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Phytochemical screening and proximate composition of *cassia hirsute* seeds

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ABSTRACT
The phytochemical screening, proximate composition, mineral elements and quantitative assay of anti-nutrients of the seeds of *Cassia hirsute* have been evaluated. The brown seeds have pungent smell and are bitter and unpleasant to taste. The phytochemical screening indicated the presence of alkaloids, tannins, saponins, phenols, sterols, triterpenes, glycosides and carbohydrates; phlobatannins and flavonoids were absent. The proximate composition of the seeds was 8.7% moisture, 7.7% ash, 7.0% fat, 10.5% protein, 13.2% fibre and 52.9% carbohydrate with estimated caloric value of 316.20 kCal/100g sample. The mineral elements determined in the seeds include K, Ca, Na, Fe, Mg and P, heavy metals such as Pb, Cr and Cu were not present. The antinutrient assay revealed that the total tannins, oxalate and phytic acid contents were high but hydrocyanic acid was not detected. The high values of these toxic materials suggest that the seeds are not suitable for consumption as food, but the solvent extract may be suitable as fumigant to dispel insects, rodents and snakes.

Introduction

Some plant seeds and other parts of plants are commonly consumed as food or are used for medicinal preparations for health care delivery in Nigeria. The nutrient components of these plants parts are well known but the active components which make them efficacious in medicinal application are still under investigation. There is a renewed interest in herbal medicine because many communities still depend greatly on herbs for health care delivery.

Locally, it is known that the root extracts of *Aristochia spp* (Snake worth) are used as antidote for snake or scorpion bite poisoning. It is also reported that the leaf extracts of *Byrophyllum pinatum* (Ndodop) are used as cure for eczema and preventive treatment for catarrh, cough and epilepsy; also extracts of the leaves and fruits of *Krelancheu spp* are used as antihelmintic agent in Akwa Ibom State of Nigeria.

A large proportion of the world's population depends on traditional medicine because of the scarcity and high costs of orthodox medicine [1, 2]. Medicinal plants have provided the modern medicine with numerous plant-derived therapeutic agents [3, 4]. Natural products play a dominant role in the development of novel drug for the treatment and prevention of diseases [5, 6].

A number of authors have reported that the effectiveness of plant parts used as food or medicine depends on the nutrients and bioactive components such as alkaloids, tannins, saponins, flavonids, essential oils and other similar components [7-9].

Cassia spp are believed locally to have some repellent activity on both snakes and insects; besides they are extensively used in many herbal preparations; the leaf extracts from *Cassia alata* are used as antifungal lotion while those from *Cassia auriculata* , *Cassia fistula, Cassia angusifolia* are used as laxatives [10]; though *Cassia hirsute* is believed to have similar effects as other *Cassia species,* no work has been reported on it.

A detailed study on the extracts of *Cassia nigricans* Vahl leaves, an active constituent 1, 6, 8- trihydroxy-3-methylanthraquinone (emodin) was *isolated* [11]. Ayo and his co-workers [12] isolated a steroidal ester; hydroxyestranic acid, ethyl ester, from the methanol extract of the leaves, possessing potent antimicrobial activity Besides anthraquinones, luteolin and the steroidal ester, sitosterol acetate, heptadecanoic acid, 14-methyl ester; and a toxic principle, bis -2- ethylhexyl phthalate were identified in the methanol extract of the leaves of *C. nigricans* Vahl [13].

Pharmacological properties of *Cassia nigricans* Vahl against human and veterinary diseases has been investigated [14]. *C. nigricans* Vahl is used in Ghana to control insect pests of stored grains and legumes [15].

Emodin isolated from the ethyl acetate extract of the leaves of *C. nigricans* Vahl was tested against common pathogenic microorganisms. The results demonstrated that emodin significantly inhibited the growth of all the microorganisms [16]. Species of *Cassia* are rich sources of polyphenols, anthraquinone derivatives [17, 18]

The plant *Cassia hirsute* is a perennial hairy scrub usually growing among crops and weeds in the gardens. The seeds are stored in cylindrical re-curved pods, each containing numerous brown seeds with hard testa. The aim of this study is to determine both the nutritive and bioactive components present in the seeds of *Cassia hirsute.*

The work presented here involved the phytochemical screening, the proximate analysis; determination of mineral elements and anti-nutrient/toxic substances.

