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Short Communication

Phytochemical Analysis and Acute Toxicity of Aqueous Extract of Dried Calyces of *Hibiscus Sabdariffa* Linn in Albino Rats

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ABSTRACT

Herbal medicines remain an important component of primary healthcare in many communities worldwide. This study evaluated the qualitative phytochemical constituents and preliminary acute oral toxicity of an aqueous extract of dried calyces of *Hibiscus sabdariffa* L. collected from Maiduguri, Borno State, Nigeria. The dried calyces were extracted using the reflux method and qualitative phytochemical screening was performed using standard procedures. A preliminary acute oral toxicity assessment was conducted in albino rats using selected oral dose levels of 2000, 3200, and 5000 mg/kg body weight, with animals observed for mortality and signs of toxicity over 14 days. The aqueous extract yielded 37.5% (w/w). Phytochemical screening revealed the presence of carbohydrates, cardiac glycosides, and saponins, whereas alkaloids, flavonoids, tannins, anthraquinones, cardelonides and terpenoids were not detected. No mortality or overt signs of acute toxicity were observed in the animals administered the extract at any of the doses tested. The absence of mortality at the highest dose tested (5000 mg/kg body weight) was consistent with an acute oral LD_{50} greater than 5000 mg/kg under the conditions of the present preliminary assessment; however, this represents a limit-dose finding rather than a statistically calculated LD_{50} point estimate. The findings suggest that the aqueous extract of *H. sabdariffa* calyces has low acute toxicity under the conditions evaluated. The small number of animals and the preliminary nature of the assessment limit the generalizability of the findings. Further studies involving repeated dose toxicity, comprehensive toxicological evaluation, and pharmacological investigations are warranted.

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1.0 INTRODUCTION

The use of medicinal plants in disease management has increased due to concerns over adverse drug reactions and antimicrobial resistance. The World Health Organization estimates that over 80% of the global population relies on traditional medicine for primary healthcare, particularly in developing countries (Kamboj, 2000). Among widely utilized medicinal plants is *Hibiscus sabdariffa* Linn (family Malvaceae), commonly known as roselle. Figure 1 shows the morphological features of *Hibiscus sabdariffa* while Figure 2 represents the dried calyces of *Hibiscus sabdariffa.*

Hibiscus sabdariffa is cultivated for its calyces, leaves, and seeds, and is widely consumed as a beverage and herbal remedy (Da-Costa-Rocha *et al*., 2014). The dried calyces are traditionally used in the management of hypertension, cardiovascular disorders, and metabolic conditions (Christian *et al.,* 2006; Inuwa *et al.,* 2012). Previous phytochemical investigations have shown that *H. sabdariffa* contains anthocyanins, organic acids, flavonoids, and other bioactive constituents responsible for its antioxidant and pharmacological activities (Ali *et al*., 2005; Goda *et al.,* 1997).

Despite its extensive use and documented pharmacological potential, phytochemical composition may vary depending on geographical location and extraction solvent (Ajiboye *et al.*, 2011; Obouayeba *et al.,* 2015). Therefore, safety evaluation and localized phytochemical assessment remain essential, particularly for standardization and therapeutic application (Njinga *et al.,* 2020).

This study aimed to determine the phytochemical constituents and to assess the preliminary acute toxicity of the aqueous extract of dried calyces of *H. sabdariffa* Linn in albino rats.

MATERIALS AND METHODS

Plant Collection and Identification

Dried calyces of *Hibiscus sabdariffa* Linn were purchased from Baga Road Market, Maiduguri, Borno State, Nigeria. Botanical identification and authentication were carried out by a plant taxonomist in the Department of Biological Sciences, University of Maiduguri, Borno State Nigeria and a voucher specimen with the name and number; *H. Sabdariffa* 17/092/DVM was deposited in the Department of Veterinary Pharmacology and Toxicology laboratory, University of Maiduguri.

Extraction Procedure

Two hundred grams (200 g) of powdered dried calyces were extracted with distilled water using the reflux extraction method as described by Lin. *et al*., (1999) and Usman *et al*., (2009). The extract was filtered and dried in an oven at 40°C. The dried extract was weighed to determine percentage yield using the formula:

$$\text{Yield (\%)} = \frac{\text{weight of extract(mg)}}{\textit{weight of dried materials (mg)}} \text{x}100$$

Phytochemical Screening

Qualitative phytochemical analysis was carried out using standard phytochemical screening methods (Brain *et al.,* 1975; Vishnoi, 1979; Markham, 1987; Sofowora, 2008; Evans, 2009) to determine the presence of carbohydrates, alkaloids, flavonoids, cardiac glycosides, saponins, tannins, anthraquinones, and terpenoids. Cardenolides, a subclass of cardiac glycosides, were specifically assessed using the Keller–Kiliani test as described by Trease and Evans (1989).

