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Phytochemical and Anti-Diarrheal Evaluations of the Crude Ethanol Extracts from *Cassia singueana* Del. and *Parkia biglobosa* Jacq. ex. G. Don Using Charcoal Transit in Rats

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Abstract

Plant as drug has been in existence since the period of Man's civilization across the World. Modern scientific techniques have led to isolation and identification of thousands of phytochemicals, many of which had served as chemical leads for the development of therapeutic drugs against several diseases. This study was aimed to reveal the phytochemical contents of *Cassia singueana* (rootbark) and *Parkia biglobosa* (stembark) using standard procedure and their charcoal transit mobility levels in rats. The phytochemical analysis of the extracts revealed the presence of phytocompounds like alkaloids, anthraquinones, cardiac glycosides, flavonoids, terpenoids and saponins. The oral acute lethal dose (LD_{50}) in rats were calculated to be 3807.89 mg/kg bd. wt. and 5000 mg/kg bd. wt. respectively for *Cassia singueana* and *Parkia biglobosa.* The charcoal mobility test was carried out using rats in accordance with standard procedures and results of the charcoal transit study revealed that the mean percentage distance travelled by the control 5 mL/kg bd. wt. (normal saline) and atropine sulphate 5 mg/kg bd. wt. were 0.00% and 88.99% respectively. On the other hand, the crude extracts of *Cassia singueana* and *Parkia biglobosa* travelled a mean percentage distance of 52.88% and 52.12% at 300 mg/kg bd. wt. 21.71% and 58.09% percentage travel were recorded at 600 mg/kg bd. wt. and 19.81% and 100% at 1200 mg/kg bd. wt. respectively. The *Cassia singueana* crude extract acted in non-dose-dependent manner while *Parkia biglobosa* acted in dose-dependent manner. Thus, *Cassia singueana* revealed the highest effects at low dose while *P. biglobosa* was effective at high dose in rats. Therefore, the use of both extracts as anti-diarrheal agents in folklore medicine have a scientific support due to the resistance of the flow of the charcoal transit in rats.

Keywords: *Cassia singueana* Del., *Parkia biglobosa* Jacq. ex. G. Don., charcoal, rat, rootbark, stembark.

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Introduction

The frequent movement of bowel linked with abnormal excretion is often referred to as diarrhea. The secretions that occur as a result of diarrhea are classified as; watery diarrhea, bloody diarrhea and insistent diarrhea (usually last beyond two weeks) [1]. The major problem associated with this disease is dehydration which if persist may lead to mortality especially in infants and children [2,3]. Globally about 1.7 billion mortalities are recorded annually as a result of the disease, out this number about five hundred thousand (500,000) are children under the age of five [1]. Thus, indicating that the treatment options are not enough to prevent death [4].

Plant as drug has been in existence since time immemorial from across the continents and have provided the modern medicine with a lot of plant derived therapeutic agents [1]. They are affordable, readily available and comparatively effective in the treatment of diseases. Africans and Asians rely heavily on them as a source of medication due to the number of bioactive principles inherent in them [5]. Many plants were reported in literature to possess antidiarrheal property [6]. Hence, the need to screen and validate the ethnopharmacological claims using modern scientific techniques to isolate and identify thousands of phytochemicals that may likely serve as chemical lead for the development of chemotherapeutic drugs against several diseases.

The two medicinal plants under study belong to Fabaceae. It is the third largest of the angiosperm family after Orchidaceae (Orchids) and Asteraceae (Sunflowers). The distributions of Fabaceae cut across tropical and temperate regions of the world and even aquatic region. The family is divided into three sub-families, *Caesalpinioideae*, *Mimosoideae* and *Papilionoideae*. However, the *Caesalpinioideae* consist of diverse group with 162 genera and 3,000 species among which is *Cassia* [7].

Cassia singueana is a deciduous shrub or small tree with a light, open crown which can grow between 4-15 metres tall with bole of about 15cm in diameter [8]. The root of *Cassia singueana* was reported to contain anthraquinones and terpenes [9]. *Cassia Singueana* was called Rumfu in Hausa, Rumfuhi in Fulani, Tugelele in Kanuri and Shadarat al Bashini in shuwa Arab. This plant has many medicinal uses throughout Africa. Extracts of the rootbark was reported to possess significant analgesic, antipyretic, antimicrobial, anthelmintic, antiplasmodial and antidiabetic activity [8, 10]. A decoction of the root is used to treat wounds and as a remedy for diarrhea, convulsion, dementia and sexually transmitted diseases (STDs). The roots are used to treat venereal disease, stomach complaints and as a purgative. The ash of burnt root is eaten mixed with porridge to cure abdominal pain [11, 12].