Materials and Methods

The dry seeds of *Cassia hirsute* collected from a garden in the Housing Estate, Uyo, Akwa Ibom State of Nigeria, were oven dried at 50^{o}C for 4 h. They were ground, sieved into powder and stored in amber bottles, labeled CHP and kept in a dark dry place for analysis.

The extraction and other tests were carried out according to specifications shown below:

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Phytochemical Screening

Quantitative tests for seed alkaloids, glycosides, sterols, triterpenses, phenol, tannins, phiobatannins and flavonids were cried out as below:

Alkaloids

2.0g of the *Cassia hirsute* powder (CHP) was boiled in 25ml of 50% ethanol acidified with 5% H_2SO_4 for 5 minutes in a water bath, cooled and filtered. Then 20ml of the filtrate was transferred into a separating funnel and made alkaline with 5.0ml dilute ammonia solution. The alkaline mixture was separated by shaking with 3 successive 2.5ml chloroform.

The combined chloroform extract was acidified with 5.0ml of dil. H_2SO_4 and tested for alkaloids using Wagner's [9], Mayer's and Hagger's reagents [18].

Glycosides

0.1g of CHP was boiled in 100ml distilled water for 15 minutes in a water bath and then filtered and tests were carried out for hydrolysis and free reducing sugar reaction. Borntrager's test for anthracene, glycosides [19] and Kedde's test for cardenolides were carried out [20].

Sterols and Triterpenses

2.0g of the CHP was refluxed in 25ml of chloroform for 1 h and the extract was used for;

i) Liebermann-Burchard test and

ii)Selkowski test for steroidal incleus [7].

Phenols

0.2g of CHP was boiled in 10 ml distilled water. The mixture was filtered and to 5ml of the filtrate was added 3 ml of 1% $FeCl_3$ in pyridine and the colour was noted [21].

Tannins

10g of the CHP was boiled in 50ml distilled water and the filtrate used for the following tests:

i) Ferric chloride test

ii)Lead acetate test and

iii) Vanillin- HCl test [22].

Phlobatannins

0.5g of the CHP was mixed with 5ml distilled water and boiled in 1% HCl for two minutes. The colour change was noted.

Flavonoids

0.2g of the CHP in 10ml ethyl acetate was heated in a water bath to boiling, it was cooled and filtered. The filtrate was used for frothing, emulsion and haemolysis tests [23].

Proximate Analysis

The moisture content, the ash, the crude fibre, the fat, the protein and the carbohydrate content were determined by the methods described by [24]. The carbohydrate being obtained by the difference between the total dry matter (100%) and the sum of the percentage amount of crude fibre, protein, fat and ash.

Determination of Mineral Element

3.0g of the CHP was charred in a pre-weighed crucible and finally ashed in a muffler furnace at 500^{o}C for 4 h. After cooling to room temperature 10 ml of nitric acid (1:1) was added to the ash and then digested on a steam-bath until the volume of the solution was reduced to half. It was then transferred quantitatively to 100ml mark by addition of distilled water. The solution was used to determine all the mineral elements present in the sample, spectrophotometrically.

Determination of Toxic Compounds

Standard methods were used in determining the toxic compounds present in the sample. Total oxalate was determined by the Dye method [25], tannin was quantitatively determined by Burn's method [22] while phytic acid was determined by McCance method [26], hydrocyanic acid content was obtained by titrating alkaline steam distillate obtained from 24 h water-soaked CHP with 0.02 M $AgNO_3$ using appropriate indicators.

Results and Discussion

Phytochemical Screening

The bioactive substances shown in Table 1 were sterols, triterpenes, saponin, tannins and phenols which were present in high concentration; alkaloids and glycosides were moderately present while protein, flavonoids, anthracene glycosides were present in low concentration. The extract had pungent smell. The plant palleluchemicals of saponins, phenols, sterols and triterpenes with pungent and repulsive effect could be responsible for such characteristic smell in the seeds, hence effective as snake repellant in an environment containing cassia plants. The high concentration of phenol could contribute to the seed being poisonous to animals hence it is unsuitable for use as food. The presence of alkaloid commonly used in drug preparation may account for the medicinal use of the cassia seeds.

