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Foaming Characteristics of Saponins of Leaves of Hibiscus Sabdariffa Linn (Red Variety)

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Abstract:
The aim of this study was to determine the foaming characteristics (foam power and foam stability) of saponins of the leaves of Hibiscus sabdariffa Linn. The preliminary screening for saponins and phytochemical screening were carried out to determine the probable presence of saponins and other secondary metabolites. The results showed the probable presence of saponins in the leaves of Hibiscus sabdariffa Linn. Cold maceration extraction method was conducted and yields were obtained from the extracts i.e. aqueous, methanol, and n-butanol. The methanol extract possessed the highest yield of 46.53 % compared to aqueous and n-butanol extracts with 8.15 % and 0.69 % respectively. The foam heights and foaming times of aqueous, methanol and n-butanol extract and that of synthetic detergents (Tween 80 and Triton X-100) when subjected to statistical analysis (ANOVA) showed significant decreases (at $p<0.05$) between the foam heights (H_0, H_1, and H_5) and foaming times (Tc) at varying concentrations. The foam power of n-butanol extract at 0.5 % was the highest with values of 40.47 % indicating that it was not stable when compared with the values of 87.28 % and 73.57 % for Triton X-100 and Tween 80 respectively. Therefore, saponins of the leaves of Hibiscus sabdariffa Linn. may be of limited use in the detergent industries but relevant in related industries. It is recommended that the foaming properties of the crude saponins would be more enhanced when subjected to further purification processes to meet the standards of Triton X-100 and Tween 80.

Keywords: *Saponins, Hibiscus sabdariffa Linn., secondary metabolites, foam power, foam stability, metastable*

1. Introduction

Saponins are secondary metabolites synthesized by many different plant species [1]. Their name is derived from Latin word '*sapo*' meaning soap, due to their surfactant properties which allow forming stable soap-like foam upon shaking in aqueous solution [2, 3]. Saponins are recognized by their ability to produce a soapy lather when shaken with water. They are widely distributed in nature and reported to be present in 500 genera of plants. All saponins are polar in nature and are freely soluble in water but insoluble in non-polar solvents. Saponins on hydrolysis yield an aglycone known as 'sapogenin' and a glycone known as sugar. A wide variety of plants belonging to the families of *Liliaceae, Dioscoreaceae, Solanaceae, Sapindaceae* and *Agavaceae* are the major sources of saponins. However, a few neutral saponins have also been isolated and characterized from animal sources. They are found in oats, peppers, aubergine, tomato seed, alliums, asparagus, yam, fenugreek, yucca and ginseng [4], millet, guinea corn, groundnut, sweet potatoes and cassava [5] and neem [6].

Saponins have many medicinal uses including, antimicrobial, anti-tumor, insecticidal [7] hepatoprotective, haemolytic [8] and anti-inflammatory activities. They also decrease blood cholesterol level and may be used as adjuvant in vaccines [9, 10, 11, 12, 13, 14, 15]. In addition, saponins are used in the preparation of soaps, detergents, fire extinguishers, shampoos, beer and cosmetics [16]. Many saponins exhibit haemolytic activity, have a bitter taste and are toxic to fish [17].

Hibiscus sabdariffa Linn. is a species of hibiscus, native to the old world tropics, used for the production of bast fiber and as an infusion (herbal tea). It is an annual or perennial herb or woody-based sub-shrub, growing up to 2-2.5 m (7-8 ft) tall. The plant is widely cultivated for its strong fibers and is well known for its edibility and medicinal properties. Though the calyx is the most frequently used portion of the plant, the leaves and seeds are often made into salads, curries and potherbs [18]. The plant is rich in vitamins, natural carbohydrates, proteins, tannins, gums and other antioxidants including minerals [19].

The crop is mainly grown as a vegetable from the savannah and semi-arid areas in Africa, while its use as a fibre crop is mostly in southern Asia. Formerly, it was traditionally cultivated in Nigeria for its leaves, seeds and stems but is now being grown commercially for its calyces [20]. *Hibiscus sabdariffa* Linn. is widely grown in northern parts of Nigeria,

where the dried calyx is used for making a popular *'zobo'* drinks [21]. [22] reported that there are three common varieties of *Hibiscus sabdariffa* Linn. grown in Nigeria. Two of these varieties have red calyces while one has green calyces. The green variety is more predominant in the Southern part of Nigeria but the green variety is scarce while the other two red varieties are predominant in the Northern part of this country. However, the green variety is also common in the Northern part of the country. The calyces from these varieties have a number of uses and promising prospects for industrial purposes [23].

2. Materials and Methods

2.1. Methods

2.1.1. Sample Collection and Treatment

Preliminary screening for saponins was carried out on fresh leaves of *Hibiscus sabdariffa*, Linn. The sample was collected from the campus of the University of Maiduguri and authenticated by the Botany section of the Department of Biological Sciences. The plant was separated into different parts and the leaves were air dried under shade. Further drying was carried out in the open until it was completely dried. The dried leaves were milled into a fine powder using an electric blender.