Parkia biglobosa on the other hand is commonly referred to as African locust bean tree. The plant is a dicotyledonous deciduous, perennial angiosperm

of the family Fabaceae - *Mimosoideae* that grows between 7 and 20 metres high and sometimes up to 30 metres [13]. The bark soaked in alkahol is used in some communities for anti-diarrhoeal properties and as an effective anti-snake venoms preparation [14, 15]. The plant is locally called *Runo* [Kanouri]; *Dorawa*, [Hausa] *Ogiriokpi* [Igbo]; *Narghi* [Fulani]; and *Nonna* [Babur Bura] [16]. It is a multipurpose tree used for many purposes such as food, folk medicine, fire woods and ornamentals etc. especially in the West African. The bark was reported as viable remedy for fever, hypertension and wounds; such as ulcer and snake bite [15]. The stembark decoction taken as remedy for the treatment of malaria, inflammatory diseases and infections due to diarrhea [17]. The purpose of this study was to investigate the phytoconstituents and the effects of these extracts on gastrointestinal charcoal transit in Wistar rats.

Materials and Methods

Sample Collection and Identification

The samples of the two plants were identified by a Taxonomist in the herbarium of the Department of Botany, Faculty of Life Sciences, University of Maiduguri, Nigeria as: *Cassia singueana* and *Parkia biglobosa* (Figure 1); where the voucher specimen numbers: 18.CH002 and 16.CH014 were respectively deposited. The *Cassia Singueana* rootbark and *Parkia biglobosa* stembark were both collected from Biu Local Government Area, Borno State, Nigeria. The plant materials were shade dried and pulverized into coarse powder using wooden mortar and pestle.



(a)



(b)

Figure 1: Aerial view of: (a). *Cassia singueana* tree (b). *Parkia biglobosa* tree

Plant Extraction

Three hundred grams each of powdered *Cassia singueana* (rootbark) and *Parkia biglobosa* (stembark) were extracted independently with 80% ethanol for 4 hours using reflux method. The combined concentrate of each extract was

independently filtered using Whatman No. 1 filter paper and concentrated to dryness on evaporating dish. After complete dryness, the extracts were then subjected to qualitative phytochemical screening, acute toxicity study and charcoal transit studies in rats.

Phytochemical Screening

Qualitative phytochemical evaluations of the secondary metabolites present in the rootbark and stembark extracts of *Cassia singueana* and *Parkia biglobosa* were evaluated using standard procedures as described earlier [18-22].

Animals Studies

All experiment on laboratory animals in this study followed the standard procedures for the treatment of animals according to the International Guiding Principles for Biomedical Research involving Animals [23] as certified by the Animal Ethics Committee of the Faculty of Pharmacy, University of Maiduguri with approval number FP/022018/TETFP03. Forty-five (45) adults healthy Wistar strain rats used for this study weighed between 230-260 g were obtained from Animal House, Department of Veterinary Pharmacology, Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri. They were kept in well-ventilated plastic cages at approximately 12/12 hour (night/day cycle), humidity 65±5% to acclimatize to laboratory condition before the commencement of the experiment.

Acute Toxicity Studies (LD_{50})

Acute Toxicity Studies (LD_{50}) determination was conducted using the method described by Lorke [24]. In the initial phase the rats were divided into three groups of three rats each and treated with the extracts at doses of 10, 100 and 1000 mg/kg bd. wt. and observed for 24 hours. In the second phase, 3 rats were divided in to 3 groups of 1 rat each and extract administered accordingly; the final LD_{50} value calculated using the formula presented by Lorke [24]. The value of the LD_{50} was calculated by the formula:

$$LD_{50} = \sqrt{(D0 \times D100)}$$

D_0 = Highest value that showed no mortality

D_{100} = Lowest dose that produced mortality.

Charcoal Transit on Rats

Charcoal transit was carried out using the method described by Salawu *et al*. [25]. Adult healthy Wistar rat of either sex were fasted for 12 hours prior to the commencement of the study and were randomly divided into five groups each containing three rats. Each rat was given 1 mL of castor oil orally to induce diarrhoea. At predetermined times; an activated charcoal suspension was administered by oral gavage. One hour following treatments, each group was given 0.2 mL of charcoal meal (10% charcoal suspended in 5% gum *acacia*); and after a duration of thirty minutes, the rats were euthanized and the small intestine of each rat was carefully separated from the mesenterium to avoid

