# PHYTOCHEMICAL SCREENING AND DETERMINATION OF SOME ELEMENTS IN THE POD PULP AND STEM BARK EXTRACT OF *CASSIA SIEBERIANA* DC

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*Abstract*
This study is on phytochemical screening and determination of some elements in the pod pulp and stem bark of cassia sieberiana. People often take the pod pulp of cassia sieberiana. The phytochemical constituent was done by standard procedure for tannins, saponins, terpenoid, flavonoids, steroid, phlobatannins, carrdiac glycosides and reducing sugar. It also determines the elemental content of the plant using Atomic Absorption Spectrometer and Flame photometer. The result of this study revealed that tannins, saponins, flavonoids, steroids, phlobatannins, terpenoid, cardiac glycoside and reducing sugar were all present. Also, Na, K, Cu, Zn, Pb and Mn were present in the pod pulp and stem bark of cassia sieberiana. The results show that this plant contains elements of vital importance in man's metabolism that are needed for growth and development, prevention and healing of diseases. It is hoped that novel drugs could be developed from this plant.

*Keywords:* phytochemical, elemental content, cassia sieberiana

## *Introduction*

Phytotherapy or Herbalism is a traditional medicinal practice base on the use of plant and plant extract. It is also known as Botanical medicine. (www.free encyclopedia. com, 2010). The use of medicinal plant as a source for relief from illness can be traced back over 500 millennia to written documents of early civilisation in China, India and near east, but it is doubtless an art as old as mankind (Thomson, 1978; Stockwell, 1988). A wide range of medicinal plant part is use for extract as raw drug and they possess varied medicinal properties. The different part used include root, stem, flower, fruit, twigs exudants and modified plant organs. It is to this effect that work was done on the pod pulp and stem bark of *Cassia Sieberiana* DC.

*Cassia Sieberiana* DC belongs to the family leguminosae-Caesalpinioideae and grows up to10-20m high with drooping branches. The pods are cylindrical smooth, 40-60cm long and about 1.5cm in diameter; it is indehiscent and dark brown in colour. The plant was described by Gledhill (1991) as an open savannah tree found in drier area of forest and thickets. The plant is widely distributed in southern Sahel and Sudan savannah from Senegal to Cameroun as far as Sudan and the Republic of Congo (Michael, 2004). It is also found in most part of Nigeria, in the North-West, it is found in places like Zamfara, Zurumi and Sokoto. It is found widely distributed in Yobe, Borno, and some part of Adamawa state in the North eastern part of Nigeria. It is mostly found in Agodi in Ibadan and Awka in the south west and south east respectively (Keav *et al*, 1964). Its English names are drum stick, Africa laburnum (Dalziel, 1987). Its vernacular names in Nigeria include 'Marga' in Hausa, the Fulani people called it 'Margaje' and in Yoruba it is called 'Ifo' (Toma *et al*, 2009).

The fruits, seed, leaf and root bark are use for the treatment of fever, jaundice, ache, gonorrhoea, pile, ulcer, debility, diuretics, arthritis and rheumatism. It is also used as

vermifuge and ear treatment (Burkil, 1985; Shahina, 1989).

Medicinal value of this plant lies in some chemical elements that produce definite physiological action on human body. The most important of these bioactive constituents of the plant are alkaloid, tannins, flavonoids, and phenolic compounds making it suitable for antibiotic, bacteriostatic, purposes (Bajaj, 1988).

This study investigated some elements such as Sodium (Na), Potassium (K), Lead (Pb), Copper (Cu), Manganese (Mn), Zinc (Zn). Elements in the stem bark and pod pulp of *cassia sieberiana* was determined as certain elements at elevated levels are toxic, such an assessment is helpful in regulating their use.

Also, attempt was made to carry out the phytochemical screening of the extract from the pod pulp and stem bark of the plant.

## *Materials and Methods*

### *Sample Collection and Identification*

The plant was collected in Adamawa State University Campus behind Fisheries Department. Identification and authentication was done by Elder Ibrahim Till, a plant taxonomist in the department of forestry, Adamawa State Environmental Resource Centre, Mubi. The specimen number to the plant was 074. The pod pulp and stem bark was dried, ground to powder using pestle and mortar. It was stored in cellophane bag at room temperature until required for the experiment.

### *Extraction Technique*

The extract of the sample was prepared by soaking 100g of the dried powdered sample in 200ml of distilled water for 24h. The extract was filtered using Whitman filter paper No 42 (125mm).

