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Toxicity and phytochemical analysis of petroleum-ether, ethanolic and aqueous extracts of *Ceratotheca sesamoides*

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ABSTRACT

This research investigated the toxicity and phytochemical analysis of aqueous, ethanol and pet ether extract of *Ceratotheca sesamoides* leaves in albino wistar rats. Qualitative and quantitative phytochemical screening of *Ceratotheca sesamoides* were carried out. The result of the toxicity test revealed that aqueous *extracts of Ceratotheca sesamoides* leaf did not show any fatality with dosage range (300 mg-5000 mg). This shows that the LD_{50} is greater than 5000 mg/Kg body weight. The phytochemical investigation shows the presence of glycoside, carbohydrate and alkaloid in all the extract and only flavonoid was found in Aqueous and ethanol extracts. This shows that *C. sesamoides is safe for consumption and is rich in* polyphenols, flavonoids and tannins.

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Introduction

Ceratotheca sesamoides commonly known as false sesame is an annual vegetable crop [1] belonging to the Pedaliaceae family. The Pedaliaceae has 16 genera and 60 species largely in Africa, Indo-Malayan region, and tropical Australia [2]. The plant, *Ceratotheca sesamoides* is described by many hairs on the stem and petiole, with leaf margin; the flowers are pink with dots purple or brown and sub erect growth habit. The *Ceratotheca sesamoides* is normally cultivated in Savannah or semi-arid areas of Africa. The native tribes of the savanna ecological zones of Nigeria relish false sesame known as 'eku' or

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‘bunga’ in Yoruba and 'karkashi' in Hausa [3]. The leaves are eaten raw or cooked with starchy morsels prepared from cereals, cassava and yam.

The plant has the following medicinal activities: Anti-oxidant, anti-inflammatory, anti-hypertensive, cytotoxic, anti-tumour, antiviral [4] parasitic infections, diarrhea and dysentery and insecticidal activities [8]. It is also one of the sources of protein, minerals and vitamins [3], *Ceratotheca sesamoides* has been used to treat childhood diseases such as measles [6], the steeped and the slimy liquid of the leaves are dropped into the eye for the treatment of conjunctivitis [7]. The leaves of *C. sesamoides*is used in southern part of Kaduna state to manage infective hepatitis but very little has been done to determine its efficacy. Thus, the study seeks to investigate the Phytotochemical content, Total polyphenol and total flavonoid content of the aqueous, ethanolic and petroleum ether leaves extracts of *Cereatotheca sesamoides*.

General uses of *Ceratotheca sesamoides*: The mucilage is occasionally used as an emollient and lubricant [7]. The leaves are eaten as food with solid such as tuwo and fufu.

At present only a few documents are available about the history of this plant and there is lack of effective scientific research about the plant *Ceratotheca sesamoides.* The plant indicates a potential to have antiviral properties [4] and is used in the southern part of Kaduna state to manage hepatitis virus, but has not been scientifically proven. It is easy to cultivate and it thrives well in all manner of soils even with minimal water supply. Present study therefore, is to determine the toxicity and phytochemical analysis of *Ceratotheca sesamoides* leave plant extract in order to ascertained the safety use of the plant while the knowledge of the phytochemicals will give an inside of the type of phyto-metabolite readily available in the plant.

Materials and Methods

Plant samples

C. sesamoides fresh leaves was sourced from Kafanchan, Kaduna state and authenticated at the Biological Science Department ABU, Zaria and specimen with voucher number 2859 kept at the Department herbarium unit.

Plant extracts preparation

The plant sample collected was thoroughly cleaned, shade dried and powdered by grinding in mortar and pestle. About 500 grams of the powdered leaves was soaked in 2500 mLs of Aqueous, Ethanol and Pet-ether solution for 24 hours at room temperature and filtered. The extracts was concentrated by drying in a water bath maintained at a temperature of 40^{o}C until a residue was obtained and was kept in a sealed container refrigerated at 2-4^{O}C until when required.

