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Physicochemical and Phytochemical Evaluation of leaves of *Cassia singueana* Del.

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Cassia singueana leaves possess great therapeutic values in the traditional system of medicine and some of these have been scientifically established thereby making it commercially viable. This research investigated the physicochemical and phytochemical characters of the powdered leaves of *Cassia singueana* used as a crude drug. The physicochemical parameters along with organoleptic evaluation and Phytochemical screening of aqueous, methanol and petroleum ether extracts of *Cassia singueana* were carried out. The moisture contents, total ash, acid insoluble ash, water and alcohol soluble extractive values were found to be 4.35 ± 0.21, 7.28 ± 0.06, 0.86 ± 0.03, 22.41 ± 0.45 and 36.52 ± 0.81 % (w/w) respectively. The leaf powder had a distinct characteristic odour and a slightly bitter taste with an astringent and nauseous sensation to the tongue. The phytochemical screening revealed the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, proteins, saponins and tannins. The results of this study show that the crude drug is in good preservative condition with negligible extraneous materials and the biological activity of *C. singueana* leaves is as a result of the individual and/or synergetic activities of its various phytochemical constituents.

Keywords: *Cassia singueana,* physicochemical, phytochemical, crude drug, traditional medicine

1. Introduction

The use of traditional medicine is the oldest form of healthcare known to human race and has been used in all traditions throughout history (Kunle *et al.,* 2012). The cultural aspects of herbalism in different countries and societies play an important role to the diversity of worldwide herbal use (Dwivedi *et al.,* 2014). Evaluation of drugs means confirmation of its identity and determination of its quality, purity and detection of nature of adulteration (Kumar *et al*., 2013).

Figure 1**:** *Cassia singueana* in its natural habitat

Cassia singueana Del., (Figure 1) commonly known as Runhu in the Hausa speaking language belongs to the family *Caesalpinioideae*. It is a woody annual herb or under shrubs between 1.2 and 1.5m high with small yellow flowers. It is widely spread in India and tropical Africa including northern Nigeria, especially in cultivated or old clearings, by the road side and open grassy areas (Ior *et al*., 2015).

In traditional medicine the leaves of *Cassia singueana* are used in the management of peptic ulcer (Ode and Asuzu, 2011), acute malaria (Ior *et al*., 2015), abdominal pain (Dambatta and Aliyu, 2011), skin cancer (Mebrahtom, 2012), urinary schistosomiases, hernia, convulsion, constipation, heartburn and snake bite (Adzu and Gamaniel, 2003). The powdered crude drug of *Cassia singueana* is sold in commercial quantity in sokoto metropolis as well as other northern states in nigeria (Dambatta and Aliyu, 2011; Ior *et al*., 2015; Uba and Baburo, 2016$_{ab}$; Uba *et al*., 2016). The aqueous extracts of *C. singueana* revealed the presence of phenolics, quinines, tannins, terpenoid, glycosides, alkaloids, anthraquinones and flavonoids (Usman *et al.,* 2018). Proximate analyses of *C.*

singueana leaves revealed that the leaves have a low moisture content with high crude protein and fat content (Usman *et al.,* 2018).

The crude methanol extract of *C*. *singueana* leaves demonstrated increased percentage preventive index against ethanol induced gastric ulcer compared to omeprazole treated rats (Ode *et al*., 2011). Ode and Asuzu (2014), reported that Luteolin isolated from the extract of *C. singueana* leaves was identified as the antiulcer agent with broad mechanism of actions. Alsiede *et al.* (2015), found that there are high contents of phenolic compounds and significant linear correlation between the values of the concentration of phenolic compounds and antioxidant activity of *C. singueana*. In a study conducted by Mebrahtom *et al.*, (2014), it was discovered that ethanol extract of *C. singueana* leaves was relatively safe to mice and demonstrated anti-malarial activity.

The *in vitro* anthelmintic screening of *C. singueana* leaves and stem, showed some effect against schistosomiasis using newly excysted tapeworms (cestodes) of *Hymenolepis diminuta* as the primary test organism (Mølgaard *et al.*, 2001). It was found that ethanol and aqueous extracts of *Cassia singueana* leaves may have some valuable anti-microbial activities against Gram positive and Gram-negative microorganisms (Adeyanju *et al.*, 2011). In a study of the long-term effects of *C. singueana* leaf extract on haematological parameters in rats, it was found that long-term exposure to *C. singueana* extract exerted no toxic effects on the hematological indices of the rats (Ode and Nwaehujor, 2010). Evaluation of the Biochemical changes in rats following chronic toxicity with *C. singueana* leaf extract revealed no toxic damage in the liver, heart or bones but possibly in the kidneys (Ode *et al.*, 2011). In a study of the levels of some heavy metals in various plants, *C. singueana* contained toxic level of Cadmium (Usman *et al.,* 2018).

