PHYTOCHEMICAL INVESTIGATION AND TOXICOLOGICAL INSIGHTS OF *CASSIA SIEBERIANA* LEAF EXTRACT: IMPLICATIONS FOR MEDICINAL USE

ABSTRACT

Introduction:*Cassia sieberiana*, a member of the Fabaceae family, has a rich history of traditional medicinal uses. This study focuses on exploring the medicinal potential of the methanol extract from Cassia sieberiana leaves. The research aims to conduct a comprehensive analysis of bioactive compounds and assess acute toxicity through LD50 determination.

Methods: Fresh leaves were collected from Opi town, Nsukka, Nigeria, and authenticated. Male Wister albino rats were acclimatized, and phytochemical screening was performed using qualitative and quantitative methods. LD50 determination followed internationally recognized protocols, employing mice models.

Results: The methanol extract exhibited a yield of 11.2%. Phytochemical analysis revealed the presence of proteins, carbohydrates, tannins, alkaloids, steroids, cardiac glycosides, saponins, flavonoids, reducing sugars, terpenoids, and quinones. Alkaloids were predominant (1770.8±74.43 mg/100g). LD50 determination showed 100% survival at 5000 mg/kg, indicating relative safety.

Discussion: The high yield suggests methanol as an effective solvent. Phytochemical composition aligns with traditional uses, and the prevalence of alkaloids supports reported medicinal applications. Low phenolic content suggests antioxidant effects, while flavonoids may contribute to anti-inflammatory properties. Saponins and tannins indicate potential for antimicrobial and purgative use. The study affirms the safety of the methanol leaf extract.

Conclusion: The study provides valuable insights into the medicinal potential of Cassia sieberiana. The significant alkaloid content, diverse bioactive compounds, and demonstrated safety in LD50 determination support its traditional uses. These findings lay a robust foundation for further exploration of Cassia sieberiana in drug development and healthcare applications.

Key words: *Cassia sieberiana*, acute toxicity, LD50 determination, traditional medicine, plant-derived compounds.

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INTRODUCTION

Cassia sieberiana, a member of the *Fabaceae* family, has long been recognized for its traditional medicinal uses across various cultures. The exploration of plant-derived compounds for their potential therapeutic applications has gained considerable attention in recent years. Among various plant parts, the leaves of *Cassia sieberiana* stand out as a reservoir of phytochemicals that may hold therapeutic promise. In this study, the methanol extract of *Cassia sieberiana* leaves

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emerges as a subject of profound interest, as it holds the promise of unlocking new dimensions of its medicinal potential.

This study embarks on a journey of exploration, aiming for acomprehensive analysis of the bioactive compounds present in *Cassia sieberiana* leaves and concurrently assessing its acute toxicity through the determination of the lethal dose (LD_{50}).

Phytochemical analysis plays a pivotal role in unraveling the intricate chemical composition of plant extracts, shedding light on bioactive compounds that may contribute to their pharmacological effects. Simultaneously, the investigation seeks to ascertain the acute toxicity profile through LD_{50} determination (Ahmad, et al., 2021), ensuring a thorough evaluation of the extract's safety. In accordance with internationally recognized protocols, this study will utilize mice models to establish the dosage at which the *Cassia sieberiana* leaf extract becomes lethal to 50% of the tested population. The LD_{50} data generated will provide essential information for evaluating the safety margins and potential risks associated with the consumption or application of the plant extract (Aprioku et al., 2014).

The convergence of phytochemical analysis and LD50 evaluation is poised to offer a well-rounded perspective on the medicinal potential of *Cassia sieberiana* methanol leaf extract. By bridging the gap between chemical composition and acute toxicity, this research not only contributes to the scientific understanding of this botanical resource but also lays the groundwork for informed decision-making regarding its utilization in various traditional and modern medicinal applications.

In the pages that follow, we present a detailed account of our methodology, results, and discussions, aiming to enrich the scientific discourse on *Cassia sieberiana* and provide a foundation for future studies exploring the intricate interplay between bioactive compounds and toxicity in medicinal plants.

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Methods

Plant Materials (*Cassia sieberiana*)

Fresh leaves of *Ceassia sieberiana* was collected from a natural habitat in Opi town, Nsukka, Enugu State, Nigeria and was authenticated at the taxonomy Unit, Department of Botany, University of Nigeria, Nsukka.

Animals

Male adult Wister albino rats were obtained from Animal House of the department of Zoology and Environmental Biology, University of Nigeria, Nsukka and acclimatized for 7 days under standard environmental conditions and were maintained on regular feed and clean water.

Phytochemical Screening: Qualitative phytochemical analysis: Chemical tests were performed on the aqueous extracts for the qualitative estimation of phytochemical components using methods defined by (Harbone, 1996; Sofowora, 1993; Trease and Evans, 1989).

