological activities are anti inflammatory, antimicrobial,
antibacterial, antiviral, antiasthmatic, anticancer, anti malarial, antituberculosis, antirheumatic and cholesterol-lowering properties.

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