In light of the medicinal uses and commercialisation of *Cassia singuena* crude drug, it is pertinent to establish some standards required for the quality control of the crude drug. Hence, the present study was carried out to investigate the physicochemical and phytochemical characteristics of *Cassia singueana.*

2. Materials and Methods

2.1 Collection and authentication of plant material

Three samples of *Cassia singueana* leaves were collected in the wild from Dange-Shuni (12°45′53.64″N, 5°25′34.716″E), Sabon Birni (13°24′11.178″N, 6°16′23.682″E) and Rabah (13°11′26.58″N, 5°38′36.018) local governments of Sokoto State, Nigeria. The fresh leaves were identified by a consultant Taxonomist in the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The voucher number; PCG/UDUS/LEGU/0001 was given to the sample.

2.2 Preparation of Plant Material

The three samples were thoroughly washed and garbled before drying in a hot air oven at 45°C. After drying, the samples were garbled again and coarsely powdered using a sterilised electric blender. Representative samples were quantitatively taken from each sample and homogenised using mortar and pestle. The homogenised sample was stored in an air tight plastic container until analyses.

2.3 Organoleptic Evaluation

The organoleptic evaluation on the leaf powder was performed to determine the sensory characters (colour, odour, smell and taste). It was done according to the methods of Chanda (2013).

a. Colour: The colour of *C. singueana* was observed under ordinary light and the hue was recorded.
b. Odour: A small portion of *C. singueana* powder was placed in a 50cm^3 beaker and the air over the material was slowly and rapidly inhaled. The strength and sensation of the odour were recorded.
c. Taste: A small portion of *C. singueana* powder was repeatedly placed on the tongue and moistened with the saliva. The true and sensual tastes were recorded.

2.4 Physicochemical Evaluation

2.4.1 Moisture Contents

Three grams of the powdered drug sample was weighed into three pre-weighed crucibles and placed into a hot air oven at 105 °C for 24 h. The samples were then cooled in a desiccator and weighed again. This was repeated until constant weight is recorded (Uba *et al.,* 2016$_b$; James, 1995). The weight lost due to moisture was calculated using equation 1.

$$Moisture\ content\ (\%\, w/w) = \frac{W1 - W2}{W1 - W0} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where**:** W0 = Weight of empty crucible; W1 = Weight of fresh sample; W2 = Weight of dried sample

2.4.2 Total Ash Value

The dried samples above were placed in a furnace at 600^{0}C for three hours. The samples were cooled in a desiccator and weighed. The total ash in the sample was determined as percentage of the initial dry weight of the sample as shown in equation 2 (Uba *et al.,* 2016$_b$; James, 1995).

$$Total\ ash\ (\%\, w/w) = \frac{W2 - W0}{W1 - W0} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where: W0 = Weight of empty crucible; W1 = Weight of crucible + dry sample; W2 = Weight of crucible + ash sample

2.4.3 Acid Insoluble Ash

The total ash obtained above was boiled with 25 mL of dilute hydrochloric acid (10%) for five minutes and filtered through an ash-less filter paper. The insoluble ash was washed with hot distilled water until the filtrate is neutral. The insoluble ash along with ash-less filter paper were taken in tarred silica crucibles and incinerated at 450^{o}C. The content was then cooled and weighed (Rani and Satish, 2014). The percentage of acid insoluble ash was calculated using equation 3;

$$Acid\ insoluble\ ash\ (\%\, w/w) = \frac{Weight\ of\ acid\ insoluble\ ash}{Weight\ of\ sample} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

2.4.4 Determination of Extractive Values

The alcohol and water-soluble extractive of *C. singueana* were determined according to the method described in the United States pharmacopeia (2005). Exactly 5g of coarsely powdered air-dried drug were macerated with 100 mL of solvent (80% ethanol and water) in a closed flask for twenty-four hours. The flask was occasionally shaken during the first six hours and allowed to stand for eighteen hours. It was then filtered rapidly; taking precautions against loss of solvent. 25 mL of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish at 105°C to constant weight. The percentage of alcohol and water-soluble extractive was calculated with reference to the air dried drug and represented as % extractive value using equation 4;

$$Extractive\ value\ \%\,(w/w) = \frac{Wr \times 100}{Ws \times 25} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

2.4.5 Determination of pH

A 1% solution of *C. singueana* was prepared with distilled water and the pH was determined using a Digital pH meter. The pH meter was calibrated with pH tablet (pH 4). At the end of a set of measurements, the reading of the aqueous solution was noted (Dwivedi *et al.*, 2014).

