Extractive Values and Diuretic Activity of Methanol Extract of Three Varieties of *Hibiscus sabdariffa* Linn (Malvaceae) in Wistar Albino Rats.

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ABSTRACT

Hibiscus sabdariffa Linn (Malvaceae) is widely cultivated in Nigeria as food and for medicinal purposes. The calyx is the most frequently used part of the plant and has been reported to have antioxidant, antihypertensive and diuretic activity. The aim of the present study is to determine the extractive values and diuretic activity of three varieties of *H. sabdariffa*. Aqueous, methanol, ethanol, chloroform and hexane soluble extractive values were determined. The effect of the extracts of *H. sabdariffa* on urine output was evaluated and the concentration of sodium and potassium in blood was also determined. The results showed that water extract had the highest extractive value (32.90%, 26.50% and 29.00%) for the three varieties (Dark red, Light red and Green) respectively. *H. sabdariffa* extract (100 mg/kg G) produced a significant ($p<0.05$) increase in urine output compared to the control. The diuretic activity of extract was comparable with the standard drug Hydrochlorothiazide (10 mg/kg). The other species of *H. sabdariffa* extract produced no significant increase in urine output. *H. sabdariffa* extract (200 mg/kg G) showed a significant ($p<0.05$) natriuretic activity compared to control. There was no significance difference in the serum potassium levels for all the three varieties at the doses employed. The study showed that water is the most suitable solvent for extraction of the calyx of *H. sabdariffa* and the green variety produced a significant increase in urine output with potassium sparing activity.

Keywords: *Hibiscus sabdariffa*, Calyx, Extractive values, Diuretic Activity, Hydrochlorothiazide.

INTRODUCTION

Medicinal plants have been used for the treatment of illness since ancient time[1]. Numerous medicinal plants are now used as a source of modern medicine[2]. They play an important role in the primary health care as a reservoir of active principles, thus they represent useful tools for discovery of well tolerated new drug[3].

Hibiscus sabdariffa Linn is commonly named as "red sorrel" or "sour tea", or "roselle" and it is popularly called "zobo" in Nigeria. It is a known medicinal plant with a worldwide fame[4] and the plant is grown in all parts of the world; it is native to West Africa or Asia and was introduced to other parts of the world such America, Philippines, Malaya, West indies and Indonesia[5,6]. It is greatly cultivated in the northern part of Nigeria, from where it is distributed to other parts of the country[7].

H. sabdariffa is an annual or perennial herb or woody-based sub-shrub, belonging to the Malvaceae family, it grows up to 2-2.5 m (7-8 ft) tall[8]. It has a deep penetrating taproot and a smooth or nearly smooth, cylindrical, typically dark green to red stems[9]. The leaves are 7.5-12.5cm long, simple, alternate, green with reddish veins and short or long petioles. Flowers are solitary, axially, 5-7cm in diameter; Calyx is thick, red prominently; Petals 5 and Stamens are numerous[8].

H. sabdariffa is mainly cultivated for its calyx, which is of three types: green, red and dark red. The plants from which the three varieties of calyxes are obtained differ in the following parameters according to Javadzadeh *et al*.,[10] and Golam *et al*., [11]. Plant height, canopy diameter, number of branches per plant, number of fruits per branch, number of fruits per plant, weight of fruits per plant, weight of fresh calyx/ plant, weight of dry calyx / plant, fruit weight, fruit length, fruit diameter, weight of fresh calyx /fruit, weight of capsule /fruit.
According to Golam *et al*.,[11] the parent varieties are the arab (dark red) and terrenganu variety while the green variety is a mutant of the parents.
Extractive values are useful for the evaluation of a crude drug. It gives an idea about the nature of the chemical constituents present in the crude drug, and it serves as a useful tool that helps to quantify the amount of chemical constituents soluble in a particular solvent[12]. It is primarily useful for the determination of exhausted or adulterated drugs. The extractive value of the crude drug determines the quality as well as purity of the drug.

Hibiscus sabdariffa plant has antiseptic, diuretic, purgative, sedative and emollient properties. The leaves in combination with ginger are used to suppress high blood pressure and in treatment of hypertension[13]. Kerharo and Adam,[14] reported that the calyx shows antibacterial activity, also, antihypertensive, hepatoprotective, antioxidant and antimutagenic activity of the dried petals have been investigated and reported[15,16,17].