### *Phytochemical Analysis*

Phytochemical screening was carried out following the procedures described by Trease and Evans (1989), Sofowara (1993) for tannin, saponin, flavonoids, terpenoids, phlobatannin, cardiac glycosides, steroid and reducing sugar.

### *Test for Tannins*

About 0.5gm of the dried sample was boiled in 20ml distilled water in a test tube and then filtered. A few drops of 0.1% Ferric chloride was added and observed for brownish green or a blue black colouration that indicated the presence of tannin.

### *Test for Phlobatannin*

Aliquot portion of the aqueous extract was boiled with 1% aqueous hydrochloric acid. Deposition of a red precipitate was an evidence for the presence of Phlobatannin.

### *Test for Flavonoid*

5ml of dilute ammonia solution was added to a portion of the aqueous filtrate followed by the addition of concentrated $H_2SO_4$. A yellow colouration that was observed indicated the presence of flavonoids. The colouration disappeared on standing.

### *Test for steroids*

2ml of acetic anhydride was added to 0.5g ethanolic extract of the sample with 2ml $H_2SO_4$. The colour changed from violet to green indicating the presence of steroids otherwise it is absent.

### *Test for terpenoids*

5ml of the extract was mixed in 2ml of chloroform and 3ml of concentrated $H_2SO_4$ was added to form a layer. A reddish brown colouration of the interface formed to show positive result for the presence of terpenoids.

### *Test for cardiac glycosides*

5ml of the plant extract was treated with 2ml glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of con.$H_2SO_4$. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides. A violet ring appeared below the brown ring, while in the acetic acid layer, a greenish ring was formed just gradually throughout thin layer.

*Test for saponin*

About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously; formation of emulsion was noticed.

*Standard test for combined reducing sugar*

1ml of the extract was hydrolysed by boiling with 5ml of dilute HCl. It was neutralised with NaOH. A mixture of equal volume of Fehlings solution A and B was added and boiled in a water bath for 2 minutes. A brick red precipitate indicated the presence of reducing sugar.

*Quantitative Determination of Phytochemical Constituents*

*Alkaloid Determination Using Harborne (1973) Method*

5g of the sample was weighed each into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allow to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one quarter of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate collected and washed with dilute ammonium hydroxide and filter. The residue was the alkaloid, which was dried and weighed.

*Tannin Determination by Van-Burden and Robinson (1981) Method*

500mg of the samples was weighed into a 50ml volumetric flask. 50ml of distilled water was added and shaken in a magnetic stirrer for 1hour. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filterate was pipette into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1M HCl and 0.008M potassium Ferro cyanide. The concentration at wavelenght of 190nm within 10 minutes of preparation was recorded using Ultra Violet spectrometer.

*Saponin Determination by Obadoni and Ochuko (2001) Method*

20gm of the sample was put into a conical flask and 100ml of 20% ethanol was added. The sample was heated over a hot water bath for 4hrs with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extract was reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer discarded. The purification process was repeated. 60ml of n- butanol was added. The combined n- butanol extact was washed twice with 10ml of 5% aqueous NaCl. The remaining solution was heated in a water bath. After evaporation the sample was dried in an oven to a constant weight; the saponin content was calculated as percentage i.e. (mass yield/initial mass) 100%.

*Flavonoid Determination by Method of Bohm and Kocipai Abyazan (1994)*

10g of the plant samples was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whiteman filter paper No. 42 (125mm). The filtrate was then transferred into a weighed crucible and evaporated into dryness over a water bath and weighed to a constant weight.

*Elemental Determination*

*Determination of K, Na. and Ca Using Flame Photometer*

Few grams of the powdered sample was placed inside a weighed crucible and heated in muffle furnace at 400°C till there was no evolution of smoke. The crucible was cooled in desiccators at room temperature. The ash totally free from carbon was moistened with conc.$H_2SO_4$ and heated on hot plate till a fume of sulphuric acid was evolved. The

crucible with sulphated ash was again heated at 600°C in muffle furnace till weight of sample was constant (3-4 hrs). One gram sulphated ash was taken into beaker which dissolved in 100ml 5% conc. HCl to obtain solution for determination of K and Na through flame photometer.
The respective concentration of the elements was determined from the standard curve. (Indrayan et al, 2005).

*Determination of Cu, Pb and Mn with Atomic Absorption Spectrometer. (Schramel et al, 1988).*

About 1.25g dried samples was transferred to the destruction tube (Kjeldal flask), 25ml $HNO_3$ was added, three boiling chips added and a funnel was placed on top of the destruction tube. The tube was heated to 100°C, 125°C, and 150°C and maintain at each interval for 1 hour, 15 minutes and 10 minutes respectively.