2.5 Extraction of Plant Material for Phytochemical Screening

The powdered plant materials (10g) each of the three plant samples were transferred separately into 250 mL conical flask and macerated with 100 mL of petroleum ether, ethanol (80%) and water respectively for 24 hours with occasional shaking. The extracts were filtered through a Whatman No. 2 filter paper, evaporated on a water bath and finally dried in an oven (Sruthi and Indira, 2016).

2.6 Qualitative Analysis

Qualitative chemical tests for detection of alkaloids, anthraquinones, proteins, cardiac glycosides, tannins, saponins, terpenoids, and flavonoids were carried out on the various extracts using standard procedures as illustrated by Sofawara (1993), Trease and Evans (2009), Sruthi and Indira (2016) & Ahmad (2012).

3. Results and Discussion

3.1 Organoleptic Properties

The result of Organoleptic evaluation (macroscopic features) is shown in Table 1. Organoleptic evaluation provides the simplest as well as quickest means to establish the identity and purity to ensure quality of a particular drug (Chanda, 2013). The organoleptic evaluation of *cassia singueana* revealed that the leaf powder is green which has a distinct characteristic odour and a slightly bitter taste with astringent and nauseous sensation to the tongue. In a study by Rani and satish (2014), the colour, taste and odour of *Cassia tora* powdered leaves is green, slightly bitter and characteristic respectively. Powdered leaves of *Cassia occidentalis* is coloured green, with a bitter taste and fetid odour (Manikandaselvi *et al.,* 2016; Rani and satish

2014). The powdered leaves of *Cassia fistula* are green with a faint characteristic odour and a slightly bitter taste (Rani and satish 2014). The above-mentioned crude drugs have similar Organoleptic feature with *Cassia singueana* with regard to colour, odour and taste; however, they may vary in their sensation to the tongue. The Organoleptic parameters can be used for first-hand identification or authentication of crude drugs in the market.

Table 1: Organoleptic Properties of *Cassia singueana* Drug Powder

Features	Observation
1. Colour	Green
2. Odour	
•Strength	Distinct
•Sensation	Characteristic
3. Taste	
•True	Slightly bitter
•sensual	Astringent and nauseous

3.2 Physicochemical Properties

The result of physicochemical parameters of *Cassia singueana* which are physical constants used in standardisation of herbal medicine is presented in Table 2.

Table 2: Physicochemical Properties of *Cassia singueana* leaf powder

S/N	Parameters	Values obtained (% w/w)
1.	Loss on drying	4.35 ± 0.21
2	Total ash	7.28 ± 0.06
3.	Acid insoluble ash	0.86 ± 0.03
4.	pH*	6.25 ± 0.05
5.	Alcohol soluble extractive value	36.52 ± 0.81
6.	Water soluble extractive value	22.41 ± 0.45

'*'= No unit.

The percentage loss on drying (LOD) was found to be 4.35 ± 0.21 % (w/w). Shade dried sample of *Cassia singueana* collected in Gombe shows higher moisture content (5 %) compared with the current sample (Usman *et al.,* 2018). Air dried drugs contain about 10-12% of moisture and this may be sufficient to activate enzymes present in the leaves and bring about decomposition of the glycoside (Trease and Evans, 2009). In a study of commercial samples of *C. singueana* leaves powder, a similar result was obtained in terms of the moisture content (Uba *et al.,* 2016; Uba *et al.,* 2016_a; Uba *et al.,* 2016_b). Shade dried sample of *Cassia singueana* collected in Gombe shows higher moisture content (5 %) compared with the current sample (Usman *et al.,* 2018). Other *Cassia* species such as *Cassia occidentalis* (10.17 %), *Cassia grandis* (18.48±0.89 %), *Cassia auriculata* (8.93±04 %) and *Cassia fistula* (7.794 %) shows higher amount of moisture content (Manikandaselvi *et al.,* 2016; Rani and Satish, 2014; Meena *et al*., 2010; Saraswathy *et al.,* 2009). The moisture content of a drug is minimised in order to prevent its decomposition either due to chemical changes or microbial contamination (Pradhan *et al.,* 2015).

