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Preliminary Study of Phytochemical Constituents and Acute Toxicity of *Hibiscus Sabdariffa* Extracts

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ABSTRACT

This study was done to assess the phytochemical constituents and acute toxicity of Hibiscus sabdariffa calyx and leaf extract. The crude extract of the plant parts were obtained after solvent percolation and drying. Then, the presence of tannins, saponin, flavonoids, alkaloids and phenol were assessed qualitatively. Also, albino rats (*Rattus norvegicus*) were used to assess the toxicity level of the plant materials, haematological and biochemical parameters of the test animal blood were also assessed. The results revealed that the two plant parts contained alkaloids, tannins, saponin, phenol and flavonoids. Also, In the acute toxicity assay, the oral lethal dose (LD_{50}) of >5000 mg/kg bw and 2236.07 mg/kg bw were recorded respectively for the calyx and leaf extract of the plant. The plant calyx and leaf extracts showed no significant ($p<0.05$) effect on the levels of red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), and haemoglobin concentration (Hb) albeit, the plant extracts had a significant increase effect on the platelet count of the rats. there was a significant increase in the alkaline phosphatase (ALP), Alanine amino transaminase (ALT) and Aspartate amino transaminase (AST) in the group treated with the leaf extract compared with the control whereas the group treated with the calyx extract had comparable level of these enzymes with that of the control. These results has lent credence to the medicinal claim of the plant parts however, the leaf of the plant should be used in moderation.

KEYWORDS: *Hibiscus sabdariffa, phtochemica, acute toxicity, haematology, biochemical*

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1. INTRODUCTION

Medicinal plant is described as any plant which contain in any of its parts some bioactive substances that may be used for therapeutic purposes or which may be used as for the manufacturing of drugs [1]. Medicinal plants contain numerous bioactive substances belonging to chemical families like alkaloids, quinones, terpenoids, flavonoids, carotenoids, sterols, glycosides, tannins, saponins and phenols. These are secondary metabolites that are generally referred to as phytochemicals.

Traditional medicine has been described as a reliable alternative approach to health care delivery throughout the world since it is cheap, easily accessible and efficacious [2]. In Africa especially Nigeria, larger percentage of the population rely on plant derived materials for the management of various diseases. Since the turn of the millennium, the interest in traditional medicine has greatly increased primarily because of the increase in the phenomenon of antibiotics resistance [3]. Other reasons which readily make plant based medicinal preparation appealing in Nigeria is the level of poverty which makes the synthetic drugs most of which are imported too expensive for the average citizens. Moreover, the majority of rural dwellers do not have access to modern health care, so they mostly depend on medicinal plant to prevent or eliminate diseases [4].

The Africa tropical rain forest is endowed with many plant species which are exploited for medicinal purposes since antiquity. One of such plants is Roselle plant (*Hibiscus sabdariffa*) which is a member of the Malvaceae family that is believed to be native to Africa. It is cultivated in Nigeria, Sudan, India, Malaysia and Taiwan [5]. It is an annual or perennial woody shrub, growing to 2-2.5 m tall. The leaves are lobed, 8-15 cm long, arranged alternately on the stems. The flowers are white to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base, fleshy and bright red as the fruit matures [6, 7]. The plant is attracting the attention of food and beverage manufacturers and pharmaceutical industry for possible use as a natural product for herbal medicine and as a colorant to replace some synthetic dyes [8].

In African folkloric medicine, it is used as a beverage that helps to lower the body temperature, to treat cardiac conditions, and as a diuretic. Also, it has been reportedly used as antibacterial, cholagogic, and anthelmintic agent as well as in high blood pressure management [9]. Most of the researches done were on the calyx of the plant whereas, there is dearth of information on the phytochemical constituents and toxicity level of the other parts of the plant particularly leaf. Therefore this study was designed to compare the phytochemical constituents and toxicity of leaf extracts of *Hibiscus sabdariffa*.

2. MATERIALS AND METHODS

2.1. Collection, Identification and Extraction of the Extracts

Fresh leaves and fruits of *H. sabdariffa* (red type) were harvested from plants in the Horticultural and Botanical Garden of Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria in June, 2018. The plant materials were then authenticated at the Herbarium section of the Department of Forest Resources Technology and voucher specimens (A-HS1137L and A-HS1137C) of leaf and calyx respectively were deposited in the same department, Rufus Giwa polytechnic, Owo. The authenticated plant materials were cleaned thoroughly with tap water and then airdried. Afterwards, the plant material were then grounded into powder with the aid of an electric grinder and were stored in clean air- tight containers, and kept in a cool, dry place until required for use. One hundred gram (100 g) of the powdered sample was soaked in 200 ml of ethanol for 48hr with intermittent stirring using sterile spatula. The plant extracts were then filtered through muslin cloth into bijou bottles and then dried using rotary evaporator at a temperature of 50°C to yield crude extracts [10].