being over-stretched and then measured in centimeter. The length of small intestine and the distance traveled by the charcoal were recorded. The animals were grouped as follows: Group 1 was treated with 300 mg/kg bd. wt. of extract, Group 2 was treated with 600 mg/kg bd. wt. of extract while Group 3 was treated with 1200 mg/kg bd. wt. of extract, Group 4 was treated with Normal saline 5 mL/kg bd. wt. and Group 5 was treated with Atropine sulphate 5 mg/kg bd. wt. All treatments were given intra-gastrically. The length of the intestine from the pyloric sphincter to the ileo-caecal junction was isolated and the distance travelled by the charcoal meal was measured in centimetre (cm) and expressed as percentage of the intestine from pylorus to caecum [26]. The position of the charcoal meal was regarded as the transit point and the mean for each treatment group was determined. For each animal, gastrointestinal transit was calculated as the percent inhibition (PI) was calculated as follows:

$$PI = \frac{\text{Mean Control - Mean Treated) group}}{\text{Mean Control Group}} \times 100$$

Statistical Analysis

The results were expressed as mean ± SEM and subjected to comparison using one-way analysis of variance (ANOVA).

Results and Discussion

The results of the phytochemical screening of the ethanol crude extracts of *Cassia singueana* rootbark and *Parkia biglobosa* stembark are presented in Table 1 below.

Table 1. Results of the phytochemical constituents of ethanol crude extracts of *Cassia singueana* rootbark and *Parkia biglobosa* stembark

Constituents	***Plant Extract***	
	Cassia singueana	***Parkia biglobosa***
Alkaloids	+	-
Anthraquinones	+	+
Cardiac glycosides	+	+
Flavonoids	-	+
Saponins	+	+
Tannins	+	+
Terpenoids	+	+

Key: + = Present – = Absent

Table 2. Effect of Rootbark Extract of *Cassia singueana* on Charcoal Transit in Rats

Treatment (mg/kg)	**Mean total length of intestine (cm) (Mean±SEM)**	**Mean distance travelled by Charcoal (cm) (Mean±SEM)**	**Protection (%)**
Control	110.67±6.25	93.37±3.35[a]	0.00
300	120.60 ±4.24	44.20±4.24[b]	52.88
600	116.00±9.97	73.10±8.57[c]	21.71
1200	117.40±8.20	74.87±5.41[c]	19.81
Atropine	110.75±1.06	17.75±8.84[d]	80.99

Means with different superscript letters along the same column are significantly different (P<0.05)

Table 3. Effect of Stembark Extract of *Parkia biglobosa* on Charcoal Transit in Rats

Treatment (mg/kg)	**Mean total length of intestine (cm) (Mean±SEM)**	**Mean distance travelled by Charcoal (cm) (Mean±SEM)**	**Protection (%)**
Control	110.67±6.25	93.37±3.35 [a]	0.00
300	103.87±2.52	41.90 ±4.10[b]	55.12
600	103.47±5.23	39.13±2.27[b]	58.09
1200	97.33±5.08	0.00±0.00[c]	100
Atropine	110.75±1.06	17.75±8.84[d]	80.99

Means with different superscript letters along the same column are significantly different (P<0.05)

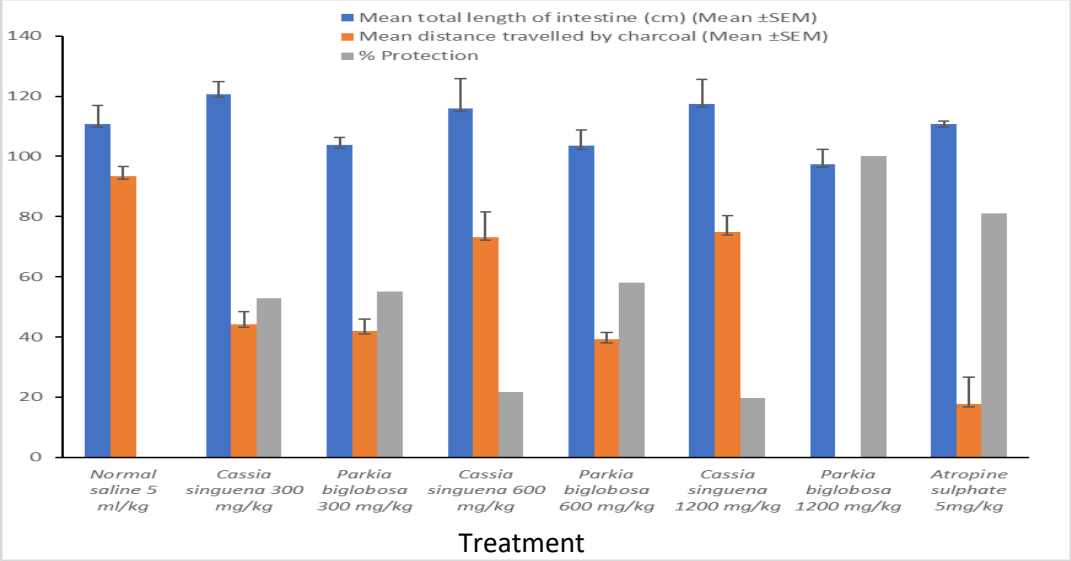


Figure 2. Comparative effects of *C. singueana* and *P. biglobosa* extracts at different concentrations of extracts and standard drug in Wistar rats