Diuretics are defined as compounds that increase urine flow[18], increasing urine volume as well as water and solute (sodium and chlorine) excretion. They act in the kidneys primarily through decreased tubular re-absorption of sodium ions and its osmotic equivalent of water in the renal tubules. Diuretics have been employed in the treatment of edema of various etiology (such as heart failure, liver cirrhosis, and nephritic syndrome) and in the management of hypertension[19]. They are often used in combination with other classes of drugs (such as calcium channel blockers, beta blockers, angiotensin converting enzyme inhibitors/receptor blockers and vasodilators). Furthermore, diuretics have the potential to lower blood pressure by causing reduced arterial resistance and/or decreasing cardiac output[20,21].

The commonly used classes of diuretics are loop, thiazide, and potassium sparing diuretics, and carbonic anhydrase inhibitors[22]. Thiazide diuretics are known to cause hypokalemia that may result in arrhythmias[23,24].
The present study aimed to determine the extractive values and diuretic activity of three varieties of *Hibiscus sabdariffa.*

MATERIALS AND METHODS

Plant collection and identification

The dried calyx of three varieties of *Hibiscus sabdariffa* were purchased from a local market in Ilorin, Kwara state. Samples of these varieties were identified by Mr. Bolu-Ajayi and authenticated at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Nigeria where voucher specimens (Green (UILH/001/127), light red (UILH/002/646) and dark red (UILH/003/1351)) were deposited.

Preparation and extraction of plant materials

The collected calyx was carefully cleaned to remove dust and sand particles. They were further dried in a hot air oven at 30 ^{o}C and then reduced to powder using a laboratory mill and thereafter stored in a tightly covered amber coloured glass jar until required.

A 200 g quantity of each plant material was weighed separately into three different amber bottles for extraction, and were exhaustively extracted by maceration for 72 hours with 80% methanol at room temperature with occasional stirring, after which it was filtered and concentrated using rotary evaporator to obtain the methanol extracts. The percentage yield of each was determined using the formula;

$$\text{Percentage yield (\%)} = \frac{\text{Weight of solid extract}}{\text{Weight of powdered leaves used}} \times 100$$

Determination of extractive values of *H. sabdariffa*

The dried powdered plant material of *H. sabdariffa* (L) was extracted with water, methanol, ethanol, chloroform and hexane. A 5 g quantity of the coarsely powdered plant material was weighed into a dry 250 ml conical flask. Then the flask was filled with different solvents (100 ml) separately. The flasks were corked and kept aside for 24 hours at room temperature, shaking frequently. The extract was filtered through Whatmann No. 1 filter paper into a 50 ml measuring cylinder.

After the filtrate was obtained, it was then transferred into weighed porcelain dishes, evaporated to dryness on a water bath and then dried in an oven at 50 ^{o}C to constant weight and the final weight determined[25]. The extractive value was calculated in percentage using the following formula;

$$\text{Extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} * 100$$

Experimental animals

Wistar albino rats (110-150 g) of both sexes, obtained from the Animal house of the Ladoke Akintola University of technology, Ogbomosho, Oyo state, Nigeria were used. The animals were kept in the animal house of Department of Pharmacology and Toxicology in a well-ventilated room at a controlled temperature and 12 h light/dark cycle. They were fed with standard rodent pellets (Livestock Feed plc., Lagos, Nigeria) and water *ad libitum*. The animals were treated in accordance with the guideline for the use of animals by University Ethical Review Committee, University of Ilorin, Ilorin, Nigeria. Ethics clearance with reference: UERC/ASN/2019/1605 was obtained.

EVALUATION OF DIURETIC ACTIVITY

Diuretic activity was determined following the method used by Lahlou *et al*., [26] with slight modification.

The animals were randomly divided to eight groups of six animals each and treated as follows; Group I : 100 mg/kg methanol extract of dark red calyx of *H. sabdariffa* (MD), Group II: 200 mg/kg MD, Group III:100 mg/kg methanolic extract of light red calyx of *H. sabdariffa* (ML), Group IV: 200 mg/kg ML, Group V: 100 mg/kg methanolic extract of green calyx of *H. sabdariffa* (MG), Group VI: 200 mg/kg MG, Group VII: 10 mg/kg Hydrochlorothiazide (positive control) and Group VIII: 0.2 ml 0.9% normal saline.