2.2. Laboratory animals

The albino rats (*Rattus norvegicus*) weighing between 150 and 200g used in this study were obtained from Department of Animal Production and Health, Federal University of Technology, Akure and were acclimatized and maintained in the animal house of the Science Laboratory Technology Department of Rufus Giwa Polytechnic, Owo, Nigeria.

2.3. Qualitative phytochemical screening

The extracts of the different plant parts were subjected to qualitative phytochemical analysis for the presence of tannins, saponin, flavonoids, alkaloids and phenol were carried out on the extracts using standard procedures as described by [10, 11].

2.3.1. Test for tannins

1ml of extract was boiled in 20ml of water in a test and then filtered. A few drops of 0.1% ferric chloride was added and observed green or a blue – black coloration which confirmed the presence of tannin.

2.3.2. Test for saponin

About 5ml of the extract was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion which confirmed a positive presence of saponins.

2.3.3. Test for flavonoids

A 3ml portion of 1% Aluminum chloride solution was added to 5ml of each extract. A yellow coloration was observed indicating the presence of flavonoids. 5ml of dilute ammonia solution were added to the above mixture followed by addition of concentrated H2SO4. A yellow coloration disappeared on standing. The yellow coloration which disappeared on standing indicating a positive test for flavonoids.

2.3.4. Test for alkaloids

A 1ml portion of the extract was stirred with 5ml of 1% aqueous HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1ml of the filtrate was treated with a few drops of either Mayer‟s reagent (Potassium mercuric iodide- solution gave a positive test for alkaloids.

2.3.5. Test for phenol

A 5ml portion of the extract was pipetted into a 30ml test tube, and then 10ml of distilled water was added to it. 2ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added and left to react for 30min.The development of bluish-green colour was taken as a positive presence of phenol.

2.4. Acute Toxicity Study

The animals were divided into 7 groups of five animals per group; control group and 6 treated groups. They were maintained on standard rat feed (Top feed, Warri, Nigeria) with water and allowed to acclimatize for seven days to the laboratory environment before the experiment. After an overnight fast, the control group received 0.3 ml sterile distilled water while each treated group received 10 mg/kg, 100 mg/kg, 1000 mg/kg, 1,500 mg/kg, 2,250 mg/kg, 3,500 mg/kg and 5,000 mg/kg body weight administered orally with the aid of a feeding needle connected to syringe at stated doses in appropriate volume of sterile distilled water [12]. The animals were observed for signs of toxicity including writhing, paw-licking, stretching, respiratory distress, diarrhoea and mortality for the first critical 4 hours and thereafter at 3 hour interval for 24 hours. The oral median lethal dose (LD_{50}) was calculated as the geometric mean of dose that caused 0% (a) and 100% (b) mortality respectively [13] using the formula; LD50= $(ab)^{1/2}$.

Twelve (12) mature male *albino* rats were used for this part. Animals in group I (control) received an equivalent volume (0.34 ml) of distilled water. The test groups II and III received orally, 500 and 250 mg/kg body weight of leaf and stem bark extract respectively representing ten percent of each extracts' LD_{50} for a period of 21 days. The extract was dissolved in distilled water as vehicle and delivered in 0.45 and 0.30 ml to group II and III respectively. After which they were fasted overnight before being sacrificed 24 hr after the last administration using standard protocols.

2.5. Determination of Haematological Parameters

At the end of the treatment period, the animals were anaesthetized in chloroform vapour and the blood collected via cardiac puncture into a plane tube. Heparinized test tubes were used to collect blood samples for haematological indices assay. White blood cells (WBCs), Red blood cells (RBCs), packed cell volume (PCV), platelets and Haemoglobin (Hb) were assayed by automated techniques using the Sysmex (Sysmex K21, Tokyo, Japan) automated machine respectively.

2.6. Determination of Blood Biochemical Parameters

Test kits for estimation of serum alanine amino transferase (ALT), alkaline phosphatase (ALP), aspartate amino transferase (AST), alkaline phosphatase (ALP) were obtained from Teco Diagnostics, USA.

2.7. Data Analysis

Data were presented as mean±standard error (SE). Significance difference between different groups was tested using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range

Test using SSPS window 10 version 25.0 software. The significance was determined at the level of P<0.05.

3. Results and discussion

The result of the qualitative phytochemical screening of the plant extract is presented in Table 1 where it was revealed that the two plant parts contained alkaloids, tannins, saponin, phenol and flavonoids with varying degree of reactivity. The presence of the various secondary metabolites in the plant materials justified their traditional uses in the treatment of various ailments and phytomedicines. Earlier researchers have reported the presence of these phytochemicals in the *H. sabdariffa* [7,13]. These plant metabolites have been reported to possess physiological functions. They are known for their antioxidant abilities, capacity to transfer electrons, quenching of free radicals and chelatin abilities, activate antioxidant enzymes, reduce alpha tocopherols radicals and inhibit oxidases [14].