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Test for Tannins: Into a test tube containing 20mls of water, 0.5g of the dried powdered sample was added and then filtered, after which 0.1% ferricchloride (few drops) was added and detected

for a brownish green or a blue-black coloration to confirm the existence of tannins (Trease and Evans, 1989).

Test for Saponins: Inside a water bath, 2g of the powdered samples were boiled in 20mls of water, it was filtered and 10mls of the filtrate was mixed with 5mls of water and rocked for a stable persistent froth. The frothing was there after mixed with 3 drops of olive oil and observed for the emergence of an emulsion. (Trease and Evans, 1989).

Test for Flavonoids: Three methods were used to determine the existence of flavonoids: To a portion of the aqueous filtrate of each plant extract, 5ml of dilute ammonia solution was added, concentrated H_2S0_4 was also added immediately and observed for a yellow coloration in each extract which shows the existence of flavonoids. The yellow coloration on standing disappeared. (Trease and Evans, 1989). To a portion of each filtrate, few drops of 1% aluminium solution were added and checked for a yellow coloration to develop, which indicates the existence of flavonoids. A portion of the individual powdered plant parts was warmed up in 10ml ethyl acetate over a steam bath for three minutes. The mixture was filtered and 4ml of the filtrate was rocked with 1ml of dilute ammonia solution and observed for a yellow coloration to develop, an indication of theexistence of flavonoids.

Test for Steroids: To 0.5g of each aqueous extract, 2ml of acetic anhydride was added with 2ml H2S04. The colour converted from violet to blue or green in some samples showing the existence of steroids (Harbone, 1996).

Test for Cardiac Glycosides(Keller-Killani test): 2ml glacial acetic acid (comprising a drop of ferric chloride solution under layered with 1ml of concentrated H_2S0_4) was used to treat 5mls of extracts. A brownring on the interface suggests a deoxy sugar features of cardiac glycosides. A violet ring may occur below the brown ring, while in the acetic acid layer, a greenish ring may develop all around the thin layer (Harbone, 1996).

Test for alkaloids: A 0.5g sample of the extracts was mixed with 5ml of 1% aqueous hydrochloric acid on a steam bath. 1ml of the filtrate was mixed with a few drops of Dragendorff's reagent. Turbidity with this reagent is a proof of the existence of alkaloids in the extract (Harbone, 1996).

Quantitative analysis of phytochemicals:

Cyanogenic glycosides: To 2g of the different plant parts, 5ml of alkaline picrate was added, the mixture was incubated in a wath bath for five minutes and the absorbance was read a 490nm (Onwuka, 2005).

Saponins: 5ml of the extract were dissolved in a solution of methanol/ water in the ratio 1:1. They were further dissolved in 80% methanol. 2ml ethanol was added, properly rocked, placed inside a water bath of 60^{o}C to warm gently for ten minutes. The solutions were filtered and the absorbance read at 544nm.Narendra et al., (2013).

Phenols: 5g of the extracts were boiled with 50ml of ether for five minutes and filtered, 5ml of the filtrate, pipette into a conical flask, and 10ml of distilled water was added. 2ml of ammonium hydroxide was added alongside 5ml of alcohol. They were allowed to stand for thirty minutes for full colour to improve. The absorbance was read at 505nm. Edeoga et al., (2005).

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Alkaloids: To 2g of the plant extracts, 5ml of phosphate buffer solution of pH 4.7 was added, followed by the addition of 5ml of bromocresol green solution and 4ml of chloroform. The solution rocked and there after filtered. The absorbance was read at 470nm. Narendra et al., (2013).

Steriods: To 1g of plant extracts, 2ml of $4NH_2SO_4$ and 2ml of 0.5% iron(III)chloride were added followed by the addition of 0.5ml of 0.5% potassium hexacyanoferrate(III) solution. The mixtures were warmed up in a water bath at a temperature of 70^oC for thirty minutes and rocked occasionally. Thereafter, they were filtered and the absorbance was read at 780nm (Trease and Evans, 1996).

Flavonoids: To 2g of the extracts, 0.3ml of 5% $NaNO_2$solution was added after five minutes. On the sixth minute, 2ml of 1M NaOH added and the volume made up to 2ml with distilled water, the solutions were well rocked and filtered. The absorbance was read at 510nm (Boham and Kocipai, 1994).

Tannins: To 5g of the samples 50ml of distilled water was added, the mixtures were rocked with a mechanical shaker for one hour and filtered into a volumetric flask. 5ml of the filtrate was pipette into a test tube and rocked with 2ml of 0.1M $FeCl_3$ in 0.1NHCl and 0.008M potassium ferrocyanide. Theabsorbance was read at 120nm (Van-Burden andRobison, 1981).

