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# Phytochemical Analysis and Acute Toxicity Study of *Cassio occidentalis* Leaf Extract in Albino Wistar Rats

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## Abstract

The extract of *Cassio occidentalis* is externally applied on healing wounds, sores, and itch, skin diseases, and bone fracture, ringworm and throat infection. Other uses of the plant include diuretics, laxative, anti-bacteria, and anti-inflammatory, anti-fungal etc. The phytochemical and acute toxicity (LD50) of the leaf extract of *Cassio occidentalis* were investigated. The result revealed the presence of alkaloid, flavonoid, tannins in small quantity, saponins, anthraquinones in minute while cardiac glycoside, steroids and terpenoids were not found. Also, the acute toxicity of *Cassio occidentalis* is greater than 5000 mg/kg body weight (LD50 > 5000 mg/kg). From the result, it revealed that the phytochemical constituents; alkaloid, flavonoid, tannins, saponins, anthraquinones may contribute greatly to the effects of the plant and the oral consumption of the leaf extract may not be considered toxic.

***Keywords:*** *Cassio occidentalis; Phytochemical Analysis; Acute Toxicity*

## Introduction

Current trends in drug development process are focused on natural sources, especially sources of plant origin due to some proven correlation between the folkloric medicinal uses of some of these plants to biological activity. The use of plant materials to prevent and treat infectious diseases successfully over the years has continued to attract the attention of scientist worldwide [1-4]. Most of the plants exhibit a variety of phyto-pharmaceuticals, which has important applications in the fields of agriculture, human and veterinary medicine. This plays a major role in developing novel drugs for the treatment and prevention of diseases [5].

*Cassia occidentalis*, commonly known as Coffee Senna is a small erect herb that can grow up to 2m tall and found abundantly in rainforest and tropical areas of the world. It belongs to the family Leguminosae. Botanically, is classified as both *Cassia occidentalis* and *Senna occidentalis* [6]. *Cassia occidentalis* has a rich history in natural medicine and the parts of the plants used in herbal medicine include Root, Leaves and Seeds. Its seeds found in long seed pods are sometimes roasted and made into coffee like beverages. The leaves have ethno medical importance. Paste of the leaf is externally applied on healing wounds, sores, itch, skin diseases, bone fracture, ringworm and throat infection. Other uses of this plant include Diuretics, Laxative, Anti-bacteria, anti-inflammatory, anti-fungal etc [7].

The *Cassia* genus comprises some species of trees, shrubs, vines and herbs with numerous species growing in the South American rainforest and tropics. Many species have been medicinally, and these tropical plants have a history in natural medicine as purgative and laxatives [8]. It exhibited a lot pharmacological important such as antimicrobial, anthelmintic, insecticidal, antioxidant, antianxiety, antidepressant, antimutogenic, antidiabetic, wound healing, hepatoprotective, renoprotective, sun protective, smooth muscles relaxation, immune-modulating, anti-inflammatory, analgesic, antipyretic and other effects [9]. Therefore it is very important to have sufficient knowledge regarding the medicinal plant, not only because of their widespread uses but also because they have the potentials to cause toxic reactions or interact with other drugs [10].

The active principle of many drugs found in plants is phytochemical [11]. The medicinal value of these phytochemicals is because of the presence of chemical substance that produces definite physiological action on the human body [12]. Some of the valuable chemical constituents are Alkaloids, tannins, saponins, glycosides, flavonoids, phosphorus and calcium for cell growth, replacement, body building [12]. Taiwo., *et al.* [13] in their study of the biocidal and phytochemical properties of leaf extract of *Cassia occidentalis* reported that the

phytochemical constituents of (n-hexane and dichloromethane) extract of *Cassio occidentalis* show the presence of saponins, flavonoids, tannins, Anthraquinones only. As the flavonoids are present, it might be responsible for its anti-inflammatory properties. Chinese folkloric medicine contains flavonoids which has anti-inflammatory effect on both acute and chronic inflammation [4]. Alkaloids are known for decreasing blood pressure, balancing the nervous system in case of mental illness and antimalarial properties [14]. Tannins help in wound healing and anti-parasitic. Presence of terpenes suggests possessing anti-tumor and anti-viral properties (Ilodigwe., *et al*. 2014). Egharevba., *et al.* [6], in their study of phytochemical analysis and broad spectrum antimicrobial activity of *Cassia occidentalis* reported the presence carbohydrate, saponins, terpenes, sterols, flavonoids, alkaloids, phenols, resin, balsam, cardiac glycoside, phlobatannins, anthraquinones. Nuhu and Aliyu [15], reported the presence of tannins, flavonoids, saponins, *Cardenolides*, in the study of effects of *Cassia occidentalis* aqueous leaf extract on biochemical markers of tissue damage in rats.