Table 1: Phytochemical constituent of *H. sabdariffa* extracts

Phytochemicals	Calyx	Leaf
Alkaloids	+++	++
Tannin	+++	+++
Saponins	++	+++
Phenols	+++	++
Flavonoids	++	+

Key: += weak reaction, ++= moderate reaction, +++ = strong reaction.

In the acute toxicity assay, as presented in Table 2, the oral lethal dose (LD50) of >5000 mg/kg bw and 2236.07 mg/kg bw were recorded respectively for the calyx and leaf extract of *H. sabdariffa* plant. There were no symptoms of toxicity in rats treated with the calyx extract at all the dose used even at the highest dose of 5000 mg/kg bw whereas at doses above 1000 mg/kg bw of the leaf extract, some symptoms of toxicity were observedsuch as salivation, wobbling gait, weakness, pupil dilation, tremors, wet stool, spasm and death among the rats. These observations are in agreement with earlier report on the safety of the calyx extract of *H. sabdariffa* and the ethnobotanical information on the plant [14, 15]. The leaf of the red type of hibiscus is not usually consumed unlike the green variety. Even the green variety have to be passed through some treatment like boiling, alkaline fermentation and sun drying to probably to reduce the toxicants that are present in the plant. The results indicate a possible toxicity of the leaf of the *H. sabdariffa* at low concentrations if consumed over long period of time. Earlier, Clarke and Clarke [16] reported that any compound or drug with oral LD_{50} estimates greater than 1000 mg/kg body weight could be considered to be of low toxicity and safe. Hence, the leaf extract of the plant may be safe for consumption but should be used with caution as over consumption of the plant leaf may lead to fatal implications.

Table2: mortality in lethal dose (LD_{50}) determination for *Hibiscus sabdariffa* calyx extract

Dose (mg/kg bw)	Calyx		Leaf	
	No of death	Percentage mortality	No of death	Percentage mortality
Control	$^0/_4$	0	$^0/_4$	0
10	$^0/_4$	0	$^0/_4$	0
100	$^0/_4$	0	$^0/_4$	0
1000	$^0/_4$	0	$^0/_4$	0
1500	$^0/_4$	0	$^1/_4$	25
2250	$^0/_4$	0	$^1/_4$	25
3500	$^0/_4$	0	$^2/_4$	50
5000	$^0/_4$	0	$^4/_4$	100
LD_{50}		> 5000 mg/kg bw		2236.07 mg/kg bw

The results of the effect of *H. sabdariffa* extracts on the haematological indices of albino rats is presented in Table 3. The plant calyx and leaf extracts showed no significant (p<0.05) effect on the levels of red blood cells (RBC), white blood cells (WBC), packed cell volume (PCV), and haemoglobin concentration (Hb) albeit, the plant extracts had a significant increase effect on the platelet count of the rats. The non increase in the level of all the major blood parameters in the treated rats as compared with the control suggests that the plant parts may not contain agents that can interfere with haematopoietic system of the rats. This disagrees with earlier report that plant materials rich in saponins may impede blood production when consumed in large quantity because saponin has been reported to reduce haematological parameters probably due to lysis of blood cells or suppression of blood cell synthesis [17].

Table 3: Effect of *H. sabdariffa* extract on haematological indices of albino rats

Parameter	Control	Calyx	Leaf
RBC	4.97±0.01^a	5.07±0.00^a	4.82±0.01^a
WBC	6.73±0.10 ^{a}	6.82±0.08 ^{a}	7.03±0.01 ^{a}
HB	12.05±0.05^a	13.89±0.09 ^{a}	11.59±0.03 ^{a}
PCV	37.83±0.02^a	38.63±0.06 ^{a}	36.81±0.02 ^{a}
Platelets	159.67±5.18^a	170.23±2.01^b	176.05±6.09 ^{b}
Neu (%)	31.16±0.08^a	29.03±0.02 ^{a}	30.12±0.00 ^{a}
Lymp (%)	47.21±0.01^a	45.37±0.15 ^{a}	49.13±0.05 ^{a}
Mono (%)	19.79±0.07^a	20.41±0.04 ^{a}	20.98±0.12 ^{a}

Values are Mean±S.E.M, Values followed by different alphabet across the rows are significantly different at *P<0.*05

However, the increase in platelets in the treated group may be due to stimulation of the production and secretion of thrombopoetin by the extracts. Thrombopoetin is known for regulating platelet production, thus *H. sabdariffa* leaf extracts may possess some haemostatic properties [8, 17].