Experimental Animals

Three healthy adult albino rats, comprising two females and one male, weighing 170, 181, and 189 g, respectively, were used for the preliminary acute oral toxicity assessment. The individual body weight of each animal was recorded immediately before administration of the test extract, and the dose administered to each animal was calculated according to its respective body weight.

Ethical approval for the study was obtained from the Ethics Committee of the Faculty of Veterinary Medicine, University of Maiduguri, under approval number AUP-R002/2022. All applicable institutional and other relevant guidelines governing the care and use of laboratory animals were strictly adhered to throughout the study.

Acute Toxicity Study

Three healthy adult albino rats, comprising two females and one male, were used for a preliminary acute oral toxicity assessment. The study was conducted with reference to the principles of OECD Test Guideline 425, particularly its emphasis on minimizing animal use and systematic observation for signs of toxicity. However, the study design differed from the standard OECD TG 425 Up-and-Down Procedure in several respects, including the use of both sexes and predetermined dose levels. Therefore, the present experiment was not considered a fully OECD TG 425-compliant study, and the findings were interpreted as preliminary evidence of acute toxicity rather than as a definitive determination of LD_{50}.

Three predetermined oral dose levels of 2000, 3200, and 5000 mg/kg body weight were used to provide an initial assessment of the tolerability and potential acute toxicity of the extract at relatively high exposure levels. The doses were predetermined and were not selected sequentially based on the response of the preceding animal. Accordingly, the procedure was not a standard OECD TG 425 Up-and-Down Procedure.

The first rat, a female, received 2000 mg/kg body weight of the extract orally. The second rat, also a female, received 3200 mg/kg body weight, while the third rat, a male, received 5000 mg/kg body weight orally. Each animal was closely observed for 48 hours following administration for mortality and signs of toxicity, including changes in behaviour, activity, posture, respiration, feeding, and other observable clinical signs. Thereafter, all animals were monitored for a total observation period of 14 days to detect any delayed signs of toxicity or mortality.

The administered dose for each animal was calculated according to its individual body weight at the time of dosing. Because only one animal was used at each dose level and the doses were predetermined rather than sequentially adjusted according to the response of the preceding animal, the study did not provide replicated dose-response data or a statistically calculated LD_{50} with confidence limits. The absence of mortality at the highest dose tested was therefore interpreted as a preliminary limit-dose finding, with the acute oral LD_{50} considered to be greater than 5000 mg/kg body weight under the conditions of the present assessment.

RESULTS AND DISCUSSION

Extract Yield

The aqueous extraction yielded 75 g from 200 g of powdered material, corresponding to a percentage yield of 37.5%.

Qualitative Phytochemical Constituent

Table 1, shows the result of the preliminary phytochemical screening of the aqueous extract of dried calyces of *Hibiscus sabdariffa* which revealed the presence of carbohydrates, cardiac glycosides, and saponins**,** while alkaloids, flavonoids, cardenolides, tannins, anthraquinones, and terpenoids were not detected**.**

Table 1

Phytochemical Constituents of Aqueous Extract of Dried Calyces of *Hibiscus sabdariffa*

Phytochemical	**Inference**
Carbohydrates	+
Alkaloids	-
Flavonoids	-
Cardiac glycosides	+
Cardenolides	-
Saponins	+
Tannins	-
Anthraquinones	-
Terpenoids	-

Key: (+) = Positive indicating the presence of the phytochemical,
(-) = Negative indicating the phytochemical is absent in the extract

Acute Oral LD_{50}

Table 2, presents the dose levels and outcomes of the preliminary acute oral toxicity assessment of the aqueous extract of *Hibiscus sabdariffa* in albino rats. No mortality or overt signs of severe acute toxicity were observed in any of the animals administered 2000, 3200, or 5000 mg/kg body weight during the observation period. Based on the absence of mortality at the highest dose tested (5000 mg/kg body weight), the acute oral LD_{50} was considered to be greater than 5000 mg/kg body weight under the conditions of the present assessment**.** This represents a preliminary limit-dose finding rather than a statistically calculated LD_{50} point estimate**;** therefore, no confidence interval was calculated.