Toxicity studies on the aerial parts, leaves, roots of *C. occidentalis* reported that various leaf and root extracts given to mice (administered orally and injected at up to 500 mg/kg) cause mortality [16,17]. Onakpa and Ajagbonna [18], reported in a study of antidiabetic potentials of *Cassia occidentalis* leaf extract on alloxan induced diabetic albino mice that acute toxicity study revealed the non-toxic nature of the *Cassia occidentalis* leaf extract. When dosed orally using lorke 1983 method, to 1500 mg/kg body weight. It is then considered that the; LD50 > 1500 mg/kg body weight *Cassia occidentalis* did not produce any hazardous symptoms or death in the acute toxicity test, showing a LD (50) higher than 5 g/kg (5000 mg/kg body weight) [19]. Vashishtha., *et al*. [20], reported the median lethal dose (LD (50)) is 1 g/kg for mice and rats. Consumption of *Cassio Occidentalis* for maintenance of health and in management of several diseases indicated its medicinal importance; the study therefore aimed to evaluate the phytochemical constituents and to determine the acute toxicity of *Cassio occidentalis* leaf extract.

## Material and Method

### Collection and authentication of the plant material

Sample Collection and Preparation Fresh mature leaf samples of *Cassio occidentalis* were collected from Nnewi, in Nnewi-North Local Government Area of Anambra State, Nigeria in the month of July 2015.

### Plant identification

The botanical identification of the plant was confirmed by Mr. Egboka Tochukwu of the department of Botany, Nnamdi Azikiwe University Awka, Anambra state in July, 2015.

### Preparation of extracts

Dried and milled leaf materials were extracted successively with Soxhlet extractor at temperature of 800C. Each of the solvent; hexane, ethyl acetate and methanol were allowed to remain in contact with the plant material for 12 hours; the extracts were evaporated to dryness using rotary evaporator.

### Phytochemical analysis

The phytochemical analysis of *Cassio occidentalis* was carried out in the pharmaceutical laboratory using standard procedures as described by Sofowora (1993), Trease and Evans [21] and Harbone [12] and the extracts were analyzed for the presence of alkaloids, resins, tannins, saponins, flavonoids, glycosides, phenols, anthraquinones, cardiac glycosides, steroids, phlobatannins, reducing sugars.

### Acute toxicity (LD50) (lethal dose)

The acute toxicity study of the leave extract was determined using modified Dietrich Lorke's (1983) method.

13 Albino wistar rats weighing 98 - 150g were obtained from veterinary lab in Nnewi, Nnewi-North, Anambra State, Nigeria. The rats were acclimatized for one week, kept in plastic cages at room temperature and fed pelleted diet and water throughout the experimental period.

### Ethical approval

Ethical approval was obtained from the Faculty of Basic Medical Sciences Ethics Committee, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

### Guide to care and use of experimental animals

The guide to the care and the use of experimental animals was followed according to the Canadian Council on Animal Care (CCAC), Ernest., *et al* [22]. It demanded the use of non-sentient methods, and noted that when an animal must be used, there is an obligation to: Provide humane care and treatment, minimize pain and discomfort and avoid unnecessary use.

### Experimental design and animal treatment

### First-phase

This will involve two phases which was represented using the table below.

In first phase, a total number of 9 adult male and female wistar rats were used. They were grouped into 3 groups of 3 animals per group.

| Groups | Animals | Dosage | Weight |
|---|---|---|---|
| Group 1 | 3 animals | 10 mg/kg | 120 |
| Group 2 | 3 animals | 100 mg/kg | 125 |
| Group 3 | 3 animals | 1000 mg/kg | 100 |

### Second phase

From the result of the phase 1, mortality wasn't recorded; the second phase was carried out with the use of total number of 4 animals. They were grouped into four groups of one animal per group and were dosed orally with *Cassio occidentalis* ethanolic leaf extract at dose levels of 2000, 3000, 4000 and 5000 mg/kg body weight respectively.

| Groups | Animals | Dosages | Weights |
|---|---|---|---|
| Group 1 | 1 animal | 2000 mg/kg | 100 |
| Group 2 | 1 animal | 3000 mg/kg | 122 |
| Group 3 | 1 animal | 4000 mg/kg | 110 |
| Group 4 | 1 animal | 5000 mg/kg | 100 |

The animals were constantly monitored for the next 2 hours and over a period of 24 hours for behavioural changes and mortality.