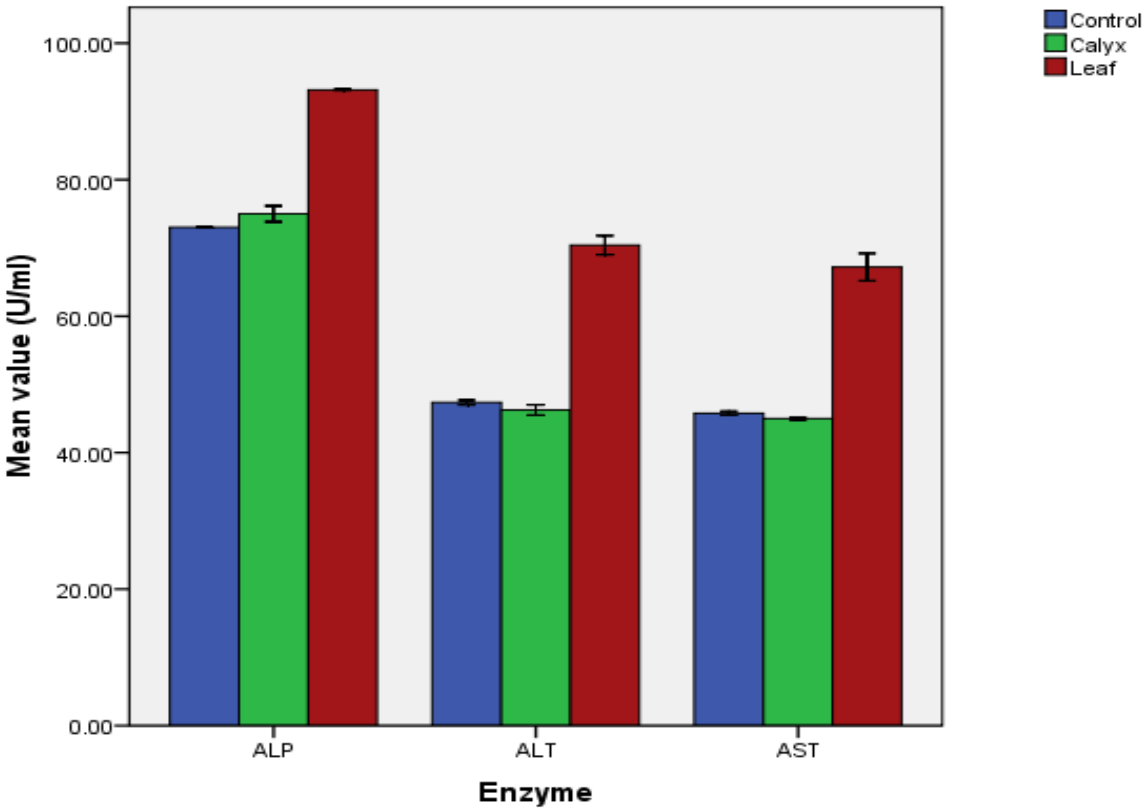

Figure 1: Effect of *H. sabdariffa* extract on blood biochemical parameters of rats

Key: I: Control Group, II: Group given Calyx extract 500 mg/kg bw, III: Group given leaf extract 250mg/kg bw.

In the blood biochemical assay, there was a significant increase in the alkaline phosphatase (ALP), Alanine amino transaminase (ALT) and Aspartate amino transaminase (AST) in the group treated with the leaf extract compared with the control whereas the group treated with the calyx extract had comparable level of these enzymes with that of the control. These enzymes are biomarkers of health indices and are of importance as diagnostic tools clinical evaluation of the state of health [18]. The liver and heart release ALT and AST when under severe stress and an elevation in their plasma concentrations are indicators of liver and heart damage [19]. Hence, the symptoms of toxicity observed and the death of the test rats treated with the *H. sabdariffa* leaf may be due to possible toxicants in the leaf that may have deleterious effect on the heart and the liver. ALT is a better parameter for detecting liver damage since it is more specific to the liver [20]. Thus, the significant increase in all these enzymes in group treated with leaf extract is an indication of potential hepatotoxicity, cardiotoxicity and kidney toxicity [18, 20].

Conclusion

From the foregoing, it is concluded that the Hibiscus sabdariffa calyx and leaf extract contained alkaloids, tannin, saponins, phenols and flavonoids. Also, the calyx extract of the plant had no toxic effect on the albino rats even at high doses with the LD50 suggested to be > 5000 mg/kg bw whereas leaf extract had a prominent toxic effect on the albino rats with LD50 2236.07 mg/kg bw. Invariably, the calyx extract may be safe for application in man however the leaf extract should be used with great caution as high doses may have fatal implication.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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