The tube was heated to 200°C and $HNO_3$ was added. The mixture was concentrated to about 5ml. After cooling, 1ml 30% $H_2O_2$ was added and destructed for 10 minutes. It was cooled again after adding 3ml 30% $H_2O_2$ and destructed for 10 minutes. 25ml water was added and heated till boiling. The whole sample was cooled, filled up to the mark, mixed and settled for at least 6 hours. The absorbance of the clear supernatant was measured and the various concentrations determined from the standard calibration curve.

*Statistical Analysis*

All Data in this work was expressed as mean ± SD.

*Results*

The result of the phytochemical screening of the pod pulp and stem bark of *Cassia Sieberiana* DC is presented in Table 1. The result showed that terpenoid, cardiac glycosides, steroids, flavonoids, saponins, phlobatannins, tannins and reducing sugars were all present in the pod pulp and stem bark of *C. Sieberiana* DC.

The result of quantitative estimations of the pod pulp and stem bark of *C. Sieberiana* is indicated in Table 2. The result showed that the pod pulp has the highest concentration of flavonoids (153.3±0.062) followed by saponins (37.17±0.016) with tannins having a minimal concentration (8.1±0.007). Also, the result showed that saponin is present in higher concentration (27.43±0.096) in the stem bark as compared with the pod pulp followed by flavonoid (23.33±0.222). Alkaloids have the lowest concentration (2.07±0.002) in the stem bark.

Elemental analysis was carried out on the pod pulp and stem bark of *cassia sieberiana using* Atomic Absorption Spectrophotometer. A total of 6 elements: Na, K, Zn, Cu, Mn, and Pb were identified in this plant and their concentrations were estimated as shown in Table 3. The result reveals that Mn was found to have the highest concentration in both the pod pulp and stem bark of the plant ($2.410\pm6.67\times10^{-7}$ and $1.653\pm2.22\times10^{-5}$ respectively) and Zn has the lowest concentration ($0.125\pm2.22\times10^{-7}$) in the stem bark. The concentration of K, Na, and Pb is greater in the stem bark when compared with the pod pulp.

*Table 1: Result of the phytochemical analysis*

| | Pod pulp | Stem bark |
|---|---|---|
| Terpenoid | + | + |
| Cardiac glycosides | + | + |
| Flavonoids | + | + |
| Saponins | + | + |
| Phlobatannins | + | + |
| Steroids | + | + |
| Tannins | + | + |
| Reducing sugars | + | + |

+ = present

*Table 2. Result of Quantitative estimation*

| | Pod pulp % | Stem bark % |
|---|---|---|
| Flavonoids | 153.33±0.062 | 23.33±0.222 |
| Tannins | 8.10±0.007 | 8.07±0.009 |
| Saponins | 37.17±0.016 | 27.43±0.096 |
| Alkaloids | 10.13±0.016 | 2.07±0.002 |

*Table 3: Result of Elemental Analysis.*

| Elements | pod pulp (ppm) | Stem bark (ppm) |
|---|---|---|
| Na | $0.326\pm1.55\times10^{-6}$ | $1.001\pm6.67\times10^{-7}$ |
| K | $1.505\pm1.49\times10^{-5}$ | $2.504\pm6.89\times10^{-6}$ |
| Cu | $0.426\pm6.67\times10^{-7}$ | $0.303\pm2.22\times10^{-5}$ |
| Zn | $0.251\pm8.89\times10^{-7}$ | $0.125\pm2.22\times10^{-7}$ |
| Mn | $2.410\pm6.67\times10^{-5}$ | $1.653\pm2.22\times10^{-5}$ |
| Pb | $0.876\pm6.67\times10^{-7}$ | $1.001\pm1.56\times10^{-6}$ |

*Discussion*

The result of the phytochemical screening of the pod pulp and stem bark of *cassia sieberiana* DC indicated the presence of terpenoid, cardiac glycosides, steroids, flavonoids, saponins, phlobatannins, tannins and reducing sugar. They were known to show medicinal activity as well as exhibiting physiological activity (Sofowara, 1993). The result of this study is supported by the report of Toma *et al* (2009) who also reported the presence of tannins, saponins, cardiac glycosides, steroids, flavonoids, phlobatannins, terpenoid and reducing sugar in the pod pulp. The presence of steroids and phlobatannins are of importance and interest in pharmacy due to their relationship with such compound as sex hormones (Okwu, 2001).