2.2. General Extraction Methods

2.2.1. Preparation Various Extracts

The sample (50 g) was extracted with aliquots of methanol shaken thoroughly and intermittently for maximum extraction. The extract was carefully decanted into another conical flask. The extraction was carried out until the supernatant was colourless. The supernatant was then transferred into a weighed beaker and evaporated at low temperature using a hot plate and residue stored at room temperature until required. The sample residue from methanol extraction was re-extracted with distilled water to produce aqueous extract and re-extracted again with n-butanol to produce n-butanol extract.

2.3. Phytochemical Screening of Sample

Qualitative analysis was carried out using the methanol extract [24].

2.3.1. Preparation of 10 % Stock Solution of Various Extracts

Aliquot of 0.5 g portion of each of various extracts (methanol, aqueous and n-butanol) was weighed, transferred into a mortar and ground with pestle. It was macerated with distilled water, transferred into a 5 ml volumetric flask and made up to the 5ml mark, mixed and transferred into a clean and dry sample bottle and stored in the refrigerator.

2.3.2. Determination of Foaming Activities of Various Extracts

Graded solutions of various extract (0.2 %, 0.4 %, 0.5 %, 0.6 %, 0.8 %, 1.0 %) were prepared from the stock solution of each extract separately. Each graded solution was shaken using a vortex mixer and the foam height measured at initial height of 0 min, after 1 min and lastly after 5 min. Times were also recorded for Initial time (Hi of formation of the foam height and the final time (Hc) for the complete disappearance of the foam [25] and foam power determined [26].

2.4. Statistical Analysis

The values were statistically analyzed using one-way ANOVA with SPSS v.21. The values were found to be significant at $p<0.05$. The values were compared with both standards Triton X-100 and Tween 80.

3. Results and Discussion

The preliminary screening for saponins of the leaves of *Hibiscus sabdariffa* Linn. yielded a long-lasting foam upon shaking in water depicting the probable presence of saponins. Different solvents such as distilled water, methanol and n-butanol were used for the preparation of aqueous, methanol and n-butanol extracts respectively. Methanol extract had the highest yield of 46.53 % when compared with aqueous and n-butanol with 8.15 % and 0.69 % respectively (Table 1) and pattern was in agreement with the findings of previous workers [25]. The results of the phytochemical screening indicated the probable presence of several secondary metabolites of plants including saponin glycosides, cardiac glycosides, carbohydrates, flavonoids and terpenoids (Table 2). Since the presence of alkaloids, tannins, phlobatanins, anthraquinones and cardenolides could not be detected, they may probably be absent (Table 2).The results of foam heights and foaming times at varying concentrations were statistically analyzed using ANOVA. It was found that values were significant at $p<0.05$ (Table 4-7). The foam stability R5 is the foam power and is the ratio of foam heights at 5 min to that at 0 min. The R_5 value of 0.5 % concentration of n-butanol extract was 40.47 % indicating that it was not metastable when compared with values of 87.28 % and 73.57 % for Triton X-100 and Tween 80 respectively (Table 3). Therefore, the saponins from the leaves of *Hibiscus sabdariffa* Linn. may not find in the detergent industry but others including cosmetics and food industries.

Extract	Yield (%)
Aqueous	8.15
Methanol	46.53
n-Butanol	0.69

Table 1: Results of Determination of Yields of Extract of Leaves of Hibiscus Sabdariffa, Linn. Using Aqueous, Methanol and N-Butanol

Test	Result
• **Test for Alkaloids**	
Dragendoff's Reagent	-
Mayer's Reagent	-
• **Test for Carbohydrates**	
Molisch's Test	+
Test for Monosaccharide	-
Test for Free Reducing Sugars/ Fehling's	+
Test for Combined Reducing Sugars	+
Test for Pentose	+
• **Test for Saponin Glycosides**	
Frothing Test	+
• **Test for Tannins**	
Ferric Chloride	-
Lead Acetate	-
• **Test for flavonoids**	
Schinoda's Test	+
Ferric Chloride	-
Lead Acetate	-
Sodium Hydroxide	-
• **Test for Phlobatanins**	-
• **Test for Soluble Starch**	-
• **Test for Combined Anthraquinone**s	-
Test for Combined Anthraquinones	-
• **Test for Terpenoids**	+
• **Test for Cardenolides**	
Keller-Killiani's Test	-
• **Test for Cardiac Glycosides**	
Salkowski's Test	+
Lieberman-Burchard's Test	+
Key: + = Present; - = Absent	

Table 2: Results of Phytochemical Screening of Methanol Extract of Leaves of Hibiscus Sabdariffa, Linn