The total ash value of *Cassia singueana* was found to be 7.2756 ± 0.0592 %, while acid insoluble ash was found to be 0.8578 ± 0.0334 % (w/w). In a study of commercial samples, the ash values were found to be high in *Cassia singueana* powdered leaves which indicates some level of contamination possibly by silica material (Uba *et al.,* 2016; Uba *et al.,* 2016_a; Uba *et al.,* 2016_b). In a study by Usman *et al*. (2018), the total ash content of *Cassia singueana* crude drug was found to be slightly lower than the sample in the current study could be attributed to difference in soil conditions. The ash values are present in definite amount in a particular crude drug, hence quantitative determination in terms of various ash values helps in their standardisation (Pradhan *et al.,* 2015).

The alcohol soluble and water-soluble extractive values are determined to be 36.52 ± 0.81 % and 22.41 ± 0.45% (w/w) respectively. The extractive values which include water soluble and alcohol soluble extractives indicate the amount of active constituents in a given amount of plant material when extracted with respective solvents (Dwivedi *et al.,* 2014). This result indicates that 80% ethanol extracted a higher amount of active constituent in *Cassia singueana*. It has been found that the combination of water and organic solvent may facilitate the extraction of chemicals that are soluble in water and/or organic solvent (Anwar and Przybylski, 2012; Do *et al.,* 2014; Hijazi *et al.,* 2015; Cieniak *et al.,* 2015; Waszkowiak and Gliszczyn´ska-S´wigło, 2016). In a study by Anwar and Przybylski (2012), high amount of total phenolics were extracted from *Linum usitatissimum* using 80% ethanol. In the comparison of water and methanol leaf extract of *Sarracenia purpurea*, it was found that the 80% ethanol extract generally results in significantly higher quantities of phenolics present, as well as the triterpenes (Cieniak *et al.,* 2015). Therefore, the high extractive value of 80% ethanol may be indicative of a high amount of phenolic compounds in *C. singueana*. Extractive values are primarily useful for the determination of exhausted or adulterated drugs (Swarna and Ravindhran, 2013).

The pH of 1% aqueous solution of *Cassia singueana* was found to be 6.25 ± 0.05 which is weakly acidic. Thus, traditional decoctions of *C.*

singueana may not cause any gastrointestinal irritation (Amponsah *et al*., 2016).

Preliminary Phytochemical Screening

The preliminary phytochemical screening with the different qualitative chemical tests which revealed the presence of various secondary metabolites is presented in Table 3.

Table 3: Phytochemical Screening of *cassia singueana* leaves extracts

Metabolites	Test/Reagents used	Extracts		
		Aqueous	Ethanol	Pet. Ether
Alkaloids	Wagner's test	+	+	-
	Mayer's test	-	-	-
	Dragendorff's test	-	-	-
Anthraquinones	Borntrager's test	+	-	+
Cardiac glycosides	Keller-Killiani test	+	+	+
Flavonoids	Sodium hydroxide test	+	+	+
Proteins	Biuret test	+	+	+
	Million's test	+	+	-
Saponins	Foam test	-	+	-
Tannins	ferric chloride test	+	+	-

In results of the preliminary phytochemical screening (Table 3) the ethanol and aqueous extracts showed positive results for alkaloids, tannins, cardiac glycosides, proteins, flavonoids. However, the presence of saponins was only observed in ethanol extract while anthraquinones are seen in the aqueous and petroleum ether extract. The petroleum ether extract also tested positive for the presence of flavaonoids, antraquinones, proteins and cardiac glycosides. In a similar study by Adeyanju *et al*. (2011), the phytochemical screening of *C. singueana* revealed the presence of tannins, alkaloids, glycosides and terpenes in both aqueous and ethanolic extract while flavonoids and saponnins were only detected in the ethanolic extract which agrees with the present study, except for the presence of flavonoids in the aqueous extract. Another study reported by Usman *et al.* (2018), agrees with the current findings with regards to phytochemicals present in the aqueous extract of *Cassia singueana*. The majority of drugs contain definite chemical constituents to which their pharmacological and biological activity depend on. Qualitative chemical tests are used to identify drug quality and purity (Shailesh *et al.,* 2016).

4. Conclusion

The result of the physicochemical analysis shows that *C. singueana* crude drug is in good preservative condition with minimal extraneous materials. It also shows that the herbal decoction may not cause gastrointestinal irritations with regard to its pH and the alcohol soluble extractive value is high which may be indicative of high phenolic compounds.

Phytochemical screening of *C. singueana* leaves confirmed the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, proteins, saponins and tannins in the various extracts. The biological activity of *C. singueana* leaves is as result of the individual and or synergetic activities of its various phytochemical constituents.

The outcome of the present study provides guide in identification, authentication and quality control of the plant material and can serve in the development of a monograph for the correct identification of the crude drug of *C. singueana* leaves.

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Conflict of interest

The authors declare no conflict of interest.

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