Table 2: Dose Levels and Outcomes of The Preliminary Acute Oral Toxicity Assessment of The Aqueous Extract of *Hibiscus sabdariffa* in Albino Rats

Test Sequence	Dose mg/kg	Short-term Result (48 hr)	Long-term Result (14 days)
01	2000	Survived	Survived
02	3200	Survived	Survived
03	5000	Survived	Survived

No observable clinical signs of toxicity were recorded during the observation period

The aqueous extraction of *Hibiscus sabdariffa* calyces yielded 37.5% (w/w). This yield is comparable to those reported for aqueous extracts of *H. sabdariffa* calyces in previous studies, which have documented yields ranging from approximately 23.3% to 39.0% depending on the extraction procedure and conditions (Herrera-Arellano *et al.,* 2012; Nurfaradilla *et al*., 2019; Mohamed-Salam *et al*., 2019). However, because no comparative extraction using other solvents was performed in the present study, the 37.5% yield should not be interpreted as evidence of superior extraction efficiency. Differences in solvent to sample ratio, extraction temperature, duration, and method may also influence the reported extraction yield (Brain *et al.,* 1975; Evans, 2009).

Phytochemical screening revealed the presence of carbohydrates, cardiac glycosides, and saponins. Cardiac glycosides are known for their cardiotonic properties and their ability to improve myocardial contractility, supporting the traditional use of *H. sabdariffa* in cardiovascular conditions (Christian *et al*., 2006; Inuwa *et al.,* 2012). Similar findings were reported in methanolic extracts of *H. sabdariffa* calyces (Ajiboye *et al.,* 2011).

The presence of saponins suggests possible antimicrobial and antioxidant activity of this plant extract, as saponins have been widely reported to exhibit such properties (Al-Anbari *et al.,* 2015). Saponins are also known to enhance immune responses and may contribute to disease prevention mechanisms (Sofowora, 2008).

Carbohydrates detected in the extract may contribute to the nutritional value of the plant, consistent with reports highlighting its mineral and nutritional richness (Maregesi *et al.,* 2013; Puro *et al.,* 2014).

The absence of flavonoids, tannins, and anthraquinones in the aqueous extract contrasts with findings from ethanol or methanol extracts (Ajiboye *et al*., 2011; Obouayeba *et al.,* 2015). This difference may be attributed to solvent polarity, as organic solvents are generally more efficient in extracting polyphenolic compounds (Ali *et al.,* 2005).

The acute toxicity study demonstrated no mortality or observable signs of toxicity at doses up to 5000 mg/kg body weight. According to OECD classification, substances with LD_{50} values greater than 5000 mg/kg are considered relatively non-toxic (OECD, 2000). This finding aligns with previous reports that documented no adverse

effects following administration of high doses of aqueous *H. sabdariffa* extract in experimental animals (Adedapo *et al.*, 2011; El-Sheikh *et al*., 2015).

The absence of acute toxicity supports the safe traditional consumption of aqueous preparations of *H. sabdariffa* beverages (Da-Costa-Rocha *et al.,* 2014). However, variations in toxicity profiles have been reported when different solvents were used for extraction (Jimoh *et al.,* 2013), emphasizing the importance of extraction method in safety assessment.

CONCLUSION

In conclusion, the aqueous extract of dried calyces of *Hibiscus sabdariffa* L. contained carbohydrates, cardiac glycosides, and saponins based on qualitative phytochemical screening, which may contribute to some of the biological activities reported for the plant. The preliminary acute oral toxicity assessment showed no mortality or overt signs of severe toxicity in albino rats administered 2000, 3200, or 5000 mg/kg body weight during the 14-day observation period. The absence of mortality at the highest dose tested was consistent with an acute oral LD_{50} greater than 5000 mg/kg body weight under the conditions of the present preliminary assessment; however, this represents a limit-dose finding rather than a statistically calculated LD_{50} point estimate. Therefore, the extract demonstrated low acute toxicity under the conditions evaluated. Nevertheless, the small number of animals, use of a single animal at each dose level, and deviations from the standard OECD TG 425 Up-and-Down Procedure limit the extent to which these findings can be generalized. Further repeated-dose toxicity studies, quantitative phytochemical profiling, comprehensive biochemical and histopathological evaluations, and pharmacological investigations are recommended to establish the longer-term safety profile and therapeutic potential of the extract.

CONFLICT OF INTEREST

Declaration: There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported

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Figure 1: Morphological Features of *Hibiscus sabdariffa* Plant
Photograph showing the stem, branches, leaves, and red fleshy calyces of the mature plant. The image highlights the structural characteristics useful for botanical identification

Figure 2: Dried Calyces of *Hibiscus sabdariffa*
Close-up image of air-dried calyces used for aqueous extraction. The figure illustrates the characteristic deep red coloration and shriveled texture of the processed plant material

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