

RESEARCH PAPER

**Phytochemical Screening and Proximate Analysis of Ethanolic Leaves
Extract of *Cassia tora***

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Abstract

Cassia tora is a leguminous plant belonging to the family Fabaceae and are used for the treatment of leprosy, psoriasis, ringworm, ulcers, skin diseases and as a laxative and antiasthenic. The preliminary phytochemical screening of the ethanolic extract of *Cassia tora* was carried out using standard methods while the nutritional values were studied for moisture content, ash content, crude lipids, crude fibre, crude protein and carbohydrates. The preliminary phytochemical analysis of the leaves indicated the presence of anthraquinones, flavonoids, steroids/triterpenoids, fats, tannins, alkaloids, carbohydrates, phlobatannins and glycosides. The proximate analysis of the leaves revealed that it contained crude fat 6.01%, crude fibre 7.54%, moisture 10.23%, ash content 15.01%, crude protein 27.34% and carbohydrate 33.69%. *Cassia tora* leaves could be a potential source of nutritional and mineral elements that may be useful in nutrition. The phytochemicals observed in this study explained the medicinal action of the plant encountered in its therapeutic uses. The study demonstrated that, *Cassia tora* leaves could serve as an important source of nutrition and ethno medicine for animals and humans.

Keywords: *Cassia tora*; proximate analysis; crude protein; moisture; phytochemical analysis; macro kjeldahl.

Abstrak

Cassia tora adalah tumbuhan leguminos dari keluarga Fabaceae dan digunakan untuk rawatan leptin, psoriasis, cacing, ulser, penyakit kulit dan sebagai julap dan *antiasthenic*. Fitokimia awal ekstrak etanol *Cassia tora* dilakukan menggunakan kaedah piawai manakala nilai pemakanan dikaji untuk kandungan kelembapan, kandungan abu, lipid mentah, serat mentah, protein mentah dan karbohidrat. Analisis awal fitokimia dari bahagian daun menunjukkan kehadiran *anthraquinones*, *flavonoid*, steroid/*triterpenoids*, lemak, tanin, alkaloid, karbohidrat, *phlobatannins* dan glikosida, pada analisis proksim, daun menunjukkan bahawa ia mengandungi lemak mentah 6.01%, serat mentah 7.54 %, kelembapan 10.23%, kandungan abu 15.01%, protein mentah 27.34% dan karbohidrat 33.69%. Daun *Cassia tora* boleh menjadi sumber potensi unsur pemakanan dan mineral yang mungkin berguna dalam pemakanan. Fitokimia yang diperhatikan dalam kajian ini menjelaskan tindakan perubatan tumbuhan yang ditemui dalam kegunaan terapeutiknya. Kajian menunjukkan bahawa, daun *Cassia tora* boleh menjadi sumber pemakanan dan ubat etno yang penting untuk haiwan dan manusia.

Kata kunci: *Cassia tora*; analisis proksim; protein mentah; kelembapan; analisis fitokimia; makro kjeldahl.

INTRODUCTION

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 (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. 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 (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, floras, 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).

Cassia tora is a legume that is an annual herbaceous foetid 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*. 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 (Smita & Patil, 2010; Nature Serve, 2007). 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. The leaves also possess antibacterial, antiallergic, antimutagenic and antiviral activities. 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. The plant leaves are used as decoction febrifuge for the treatment of scorpion stings, gingivitis, dysentery and diarrhea (Irwin and Dalziel, 1955; Irvine, 1961; Barneby, 1982). The present analysis was designed to evaluate the phytochemical and proximate constituents of *Cassia tora* leaves collected from Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The *Cassia tora* fresh leaves were collected from Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria. The plant was identified by U. S. Gallah from Biological Sciences Department (BSD), Ahmadu Bello University, Zaria, Nigeria (ABU) and the voucher specimen 1370 was deposited at the herbarium of BSD, ABU.

Preparation of Extract

The plant leaves were cleaned and air dried under shade for two weeks. The dried leaves were powdered using a blender. The powdered plant material (200 g) was subjected to extraction 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 mL 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 Keller-Kiliani test were adopted for cardiac glycosides test. The crude leave extract (0.5 g) was added to 2 mL 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 mL of 1% aqueous hydrochloric acid on a water bath, the filtered extract was divided into three small portions and 2 drops of Dragendorff'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 (2 g) was also examined for free and combined anthraquinones. Identification of free anthraquinones was carried out by mixing the crude extract (1 g) with 10 mL 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 mL 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 Liebermann 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 H₂SO₄ was added down the test tube. And to the latter, small quantity of the crude extract was mixed with 2 mL of

chloroform. The layer was treated with 3 drops of concentrated H₂SO₄ (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 mL of 10% sodium hydroxide solution and heating, followed by droplet of 0.7% copper sulphate solution (Trease & Evans, 1996).

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.

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

W₁ = mass of crucible with sample

W₂ = mass of crucible with dried sample

W₀ = 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).

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

M₁ = Mass of sample; M₂ = Mass of incinerated sample; M₀ = 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°C 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 ml 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 ml with distilled water. The digested sample (10 ml) was distilled using 10 ml solution of 50% NaOH (10 M NaOH) into conical flask containing 10 ml of 4% boric acid, two drops of mixed indicator were added and titrated against 0. 1 M HCl. The result is given as % nitrogen,

$$\frac{(ml \text{ standard acid} - ml \text{ blank}) \times N \text{ of acid} \times 1.401}{\text{sample mass}}$$

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.

NFE = 100 – (% crude protein + % crude lipid + % crude fibre + % ash + % moisture) weight by dry basis. Hence, energy was evaluated: {ratio of (CP×4) +(CFT×9) +(NFE×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 (P<0.05) (confidence level = 95%).

RESULTS AND DISCUSSION

The results of the preliminary phytochemical and proximate analysis carried out on ethanolic leave extract of *Cassia tora* were summarized in table 1 and table 2 respectively.

Table 1. Phytochemical screening of the leaves *Cassia tora*

Phytochemical	Ethanolic extract
Tannins	+
Phlobatannins	+
Steroids/ Triterpenoids	+
Saponins	—
Proteins	+
Fats and oil	+
Anthraquinones	+
Carbohydrates	+
Alkaloids	+
Flavonoids	+
Cardiac glycosides	+

Keys: + = present, – = absent

Table 2. Proximate composition of *Cassia tora* leaves

Sample/Parameter	A	B	C	Mean
Moisture (%)	10.67	9.65	10.37	10.23±0.52
Crude fibre (%)	8.73	6.62	7.27	7.54±1.08
Crude fat (%)	5.95	6.0	6.08	6.01±0.07
Ash (%)	15.50	15.22	14.30	15.01±0.63
Crude protein (%)	28.25	27.74	26.03	27.34±1.16
Carbohydrate (%)	30.9	34.77	35.23	33.69±2.38

Expressed as mean of three determinations ± standard deviation for three replicates experiment

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 (Rahimullah & Imran, 2015). These phytochemical constituents have been reported to be associated with different pharmacological and nutritional activities. Human 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. 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. On the other hand, proteins are macronutrients that make up 15 percent of human's body weight. This essential metabolite brakes down in the body to fuel muscle mass which helps metabolism and improve immune system (Mozaffarian 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). 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.

CONCLUSION

Analysis of the phytochemical and proximate composition of naturally occurring *Cassia tora* leaves revealed that, the leaves possess enough carbohydrate and proteins, with appreciable number of phytochemicals like anthraquinones and flavonoids. 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. Further research is ongoing to isolate and identify the bioactive compounds responsible for the observed activity.

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