Proximate Composition

Table 2 present the proximate composition of the seeds. The moistures content of the air-dry seeds was 8.76%. The crude protein, fat and fibre contents were obtained to be 10.50%. 7.00% and 13.16% respectively while the carbohydrate and ash contents were 52.80% and 7.28% respectively, the caloric value of the seeds was estimated to be 316.2 kcal/100g sample determined according to [24]. The moisture content was similar to the value obtained for other legumes [26], but the crude protein was lower than the values from other legumes e.g soybeans (40-45%) while the fat content was higher than that of *Vigna unquiculate* 2.5% [27] but lower than that of peanuts [26]. The fibre content was also high although it was lower than the value for the bark of *Cassia augustifelia* (18.11%) but higher than those of common edible seeds e.g peanut (2.9%) and *Vigna unquiculata* (1.9%) [27]. A diet high in fibre is undersirable as it can cause constipation [28]. The seeds had high ash content compared to *Arachis hypogeal* (2.5%) and *Canavalia rosa* (2.75%) [26].

Mineral Element

The levels of mineral present in the seeds of *Cassia hirsute* are shown in Table 3. The values of K and Ca were very high thus making the seeds suitable as source of dietary K and Ca, but Mg level was low. Heavy metals like lead, copper, chromium and nickel were not detected.

ND = Not detected

Toxic Substances

The level of the toxic substances is shown in Table 4. Total oxalate, tannins and phytic acid were 1730, 5850 and 1300 mg/100g respectively, being very high compared with the standard value.

Conclusion

The results obtained indicate that *Cassia hirsute* seeds contained some toxic materials, hence they are not suitable to be used as food seeds, but can be exploited for use in herbal preparation for medicinal purposes. Since *Cassia hirsute* seeds is non edible seeds, the oil obtained from these seeds can be use in the preparation of metallic soap, insecticide and biodiesel.

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Table 1: Phytochemical Screening

	Test	Observations	Inference
1	Alkaloids		
a.	Wagner's test	Reddish ppt	Alkaloids detected
b.	Hagger's test	Yellow ppt. Observed	Alkaloids detected
c.	Mayer's test	Reddish ppt.	Alkaloids detected
2	Glycosides		
a.	Hydrolysis test	Red ppt. observed	Positive
b.	Free-reducing sugar	Brick red ppt. observed	Present
c.	Borntrager's test for anthracene glycosides	Pink rose colouration	Anthracene glycosides present
d.	Specific test for cardia glycosides	A milky colloidal solution noted	Cardiac glycosides present
e.	Keller-killiani test for deoxy-sugar of cardenolides	A brown ring formation	Deoxy-sagar present
3.	Flavonoids Ammonia solution test	 Light yellow colour	 Flavonoid present
4.	Tannins Ferric chloride test Lead-acetate test	 Blue black coloration White colloidal solution	 Tannins present Tannins present
5.	Phenols Farrio chloride in 1% pyridine test	 Intense black ppt. formed	 Phenols present
6.	Phlobatannins Aqueous HCl test	 A light brown ppt. formed	 Phlobatannins absent
7. a.	Saponins Frothing test	 A stable form was formed	 Saponins present
b.	Emulsion test Haemolysis test	An emulsion observed Thin sedimentation of blood cells	Positive test Saponins present
8.	Protein Million's reagent test	 Light yellow coloration	 Protein present
9. a.	Sterols and Triterpenes Liabermannin-Burehard test for steroidal nucleus	 Browning-green coloration	 Sterols and triterpenes present
b.	Salkowaki test	A brownish layer formed	Sterols and triterpenes present
10. a.	Carbohydrate Molish's test	 Brown ring formation	 Carbohydrate present
b.	Fehling's test	Brick red ppt.	Reducing sugar present
c.	Iodine test	Blue-black coloration	Starch present

Table 2: Proximate Composition

Parameters	Percentage Air dry Weight
Ash (%)	7.78
Moisture (%)	8.76
Crude Protein (%)	10.50
Crude fat (%)	7.00
Crude fibre (%)	13.16
Carbohydrate (%)	52.80
Caloric value (kcal/100g)	316.20

Table 3: Mineral Element

Mineral Element	mg/100g Air Dry Weight
Calcium	857.14
Magnesium	9.03
Phosphorus	351.00
Iron	21.33
Potassium	1,883.33
Sodium	421.05
Copper	ND
Lead	ND
Chromium	ND
Nickel	ND

ND = Not detected

Table 4: Toxic Substances

Toxic Substances	mg/100g Air Dry Sample
Total oxalate	1730.00
Tannins	5850.00
Phytic acid	1300.00
Hydrocyanic acid	ND

ND = Not detected