Determination of Phytochemical Constituents of the Extract

Phytochemical analysis of the dried extracts (saponins, tannins, alkaloids, flavonoids, cyanogenic glycosides) was carried as described in the protocol of [10, 11, 12]

Quantitative estimation of total phenolic

Total phenolic contents of different extracts of *C. sesamoides* leaves were determined by the modified Folin-ciocalteu method according to [13]. An aliquot of 0.5 ml of each extract (1mg/mL) was mixed with 2.5 mL of Folin- Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 2mL (75% w/v) of sodium carbonate (Na_2CO_3). The tubes were vortexed for 15sec and allowed to stand for 30min at 40 °C for colour development. Absorbance was then measured at 765nm using spectrophotometer. Total phenolics content of different extracts was expressed as mg/g tannic acid equivalent using the following equation from the calibration curve.

Total phenolic contents of different extracts of C. *sesamoides* leaves were determined by the modified Folin-ciocalteu method according to [14]. About 1ml of extracts and standard solution of gallic acid (2-10mg/L) was added into 25ml volumetric flask containing 9mL of distilled water. About 1 mL of Folin- ciocalteau reagent was added to the mixture and shaken. After 5 minutes, 10mL of 7% sodium carbonate (Na_2CO_3) solution was added and the solution was diluted to volume with distilled water and mixed. After incubation for 90minutes at room temperature, absorbance against prepared reagent blank 9 distilled water) was measured at wavelength of 750nm. Total pheolic contents were presented as mg Gallic Acid Equivalent (GAC)/g of extract. All samples were analysed in triplicates.

Total phenolic content (% w/w) = $GAE \times V \times D \times 10^{-6} \times 100/W$,

GAE - Gallic acid equivalent (μg/ml), V - *Total* volume of sample (ml), D - Dilution factor, W - Sample weight (g).

Quantitative estimation of total flavonoids

Total flavonoid contents of different solvent extracts was determined by the method described by [15]. About 0.5mL of various solvent extracts (1 mg/mL) was mixed with 0.5mL of aluminium chloride prepared in (2% in ethanol). The resultant mixture was incubated for 60min at room temperature for yellow colour development which indicated the presence of flavonoid. Absorbance was measured at 420nm using UV–VIS spectrophotometer(Shemazu 2012v). Total flavonoid content was calculated as quercetin equivalent (mg/g).

C = Xx V/N

Where: C= Total Flavonoid content (mg/g), V=volume of extract taken in mL, N=weight of plant extract in g, V = 1mL and N = 0.002 g (2mg), X can be calculated from standard curve

Toxicological studies

Acute oral toxicity of the extract was determined by Standard Official Methods of Analysis of the AOAC Guideline 420 *fixed dose procedure* [16]. A sighting study was carried out by administering different concentration of the extract to different rats to determine the minimal and maximum toxic dose. The range of concentration below the minimum toxic dose was the safety dose and was used in the main study. The main toxicity study was carried out starting from the minimum toxic dose to classify the toxicity of the plant.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) with the aid of SPSS 20v for Windows. Data's obtained were expressed as mean ± standard error of mean (SEM). Difference between the various extracts were compared using DMRT. *P* value less than or equal to 0.05 ($P \leq 0.05$) was considered significant.

Result and Discussion

Toxicity test and Lethal Dose (LD_{50}) of *Ceratotheca sesamoides* aqueous leaf extract treatment of healthy albino wister rats with aqueous *extracts of Ceratotheca sesamoides* leaf did not show any fatality starting with 300mg to 5000mg. This shows that the LD_{50} is

greater than 5000mg. Other symptoms like change in eye and skin colour, lethargy, respiratory distress and muscles paralysis were not observed. This observation showed *Ceratotheca sesamoides* is non-toxic.