Results

Percentage yield

The percentage yield of the extract was 11.2%

Phytochemical Composition of C. *Sieberiana*

The study revealed that *Cassia sieberiana* contains proteins, carbohydrates, tannins, alkaloids, steroids, cardiac glycosides, saponins, flavonoids, reducing sugars, terpenoids and quinones(~~. This is shown in~~ Table 1). The quantitative compositions of some of these phytochemicals in MLECS is shown in Table 2.

Table 1. Qualitative composition of *C.sieberiana*leaves

Phytochemicals	Bioavailability

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Protein	++
Alkaloids	+++
Carbohydrates	+++
Reducing sugars	++
Saponins	+++
Flavonoids	++
Tannins	+
Cardiac Glycoside	++
Resins	+
Steroids	++
Terpenoids	+++
Phlobatannins	ND
Acidic content	+
Oil	+

Keys

+ = Present (low aAmounts)

++ = Present

+++ = Present (high amounts)

ND = Not detected

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Table 2. Quantitative phytochemical composition of MLECS

Phytochemical	Quantity Present (mg/100g)
Alkaloids	1770.8±74.43 ~~(mg/100g)~~
Carbohydrates	1354.70±0.63 ~~(mg/100g)~~
Reducing Sugars	1196.60±2.32 ~~(mg/100g)~~
Flavonoids	427.09±40.78 ~~(mg/100g)~~
Tannins	39.16±0.43 ~~(mg/100g)~~
Steriods	14.47±0.81 ~~(mg/100g)~~
Terpenoids	199.53±3.54 ~~(mg/100g)~~
Total Phenols	0.791±0.016 GAE

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Acute Toxicity Test

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Table 3a and 3b show the acute toxicity test results of MLECS ~~as described by Lorke (1983)~~. The acute toxicity study (LD_{50}) recorded 100% survival by 24 hours for all the animals that were orally fed up to 5000 mg/kg body weight was relatively safe.

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Table 3a. Acute toxicity test results of MLECS – Phase I

Group	Dose (mg/kg.b.w)	No of Deaths
1	10	0/3
2	100	0/3
3	1000	0/3

Table 3b. Acute toxicity test results of MLECS – Phase II

Group	Dose (mg/kg.b.w)	No of Deaths
1	1600	0/3
2	2900	0/3
3	5000	0/3

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DISCUSSION

The percentage yield of 11.2% makes method leaf extract of *C.sieberiana* revealed that methanol is a good solvent for the extraction of important plant secondary metabolites. The high percentage yield, with preserved integrities of the extracts is an indication of the that this method can be adapted as a standard method of extract preparation.

In the study, the result of the phytochemical screening indicated that C. *sieberiana* leaves in rich in phytochemicals (Table 1 and 2). Alkaloids, carbohydrates and reducing sugars were found to be much higher than the other phytochemical components detected. These results agrees with the reports of Archer et al., 2019who also reported that the roots and pods (fruit) pulp indicated the presence of tannins, alkaloids, saponins, steroids, flavonoids and quinones. The result of this study is also supported by the report of Awomukwu*et al*. (2015) that the presence of tannins, flavonoids and Saponins and that alkaloids were very highly present in the leaves of C. *sieberiana*. However, in contrast to the study of Barrau et al., 2005, this study revealed that the leaves did not show the presence of phlobatanins. This difference may be attributed to differences in plant parts used, the solvent used for extraction, extraction tecniques; also some plant parts found in different environment contain different phytochemicals (Elujoba, 1989). The presence of these phytochemical constituents in C. *sieberiana* provides an empirical basis for its traditional uses as phytochemicals have been reported to have medicinal uses (Tella and Ojo, 2005).

Alkaloids, tannins, saponins, glycosides and steroids derived from plants have been shown to have antimicrobial effect and pharmacological activities (Trease and Evans, 1983). The MLECS is rich in alkaloids (1770.80±74.43mg/100g). alkaloids have been known to posses pharmacological effects such as anaesthetic (Njoku and Obi, 2009), antioxidant (Nabilah *et al*, 2011), antitumour and anti-inflammatory effect (Awomukwu*et al*, 2015). Alkaloids are also known to have multiplicity of host-mediated biological activities including, anti-malarial, anti-microbial, (Tackie and Schiff, 1993). These properties of alkaloids could explain why C. *sieberiana* leaves is used to treat malaria, bilharzia (Obidah*et al*, 2009) and general body pain (Khala *et al*, 2014). *Cassia sieberiana* is also used as abortifacient (Ajayi *et al*, 2015) and this might be due to the presence of ergometrine; an alkaloid which had been shown to be widely used to induce delivery (Hogerzeil and Walker 1996). This study also showed that C. *sieberiana* has low phenolic content (0.791±0.016GAE) which agrees with report of Awomukwu*et al*. (2015) who also reported that Cassia species in general have low phenolic contents. Flavonoids ans tannins are examples of phenolics. The flavonoid and tannin content of MLECS is 427.09±40.78mg/100g and 39.16±0.43mg/100g respectively. The phenols observed in the leaf extract of C. *sieberiana* could also be responsible for the antioxidant effect ascribed to this plant (Ajayi, 2015). Flavonoids also have anti-inflammatory effect (Awomukwu*et al*, 2015), this could be the possible explanation why aqueous extracts of *Cassia sieberiana* are used locally in Northeastern Nigeria for the treatment of inflammatory conditions (Madusolumuo*et al*, 1999). The cure of some ailments ascribed to the leaf ectract of *C. sieberiana* could be as a result of its content of flavonoids, since it has been observed that asthma, lung cancer and breast cancer were lower among people consuming high dietary quercetin, a flavonoid (Knekt*et al*, 2002). Tannins