The results of the qualitative phytochemical analyses of the ethanol rootbark extract of *Cassia singueana* and stembark extract of *Parkia biglobosa* are presented in (Table 1). The results revealed the presences of carbohydrate, anthraquinone, cardiac glycoside, saponins and terpenoids in both extracts; while flavonoid was only present in *P. biglobosa* while alkaloid was only present in *C. singueana.* These results are consistent with the findings of Longange *et al*. [27], but slightly different from the findings of Fulata *et al.* [9], who reported the presence of flavonoids in *Cassia singueana* crude extract. The positive pharmacological responses observed with both extracts could be attributed to the presence of these secondary metabolites. For instance, flavonoids, tannins, polyphenols, polyterpenes, reducing sugars and saponins were reported to be responsible for the antidiarrhoeal properties of certain plants such as *Alchornea cordifolia*, *Calotropis gigantea* and *Morinda morindoides* [28-31]. The most likely mechanism for the manifestation of the effects could be due to precipitation of proteins in enterocyte and production of protein tannates that result in anti-secretion effect as suggested by Akuodor *et al.* [32].

To certify the safety of the two crude extracts, acute toxicity evaluations were deployed and the results revealed that *Cassia singueana* and *Parkia biglobosa* crude extracts have the LD_{50} values of 3807.89 mg/kg bd. wt. and 5000 mg/kg bd. wt. which could be respectively classified as moderately toxic and non-toxic on the basis of Clarke and Clarke [33] and Lorke, [24]. Furthermore, the LD_{50} result for *P. biglobosa* is in agreement with the finding of Builders *et al.* [34]. However, the LD_{50} value of *C. singueana* is slightly higher than the findings of Fulata *et al.* [9], which could likely be due to the absent of flavonoids in the extract as reported by Fulata *et al.* [35], that flavonoid have tendencies to depressed the central nervous system and respiratory function which may result in the mortality of experimental animals. Therefore, these crude extracts could be safely use acutely for oral administration in ethno-therapeutic management of diarrhea.

The results of the charcoal transit (Figure 2) study revealed that the mean percentage distance travelled by the control 5 mL/kg bd. wt. (normal saline) and atropine sulphate 5 mg/kg bd. wt. as 0.00% and 88.99% respectively. On the other hand, the crude extracts of *Cassia singueana* and *Parkia biglobosa* travelled a mean percentage distance of 52.88% and 52.12% at 300 mg/kg bd. wt., while 21.71% and 58.09% were recorded at 600 mg/kg bd. wt. and 19.81% and 100% at 1200 mg/kg bd. wt. respectively. The *Cassia singueana* crude extract acted in non-dose-dependent manner while *Parkia biglobosa* acted in dose-dependent manner. Moreover, significant differences ($P<0.05$) were observed among the test groups (both extracts) and control as shown in Tables 2 and 3, except for the groups that were administered 300 and 600 mg/kg

bd. wt. of *C. singueana* and 600 and 1200 mg/kg bd. wt. of *P. biglobosa* extract. Thus, *Cassia singueana* revealed high effects at low dose while *P. biglobosa* was effective at high dose in rats. The most likely mechanism of action of the extracts could be by triggering in digestion and enhancing the epithelial barrier integrity by limiting the amount of any substance is in contact with the surface of the entrecotes as suggested by Builders *et al.* [34]. Furthermore, the effect observed with both extracts could be as a result of the presence of a number of phytochemicals earlier implicated to be effective anti-diarrhea agents, for instance triterpenoids, saponins, flavonoids, tannins and alkaloids are reported to inhibit the release of prostaglandins, prevent intestinal motility and hydro-electrolytic secretions, which are known to be altered in diarrheal conditions [36].

In conclusion, the high inhibitory effects observed with the crude ethanolic extracts of the rootbark of *Cassia singueana* and *Parkia biglobosa* may be due to the presence of some vital bioactive phytocompounds such as anthraquinones, alkaloids, cardiac glycosides, flavonoids, saponins, tannins and terpenoids that are capable of stopping diarrhea. Moreover, the toxicity threshold of both extracts further explained their safety for biological administration and can therefore be used as anti-diarrheal agent.

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