



Pharmacognostic Characteristics of *Hibiscus sabdariffa* L. as a Means of Monitoring Quality

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Abstract

Background and objectives: *Hibiscus sabdariffa* L. (roselle) sepals, commonly known as bissap is prepared as a cold drink which is widely drunk in Ghana and across West-Africa for their medicinal and nutritional properties. The plant is known to have anti-hypertensive, anti-hyperlipidemic, anticancer, anti-diabetic and anti-inflammatory activities. For such a widely sold and used medicinal plant, it is important that simple but reliable parameters can be used to estimate the quality. This will reduce adulteration, improve quality and hence safeguard the consumer. This study was to examine simple parameters that can be employed to estimate the quality of dried whole and powdered samples of *H. sabdariffa* that are widely sold on the open West-African market. **Methods:** The parameters investigated were macroscopic, microscopic, phytochemical, physicochemical, microbiological, and fluorescence characteristics. Heavy metal and HPLC analyses were also performed. **Results:** The sepals of *H. sabdariffa* were dark red, united sepals alongside valvate aestivation. Phytochemical analysis showed the presence of saponins, tannins, alkaloids, flavonoids, and glycosides. It exhibited unique fluorescent profiles in various reagents. HPLC fingerprint showed 7 peaks eluting within 1 and 5 minutes. Arsenic, lead, mercury, and chromium were not detected but cadmium was detected which was within acceptable limits. The aerobic bacteria and fungi count were also within acceptable limits. **Conclusion:** These parameters can be used to evaluate the quality of dried sepals of *Hibiscus sabdariffa* sold on the open market before they are used in the manufacturing of beverages and medicines.

Keywords: analysis; *Hibiscus sabdariffa*; quality control; simple methods

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Introduction

The patronage of natural therapies in the treatment or management of diseases has been on a rapid increase over many years. This can be attributed to the massive contribution of plants in providing enormous, economical but effective therapeutic outcomes [1]. Poverty and limited access to orthodox medicine have pushed a high percentage of the world's population, especially in developing economies, to use medicinal plants as their primary source of health care [1]. A high number of traditional medicines are effective but lack standardization or quality control parameters

for evaluation. The assurance of the safety and efficacy of herbal drugs is based on their quality that requires monitoring the plant material from collection, through processing to the finished packaged product. To ensure the quality of phytopharmaceuticals therefore requires the assurance of their identity, purity, content assay and other chemical, physical and biological properties [2]. Standardization of herbal medicines is the means of establishing a set of inherent characteristics, measurable parameters, unambiguous qualitative and quantitative values

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that convey an assurance of quality, efficacy, safety, and reliability [3]. It ensures that the required plant material contains appropriate substances in their right amount for the desired therapeutic effect [4] and leads to the reproducibility of the quality of a particular product [5]. It thus facilitates the detection of adulteration with inferior commercial varieties, exhausted drugs, cheaper natural substances, and chemicals.

In this respect, *Hibiscus sabdariffa* L. which is widely used all over the world and especially across West-Africa and Ghana [6-9], was chosen to determine simple parameters with relevant methods which can easily be used with available instruments to estimate its quality. The sepals of *H. sabdariffa* L. (Malvaceae) [10] has been traditionally used as food, in herbal drinks, in hot and cold beverages, as a flavouring agent, as well as in herbal medicines [11]. It is commonly known as roselle in English, "Oiselle" in French, "Zobo" in Nigeria and as "Sobolo" in Ghana [11-13]. It is usually found in Asia and tropical Africa where it is cultivated as a home garden shrub. This plant is an annual woody-based subshrub, growing up to 2-2.5m tall. The leaves are deeply lobed, 8-15 cm long and arranged alternatively on the stems [12].

The flowers are pedicellate and borne singly in leaf axils. The corolla usually consists of 5 petals which are red in colour and about 3 inches in diameter. The sepals are typically red, consisting of 5 large sepals with pointed bracteoles [8,14]. The quality of *H. sabdariffa* is however known to be affected by the seed stock, local growing conditions, time of harvest, post-harvest handling and the processes of drying [11].