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act by coagulating the cell wall proteins (Trease and Evans, 1989), while saponin causes the lysis of the bacterial cells (Robinson, 1975). The presence of tannins and saponins may be the reason why the entire parts of *C. sieberiana* are used as purgatives in Burkina Faso and in the treatment of stomachache and ulcer in Senegal (Awomukwu*et al*, 2015). Saponins serve as natural antibiotics, which helps the body fight infections and microbial invasion (Okwu, 2005). Saponins have been recorded to prevent disease invasion of plants by parasitic fungi and has shown to affect urine, plasma, fecal output and liver cholestersol concentration (Awomukwu*et al*, 2015). This may be reason why the entire parts of *C. sieberiana* are commonly used extensively as diuretics (Ajayi *et al* 2015). The high percentage of saponins in the leave of the*C. sieberiana* can be attributed why it is used as an ingredient of a medicine for intestinal worms in Cote d'Ivoire due to the bitter tasting principles associated with Saponins (Awomukwu*et al*, 2015). This plant could also be a source of adjuvant since it contains saponins in high amount which is a known adjuvant used in the production of vaccines (Philip, 2006). This study showed that MLECS has very low steroid content (14.47±0.81 mg/100g). This steroids observed in the leaf extract of *C. sieberiana* could also be responsible for the anti-inflammatory effects ascribed to this plant (Nelson and Cox, 2005). This study showed that MLECS is also rich in terpenoids (199.53±3.54 mg/100g). Terpenoids have been shown to posses medicinal properties such as anti-carcinogenic (e.g. Irofulven), antimalarial (e.g. artemisimin), anti-ulcer, anti-microbial or diuretic (e.g. glycyrrhizin and pleurmutilin) activity (Xiao and Zhong, 2006). The presence of these terpenoids could thus explain also why *C. sieberiana* leave is used as antimalaria and as diuretic (Ajayi *et al*, 2015). The presence of all these biologically active compounds suggests that the plant could serve as potential sources of drugs.

In order to evaluate the acute toxicity of MLECS, the LD_{50} of the extract were investigated using mice as models respectively. The result of the oral lethal median dose toxicity study showed no death even at 5000mg/kg body weight. This result agrees with the report of Cyril et al., 2021 who showed that aqueous root bark extracts of *C. sieberiana* was also relatively safe at 5000mg/kg body weight. In contrast to this study are the reports of Tambuora*et al*. (2005), Obidah*et al*. (2009) and Traore *et al*. (2014) who report that the LD_{50} of *C. sierberiana* aqueous root bark extract (24mg/kg) and leaves extract (660mg/kg) *via* intra peritoneal route of administration. This could be explained based on the fact that the toxicity of any plant part may be dependent on the route of administration (Okpoko et al., 2020), and also the phytochemical constituents of the different plant parts and difference in the extract in the extraction solvents and or methods.

Conclusion

In conclusion, the investigation into the methanol leaf extract of *Cassia sieberiana* has revealed promising insights into its potential as a rich source of bioactive compounds. The notable percentage yield of 11.2% signifies the effectiveness of methanol as a solvent for extracting important plant secondary metabolites, highlighting the potential standardization of this method for extract preparation. The phytochemical screening results underscore the abundance of

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alkaloids, carbohydrates, and reducing sugars, aligning with existing literature on Cassia species. The substantial alkaloid content, specifically, positions *Cassia sieberiana* as a promising candidate for medicinal applications, correlating with its traditional uses in treating conditions such as malaria, bilharzia, and general body pain. Furthermore, the presence of phenols, flavonoids, tannins, saponins, steroids, and terpenoids reinforces the plant's pharmacological potential, offering a diverse array of bioactive compounds that may contribute to its reported therapeutic effects. Importantly, the LD50 determination in mice suggests a relatively safe profile for the oral administration of the methanol leaf extract, further supporting its potential as a medicinal resource. These findings collectively provide a scientific foundation for the traditional uses of *Cassia sieberiana*, paving the way for future studies to explore its specific applications in drug development and healthcare.

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