The $LD_{50}$ (lethal dose) will be determined using (the formula) relation:

⤵A×B.

Where A= The lowest dose that brought death.

B= The lowest dose that did not bring death.

## Result

| Sample | Alkaloid | Saponins | Flavonoid | Anthraquinones | Tannins | Cardiac Glycoside | Steroids | Terpenoids |
|---|---|---|---|---|---|---|---|---|
| *Cassia occidentalis* | ++ | + | ++ | + | ++ | --- | --- | --- |

***Table 3:*** *Shows the Quantitative and qualitative phytochemical analysis of cassia occidentalis is leaf extract.*

***Key:*** *---: Absent; +: Trace/mildly present; ++: Moderately present.*

### Acute toxicity testing (Lethal Dose ($LD_{50}$)

The result of the lethal dose of *Cassio occidentalis* leaf extract which was carried out using detrick lorke (1983), showed that the acute toxicity of the *Cassio occidentalis* is greater than 5000 mg/kg body weight (lethal dose > 5000 mg/kg).

## Discussion

Phytochemical analysis is very useful in the evaluation of some active biological components of some plants [23]. Phytochemical screening helps to reveal the chemical nature of the constituents of the plant extract which may also be used to search for bioactive agents that could be used in the synthesis of very useful drugs [24]. Chemical constituents of plants are known to be influenced by season, age and geographical location [25]. The result of the phytochemical screening revealed the presence of saponins, flavonoids, resins, alkaloids, anthraquinones at different quantity and absence of cardiac glycoside, steroids, terpenes and balsam. The result of the study is similar with the study by Egharevba., et al. [6], which revealed the presence saponins, flavonoids, resins, alkaloids and anthraquinones [15,26,27] and differed for the presence of carbohydrates, sterols, terpenes, cardiac glycoside [6,27] and Cardenolides [15]. The presence of these metabolites suggests great potential for the plant as source of useful phytomedicines. For instance, the presence of flavonoids and resins might be responsible for its use as anti-inflammatory recipe in Chinese folkloric medicine as some flavonoids has anti-inflammatory effect on both acute and chronic inflammation [4]. Some plants that possess alkaloids are known for decreasing blood pressure and balancing the nervous system in case of mental illness. The presence of tannins could also shows that it is an astringent, help in wound healing and anti-parasitic [6]. Alkaloids are known to possess anti-malaria property; hence the plant may be a good source of anti-malaria for which it is traditionally uses in locally [14]. Also the use of *C. occidentalis* as genital stimulant may be attributed to the presence of alkaloids. Plant containing saponins are believed to have antioxidant, anti-cancer, anti-inflammatory, and anti-viral properties [6]. Variations also occur in the distribution of constituents in the distribution of constituents in different organs of plants [25].

Tanimu and Wudil [27], reported in a study of acute toxicity of *Cassia occidentalis* that the plant did not show any hazardous symptoms or death. The result from the acute toxicity study (lethal dose (LD50) showed that the oral administration of the extract of *Cassio Occidentalis* to the dose greater than 5000 mg/kg (lethal dose > 5000 mg/kg body weight) did not show any sign of toxicity which is similar with the research of Onakpa and Ajagbonna [18], Silva., *et al.* [19], Vashishtha., *et al* [20]. The result may be an indication that the extract could be considered less toxic, especially when administered orally [28-33].

## Conclusion

Traditional use of *Cassia occidentalis* in treatment of various infectious diseases in different regions of the world can be attributed to the presence of the phytochemical constituent which is responsible for the bio-activities of the plant and the acute toxicity study reveals that the plant may be non-toxic when consumed orally. This could also play an important role in the establishing data for preparation of monograph of the plant.

## Recommendation

The need for quantitative analysis of *Cassio occidentalis* is much needed, to enable access the specific quantities of the chemical constituents which maybe be responsible for the plants efficacy.

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