Qualitative phytochemistry of different leaf extract of *Cereatotheca sesamoides* In Table 1 shows the qualitative phytochesmistry of aqueous, ethanolic and petroleum ether leaf extracts. Result from the table shows that polyphenols, flavonoids and tannins are presence in aqueous and ethanolic extract, and are absent in petroleum ether extract. For the first time, this study shows the presence of tannins, flavonoids and alkaloids in aqueous leaf extract of *Ceratotheca sesamoides.* The presence of polar phytochemical could account for antioxidant activity.

Table 1 The qualitative phytochesmistry of aqueous, ethanolic and petroleum ether leaf extracts

Extracts	Phytochemicals							
	Carbohydrates	**Steriods**	**Cardiac glycoside**	**Saponins**	**Flavonoid**	**Tannins**	**Alkaloids**	**Anthraquinones**
Aqueous	++	-	+	-	++	+	++	-
Ethanol	+	+	+	+	+	-	+	-
Pet-ether	+	+	+	-	-	-	+	-

+ = positive, ++ = very positive, - = negative

Quantitative phytochemicals of aqueous, ethanolic and petroleum ether leaves leaf extracts of *Cereatotheca sesamoides* in Table 2 shows the result for total polyphenol was found high in Aqueous extract (63.828±0.667) followed by ethanol extract and the least was in Pet-ether extract. Similar trend was also observed in Total flavonoid content as indicated in Total polyphenol. The total phenolic content was (36.45mg/gm) expressed as gallic acid equivalent per gram. While the contents of total flavonoid (66.8 mg/gm) were expressed in terms of rutin equivalent.

Fruits and vegetables in the diet have been demonstrated in epidemiological researches to be protective against some chronic diseases linked with aging such as some type of cancer,

general cardiovascular illnes, cataracts, brain and immune dysfunction [17]. The protective effects of these natural product have assign to several ingredient such as carotenoids, vitamins C and E, and phenolic component and thiol (SH) compounds [18,21]. Several research have emphasised on the biotic actions of such which are potent antioxidants and free radical scavengers in quite a number of metabolic processes [19,22]. The concern in phenolic compounds derived from plants such as garden plants and their functions in nutrition are generally increasing [23,24]. The Phenolic chemicals from plants are also well known to perform a significant function in stabilizing lipids metabolism against peroxidation and inhibiting various types of oxidizing enzymes [20]. The differences in the structure and function relationship of flavonoid structures and their substitutions influence the phenoxyl radical stability, thus affecting the antioxidant properties of the flavonoids [17]. Hence flavonoid part and phenolics compound of aeroponically grown plant were discovered to be comparable to those grown in the soil [25]. The total produce crop was, however, higher in aeroponically grown plant.

Table 2 Total polyphenol and total flavonoid content of aqueous, ethanolic and petroleum ether leaves extracts of *Cereatotheca sesamoides*

	Petroleum ether extract	**Ethanol extract**	**Aqueous extract**
Total polyphenol			
(mgGallic acid Equivalent/g)	2.612±0.25	16.884±0.167	63.828±0.667
Total flavonoid			
(mg Quercetin/g)	0.269±0.002	0.795±0.009	0.878±0.002

Mean ± SD of 3 triplicates

Conclusion

Ceratotheca sesamoides was extracted using aqueous solvent, ethanol solvent and petroleum solvent. Chemical ingredients (qualitative phytochemical analysis, quantitative polyphenols and flavonoids) were determined. Among the three different extracts of *Ceratotheca sesamoides* leaf, aqueous extract and ethanolic extracts contain polar

phytochemicals, but aqueous extract contains higher concentration of polyphenols. Further studies is required to evaluate the possible interactions of *Ceratoteca sesamoides* leaves with therapeutic drugs and or dietary components in order to clarify its possible use as traditional herb. Although studies have shown that the plant is rich in polyphenols and flavonoids, there is need to identify the specific phenolic compound and to test their biological activity.

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Conflict of Interests
On behalf of all the contributing authors, it is declared that there is no conflict of interests regarding this paper.

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