The oral route was employed for the extract and positive control. All the groups were given a salt loading dose of 2 ml 0.9% normal saline subcutaneously to ensure proper hydration one hour before administration of the extract.

The time of first urination was recorded, and volume of urine was measured after 6 of administration of extract and standard drug.

Determination of Serum electrolytes: Blood samples were taken by caudal incision and collected in dry tubes and centrifuged at 4500 rpm for 10 minutes. The serum obtained was transferred into plain sterile sample bottles and analyzed for electrolytes (sodium and potassium).

Statistical analysis

Urine output was expressed as mean values ± standard error of mean (SEM). Data was analyzed using Graph pad Prism 6.0 software. The difference between the means of the treated and control groups was evaluated using analysis of variance (ANOVA) and Tukey Multiple Comparison Test. Results were considered statistically significant at $P < 0.05$.

RESULT AND DISCUSSION

Extractive value

Table 1: Extractive value for the three varieties of *H. sabdariffa*

Solvent	Extractive value (% w/w)		
	Dark red	**Light red**	**Green**
Aqueous (Water)	32.90	26.50	29.00
Methanol	18.75	20.05	20.05
Ethanol	8.00	9.00	8.00
Chloroform	0.40	0.30	0.60
Hexane	0.50	0.50	0.50

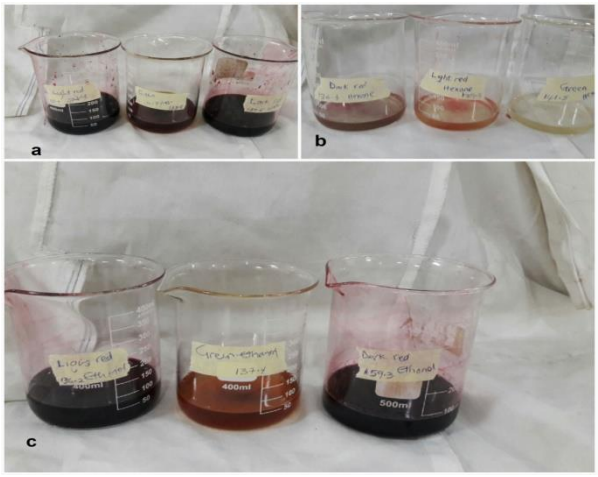

Figure 1: Colours of extract for the three varieties of *H. sabdariffa*.
Plate 1a: Pictures of the aqueous extract of the three varieties.
Plate 1b: Pictures of the hexane extract of the three varieties.
Plate 1c: Pictures of the ethanol extract of the three varieties

Effect of three varieties of *H. sabdariffa* on urine output in rats

H. sabdariffa extract (100 mg/kg G) produced a significant ($p<0.05$) increase in urine output compared to the control. The diuretic activity of extract was comparable with the standard drug Hydrochlorothiazide (10 mg/kg). The other species of *H. sabdariffa* extract produced no significant increase in urine output.

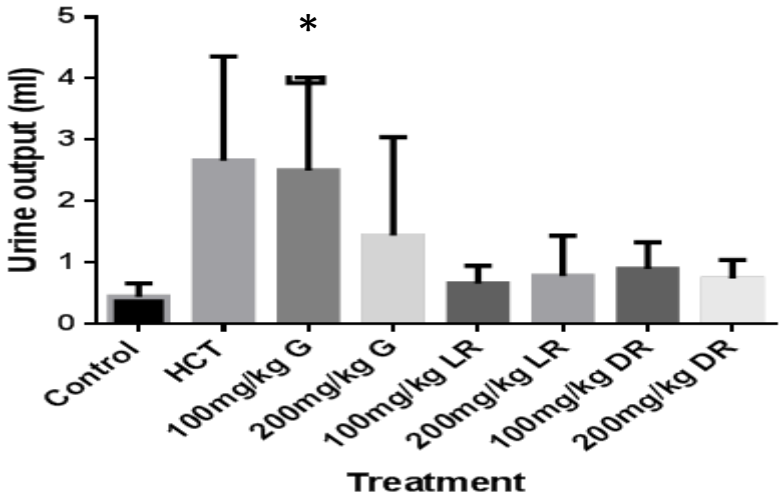

Figure 2: Effect of three varieties of *H. sabdariffa* extract on urine output in rats.
n=6, *p<0.05 vs Control (one way ANOVA, Tukey's Multiple Comparison Test).
DR: Dark red, LR: Light red, G: Green, HCT: Hydrochlorothiazide.

