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Phytochemical Investigation and Toxicological Insights of *Cassia sieberiana* Leaf Extract: Implications for Medicinal use

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 LD_{50} 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. LD_{50} determination followed internationally recognized protocols, employing mice models.

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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). LD_{50} 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 LD_{50} determination support its traditional uses. These findings lay a robust foundation for further exploration of *Cassia sieberiana* in drug development and healthcare applications.

Keywords: Cassia sieberiana; acute toxicity; LD_{50} determination; traditional medicine; plant-derived compounds.

1. 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 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 a comprehensive 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 [1], 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 [2].

The convergence of phytochemical analysis and LD_{50} 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.

2. METHODS

2.1 Plant Materials (*Cassia sieberiana*)

Fresh leaves of *cassia 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.

2.2 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.

2.3 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, [3] Sofowora, [4], Trease and Evans, [5].

2.4 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% $FeCl_3$ (few drops) was added and detected for a brownish green or a blue-black coloration to confirm the existence of tannins" [6].

2.5 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" [6].

2.6 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 H2S04 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" [6]. "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 the existence of flavonoids"

2.7 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" [3].

2.8 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 brown ring 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" [3].

2.9 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" [3].

2.10 Quantitative Analysis of Phytochemicals

2.10.1 Cyanogenic glycosides

"To 2g of the different plant parts, 5ml of alkaline picrate was added, the mixture was incubated in a water bath for five minutes and the absorbance was read a 490nm" [7].

2.10.2 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 60oC to warm gently for ten minutes. The solutions were filtered and the absorbance read at 544nm" [8].

2.10.3 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" [9].

2.10.4 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" [8].

2.10.5 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 70oC for thirty minutes and rocked occasionally. Thereafter, they were filtered and the absorbance was read at 780nm" [5].

2.10.6 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 pipetted into a test tube and rocked with 2ml of 0.1M $FeCl_3$ in 0.1NHCl and 0.008M potassium ferrocyanide. The absorbance was read at 120nm" [10].

3. RESULTS

3.1 Percentage yield

The percentage yield of the extract was 11.2%.

3.2 Phytochemical Composition of C. *Sieberiana*

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

3.3 Acute Toxicity Test

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 hour for all the animals that were orally fed up to 5000 mg/kg body weight was relatively safe.

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

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

Keys: + = Present (low Amounts); ++ = Present; +++ = Present (high amounts); ND = Not detected

Table 2. Quantitative phytochemical composition of MLECS

Phytochemical	Quantity Present
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

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

4. DISCUSSION

The extraction method for obtaining leaf extract of C. sieberiana demonstrated a substantial percentage yield of 11.2%, highlighting the efficacy of methanol as a solvent for extracting crucial plant secondary metabolites. The noteworthy yield, coupled with the maintained integrity of the extracts, suggests the potentiasl standardization of this extraction method.

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

The phytochemical screening results from the study revealed the abundance of various phytochemicals in C. sieberiana leaves, as presented in Table 1 and 2. Alkaloids, carbohydrates, and reducing sugars were notably higher than other detected phytochemical components. This finding aligns with Archer et al. [11] report on the presence of tannins, alkaloids, saponins, steroids, flavonoids, and quinones in the roots and fruit pulp of C. sieberiana. Awomukwu et al. [12] also supported these results, emphasizing the prevalence of tannins, flavonoids, and saponins in C. sieberiana leaves. However, in contrast to Barrau et al. [13] study, no phlobatanins were detected in the leaves, potentially attributed to variations in plant parts, extraction solvent, and techniques.

The presence of phytochemical constituents in C. sieberiana, such as alkaloids, tannins, saponins, glycosides, and steroids, supports its traditional uses due to the reported medicinal properties of these compounds (Tella and Ojo, 2005). The methanol leaf extract of C. sieberiana (MLECS) exhibited a significant alkaloid content (1770.80±74.43mg/100g), known for its diverse pharmacological effects, including anesthesia, antioxidant properties, antitumor and anti-inflammatory effects. Alkaloids' multiplicity of host-mediated biological activities, such as anti-malarial and anti-microbial effects, may explain the plant's applications in treating malaria, bilharzia, and general body pain.

The low phenolic content (0.791±0.016GAE) in MLECS aligns with Awomukwu et al. [12] observations on the generally low phenolic content in Cassia species. Flavonoids and tannins, subcategories of phenolics, were present in MLECS at 427.09±40.78mg/100g and 39.16±0.43mg/100g, respectively, contributing to the antioxidant and anti-inflammatory effects attributed to C. sieberiana. Tannins' action in coagulating cell wall proteins and saponins' role in lysing bacterial cells may explain the plant's use as purgatives, in treating stomachache and ulcer, and as a diuretic.

The MLECS showed a low steroid content (14.47±0.81 mg/100g) but was rich in terpenoids (199.53±3.54 mg/100g), known for various medicinal properties such as anti-carcinogenic, antimalarial, anti-ulcer, anti-microbial, or diuretic effects. The presence of these biologically active compounds positions C. sieberiana as a potential source of drugs.

To assess the acute toxicity of MLECS, the LD50 was investigated using mice as models. The results indicated no deaths even at a dosage of 5000mg/kg body weight, supporting the relative safety of the extract. This finding is consistent with Cyril et al. [14] report on the safety of aqueous root bark extracts of C. sieberiana. Discrepancies with other studies on LD50 values may be attributed to differences in administration routes, phytochemical constituents, extraction solvents, or methods.

5. 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 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 LD_{50} 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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