This may be the reason the stem bark of *C. Sieberiana* are used as herbs for breast feeding mothers to ensure their hormonal balance, since steroidal structure could serve as potent starting material in synthesis of these hormones (Okwu, 2001). The presence

of tannins and flavonoids makes it useful for immediate relief of irritating bowel disorder, arthritis, rheumatism (Gledhill, 1991). Cardiac glycosides is present in the plant which suggests its use chemotherapeutically in pulmonary troubles and in the treatment of cardiac failure due to anti-arrhythmic effects i.e. ability to increase cardiac output by increasing force contraction (Edeoga, 2005). The plant is taken as herbs for energy by many cells when transported in the blood due to the presence of reducing sugars (Toma *et.al.*, 2009). The plant terpenoid and saponin play a role in traditional herbal remedy (Sofowara, 1993).

The quantitative estimation of the percentage yield of the chemical constituents of *Cassia.sieberiana* showed that the pod pulp and stem bark was rich in alkaloids, flavonoids, tannins and saponin. This is in agreement with the report of (Toma *et al* 2009). However, in contrast to the study of (Toma *et al* 2009), flavonoids were found in higher amounts in the *pod* pulp and stem bark of the plant. This is because of the variation in bioactive compounds of the same plant due to their different environment where they are found (E*lujoba, 1989*). Thus, the stem bark and pod pulp are used as herbs because of the antioxidant effects of its flavonoids. In this study, tannins is present in moderate concentrations therefore, it does not inhibit the absorption of minerals such as Zinc, Copper and Manganese (Brune *et al, 1989*) in the body. The plant is used in traditional medicine preparations because saponin enhances the penetration of macromolecule such as protein through cell membranes (Francis *et al, 2002*). The stem bark of *C. Sieberiana* DC has a minimal amount of alkaloid hence it is used as medications for treatment of lice, rashes (www.googlebooks/ alkaloids, 2010). The presence of alkaloid and saponin suggest its bitter taste.

The result of the elemental analysis of the pod pulp and stem bark of *C. Sieberiana* shows the presence of Na, K, Cu, Zn, Pb and Mn. It is well known that trace elements play a vital role in the various biochemical and physiological processes in humans. As diabetes is a disease of metabolic abnormality, elements as such or as a component of enzymes may play a significant role in the development and control of diabetes (Naga *et al,* 2006). The elements K, Mn, Cu, and Zn are responsible for the secretion of insulin from beta cells of the islets of laangerhans and are involved in potentiating insulin action (Naga *et al, 2006*). Sodium and Potasssium are reported to be important elements for the maintenance of acid-base equilibrium and osmotic pressure of the body fluid. It has also been reported that Potassium, if taken simultaneously with Sodium prevents increase in blood pressure (Kolata, 1982). The presence of Zinc and lead in this plant suggest its role in many enzymatic processes and could be involved in working of genetic materials, proteins, immune reactions; wound healing, development of the foetus and sperm production. Further, in the prevention of diarrhoea (Hamitton *et al,* 1988). Pb is present in minute amount in the pod pulp and stem bark of this plant i.e. << when compared to normal lead level of 10microgram/gram in children and 40microgram/gram in adults (Woolf *et al*, 2007). This shows that the plant does not infer any toxicity due to lead when administered as herbs. The pod pulp and stem bark of the plant is very rich in manganese, hence, it is a useful herb for the nourishment of nerves and brain and is important in the breakdown of fats and cholesterol (Chaturvedi, 2004). Also, Cu is a factor necessary for the absorption and use of Fe in the formation of haemoglobin and helps in the formation of protective covering around nerves. This suggests the reason *Cassia sieberiana* is administered to growing children as herbs (Hamitton *et al, 1988*).

## *Conclusion*

This study on the phytochemical screening and determination of elemental

contents of the pod pulp and stem bark of *Cassia sieberiana* shows that tannins, phlobatannins, terpenoid, saponin, flavonoids, steroid, cardiac glycosides and reducing sugar were present in the plant. Also Na, K, Cu, Pb, Zn and Mn were present. The results show that this plant contains elements of vital importance in man's metabolism that are needed for growth and development, prevention and healing of diseases such as diarrhoea. It is hoped that the data obtained in this work could serve as an important resource for development of new chemotherapeutic agents and further studies in medicinal plants particularly *Cassia sieberiana*.

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