Effect of three varieties of *H. sabdariffa* on serum electrolyte in urine of rats

H. sabdariffa extract (200 mg/kg G) showed a significant ($p<0.05$) natriuretic activity in urine excretion compared to control.

Table 2: Effect of plant extract on serum electrolyte (Na+, K+) level

Treatment	Dose (mg/kg)	K+ (mmol/L)	Na+ (mmol/L)
Control	0.2 (ml/kg)	4.3±0.8	137.0±3.0
HCT	10	5.4±0.7	141.7±4.0
H. sabdariffa G	100	6.1±1.0	137.3±4.0
H. sabdariffa G	200	6.2±1.4	143.3±2.5*
H. sabdariffa LR	100	4.0±0.1	139.0±2.0
H. sabdariffa LR	200	4.5±0.2	154.0±8.0
H. sabdariffa DR	100	6.2±0.1	143.3±9.0
H. sabdariffa DR	200	4.9± 0.7	138.0±3.4

Values are expressed as mean±SEM. (n=6) *p<0.05 vs
Control (one way ANOVA, Tukey's Multiple Comparison Test)
Keys: DR- Dark red; LR- Light red; G- Green; HCT- Hydrochlorothiazide

DISCUSSION

The extractive value is a useful parameter that helps to quantify the amount of phytochemical constituent(s) in a crude drug soluble in a particular solvent[27]. From the extractive values obtained for all the solvents, it suggests that *H. sabdariffa* has more polar constituents as it produced more yield in polar solvents. The colour of the extracts varied with solvent used which suggest that there are different components being extracted into the different solvents, this is consistent with the study by Okereke *et al*.,[6] where the phytochemical analysis on extracts was obtained using different solvents for *H. sabdariffa* calyx. Water has the highest extractive value for the three varieties which justifies its use traditionally as the preferred solvent for extraction.

Calyxes of *H. sabdariffa* have been reported to contain mainly polar constituents which include: polyphenolic acids, flavonoids and anthocyanins, such as delphinidin-3-O-glucoside, delphinidin-3-O-sambubioside, and cyanidin-3-O-sambubioside[28].

Medicinal plants are often used as diuretics due to the increased side effects of modern diuretics[29] and they have been reported to be beneficial in reducing blood pressure[30].

In the present study, the diuretic activity of methanol extract of three varieties of *H. sabdariffa* was studied and demonstrated increased urinary output of varying degrees when compared with the negative control group. The 100 and 200 mg/kg of the green calyx of *H. sabdariffa* extract produced the highest urine output which was statistically significant ($p<0.05$) relative to the control, and they compared effectively with the standard drug (hydrochlorothiazide). The diuretic effect observed was not dose-dependent as the urine output produced by 100 mg/kg was higher than that produced by 200 mg/kg for the three varieties studied.

Hydrochlorothiazide directly inhibits the reabsorption of NaCl by competition with the $C1^-$ site of the co-carrier. It indirectly stimulates the reabsorption of calcium by an increase in tubular re-absorption proximal to that of sodium but their effect is low[31].

H. sabdariffa extract (200 mg/kg G) showed a significant ($p<0.05$) increase in Na^+ excretion (Table 2), and this may suggest a mechanism of action similar to thiazide diuretic. Thiazides diuretics inhibit reabsorption of sodium (Na^+) and chloride (Cl^-) ions from the distal convoluted tubules[32].

There was no significance difference in the serum potassium levels for all the three varieties at the doses employed, this is an interesting property as most diuretics are known to cause undesired electrolyte imbalance. Findings from this work therefore suggest that *H. sabdariffa* may be acting as a potassium-sparing diuretic.

CONCLUSION

Evidence from this study shows that water is the most suitable solvent for extraction of *H. sabdariffa* in order to obtain the highest yield. In addition, the study showed that the green variety possesses the highest diuretic activity with benefit of potassium sparing activity.
Further studies are encouraged to isolate the compound in the methanol extract responsible for the diuretic activity.

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