Conc. (%)	Aqueous	Methanol	n-Butanol	Triton X-100	Tween 80
0.2	5.80±0.37$^{a, b}$	4.00±0.70$^{a, b}$	1.60±0.40$^{a, b}$	74.80±2.53	41.60±1.50^{a}
0.4	6.80±0.73$^{a, b}$	4.80±0.80$^{a, b}$	4.20±0.37$^{a, b}$	89.40±6.50	48.60±1.07^{a}
0.5	5.80±1.01$^{a, b}$	4.40±0.24$^{a, b}$	6.80±0.37$^{a, b}$	93.80±0.73	45.80±2.28^{a}
0.6	10.20±1.20$^{a, b}$	5.40±0.50$^{a, b}$	6.20±0.37$^{a, b}$	89.60±1.53	46.00±2.00^{a}
0.8	7.60±1.28$^{a, b}$	5.40±0.60$^{a, b}$	6.60±0.24$^{a, b}$	99.20±2.80	55.20±4.25^{a}
1.0	5.60±2.20$^{a, b}$	5.60±0.50$^{a, b}$	8.00±0.31$^{a, b}$	100.60±6.18	58.80±5.09^{a}

Table 5: Results of Determination of Composite Value for Various Concentrations of Aqueous, Methanol, N-Butanol, Triton X-100 and Tween 80 at H_1 (Mm)

Data expressed as Mean ± SEM, (n=5), $^{a, b, c, d, e}$= Significant decrease from Triton X-100, Tween 80, Aqueous, Methanol, n-Butanol respectively at (p<0.05)

Conc. (%)	Aqueous	Methanol	n-Butanol	Triton X-100	Tween 80
0.2	4.60±0.24[a, b, e]	3.20±0.80[a, b]	0.20±0.20[a, b, c]	70.20±1.39	37.60±1.43[a]
0.4	4.60±0.24[a, b]	4.60±0.60[a, b]	2.20±0.37[a, b]	84.20±5.56	43.00±1.48[a]
0.5	4.00±0.54[a, b]	3.40±0.50[a, b]	3.40±0.60[a, b]	90.60±0.60	41.20±2.43[a]
0.6	6.80±1.31[a, b]	4.20±0.48[a, b]	3.20±0.37[a, b]	84.40±1.20	42.80±1.49[a]
0.8	5.60±0.8[a, b]	3.60±0.50[a, b]	3.40±0.40[a, b]	92.40±1.28	48.40±3.73[a]
1.0	2.00±1.00[a,b]	3.20±0.73[a,b]	4.00±0.31[a,b]	96.00± 5.59	52.60±3.95[a]

Table 6: Results of Determination of Composite Value for Various Concentrations of Aqueous, Methanol, N-Butanol, Triton X-100 and Tween 80 at H_5 (Mm)

Data expressed as Mean ± SEM, (n=5),[a, b, c, d, e]= Significant decrease from Triton X-100, Tween 80, Aqueous, Methanol, n-Butanol respectively at (p<0.05)

Conc. (%)	Aqueous	Methanol	n-butanol	Triton X-100	Tween 80
0.2	1.99±0.27[a, b]	1.64±0.22[a, b]	0.04±0.01[a, b]	13.59±1.90[b]	18.24±0.85
0.4	1.74±0.18[a, b]	1.77±0.19[a, b]	0.61±0.05[a, b]	15.94±2.84	18.76±1.00
0.5	1.71±0.20[a, b]	1.54±0.31[a, b]	0.81±0.05[a, b]	16.27±3.21	18.86±1.03
0.6	1.92±0.39[a, b]	1.85±0.25[a, b]	0.80±0.06[a, b]	16.11±3.10	18.79±0.96
0.8	2.03±0.30[a, b]	1.77±0.28[a, b]	0.89±0.01[a, b]	13.14±3.07	18.85±1.09
1.0	0.73±0.09[a, b]	1.77±0.34[a, b]	0.93±0.07[a, b]	13.63±3.21	18.89±1.14

Table 7: Results of Determination of Composite Value for Various Concentrations of Aqueous, Methanol, N-Butanol, Triton X-100 and Tween 80 at Tc (Hr):

Data expressed as Mean ± SEM, (n=5),[a, b, c, d, e]= Significant decrease from Triton X-100, Tween 80, Aqueous, methanol, n-Butanol respectively at (p<0.05). Tc- Time for complete disappearance of foam.

4. Conclusion

The results of this study showed that saponins extracted from the leaves of *Hibiscus sabdariffa* Linn. Using various reagents possessed low foam characteristics (Foam Power and Stability) Foam stability of 0.5 % methanol crude saponins extract from the leaves of *Hibiscus sabdariffa* Linn., was 40.47 %. R5 value of 40.47 % represents low foam stability for crude saponins extracted from the leaves of *Hibiscus sabdariffa* Linn., when compared with the R_5 values of the standards, Tween 80 (73.57 %) and Triton X-100 (87.28 %). It can be concluded therefore, that the saponins from the leaves of *Hibiscus sabdariffa* Linn. may not be applicable in detergent industries but may be used in related industries. It is recommended that the foaming properties of the crude saponins would be more enhanced when subjected to further purification processes to meet the standards of Triton X-100 and Tween 80.

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