Hibiscus sabdariffa sepals are commonly prepared into a local Ghanaian drink called "Sobolo". "Sobolo" is processed by boiling the sepals together with some local spices such as "Hwintea" (*Xylopiya aethiopica*) [13], "Soro" (*Piper guineense*), and "Fam wisa" (*Aframomum melegueta*), ginger (*Zingiber officinale*) [13] and sometimes pepper (*Capsicum annum*). This drink and infusions of the sepals are known to have anti-hypertensive, anti-hyperlipidemic, anticancer, anti-diabetic and anti-inflammatory activities [15,16]. The phytochemical constituents in the sepals include mucilage, pectin, niacin, xylose, proteins, fats, ascorbic acids, resins, dietary fibre minerals such as (iron,

phosphorus, calcium, magnesium, aluminium, sodium and potassium) and flavonoids [16,17].

Decoctions and infusions of *H. sabdariffa* sepals and occasionally the leaves, are taken as a cold or hot beverage in a minimum of 10 countries worldwide for the treatment of hypertension and hyperlipidemia with no reported adverse effects [6,10]. This plant is employed as a laxative and vermifuge [8]. Polyphenols derived from *Hibiscus sabdariffa* are known to have positive effects in various obesity-related conditions [10]. Anthocyanins found in high quantities in the sepals are generally considered the active ingredients for the antihypertensive and hypocholesterolemic effects. However, studies have also implicated the polyphenols and hibiscus acid present in the extracts to be the phytochemicals that are responsible for the treatment of some of the above diseases [6,10]. Derivatives of phenolic acids and flavonols, as well as phenylpropanoids have also been quantified in this plant material. The sepals are also known to be rich in polysaccharides and fiber in the form of pectins, arabinans and arabinogalactans [18].

Materials and Methods

Ethical considerations

This study was approved by the Research Proposal and Review Committee of the School of Pharmacy, University of Ghana (Ethical code RPRC 19/0002) dated 13th December, 2019.

Plant material

The fresh leaves and sepals of *Hibiscus sabdariffa* were plucked by hand from the botanical gardens of the University of Ghana in May 2019, during the rainy season. They were authenticated by Mrs. Gladys Schwinger, a taxonomist from the Department of Plant and Environmental Biology, the University of Ghana with Herbarium number PSM31/1219. Fresh leaves to be used for microscopic evaluation were stored in containers filled with glycerin for analysis. A quantity of the sepals were air-dried for 3 weeks, milled into a coarse powder and kept in well-labelled air-tight containers for further analysis.

Macroscopic evaluation

The macroscopic characteristics of the leaves, such as colour, arrangement, shape, apex base,

texture, margin, and venation were determined. The corresponding colour, odour, and texture of the sepals were determined.

Microscopic evaluation

Microscopic evaluation was done with the Leciad compound light microscope. This was performed using standard procedures recommended by the WHO guidelines on quality control methods for herbal materials, 2011 [19]. Transverse sections of *H. sabdariffa* leaves were cut and placed into different test tubes containing chloral hydrate. These were boiled for 15 minutes. The sections were then mounted in chloral hydrate under magnifications of $\times 10$ and $\times 40$ for identification of some features and ergastic cell contents according to methods described by World Health Organization (2011) [20]. Quantitative characteristics such as vein islet number, veinlet termination numbers, stomata number, and stomatal index were determined using the same protocols [19].

Powder microscopy

Small quantities of milled *H. sabdariffa* sepals were mounted in chloral hydrate and observed for various cell components. For the observation of lignified tissues, the sample was mounted in phloroglucinol and concentrated hydrochloric acid and observed under low power. Pink-stain lignified materials were identified.

Physico-chemical analysis

The physico-chemical analysis was done on the extract of the sepals by estimating the extractive values, ash values, swelling index, foaming index, moisture content, and foreign organic matter content. Total ash, acid insoluble and water soluble ash values were estimated and extractive values of petroleum ether, 50 % ethanol and water were also determined. The foreign organic matter, swelling index and the foaming index were also analyzed. Analysis was performed according to methods described by WHO, 2011 [19] and all experiments were performed in triplicates.

Preliminary phytochemical screening

Phytochemical screening was done to detect the presence of alkaloids, saponins, tannins, flavonoids, glycosides and terpenes by the methods of Khandelwal, 2002 and Harbourne, 1992 [21,22].

Fluorescent studies

Fluorescence analysis of the powdered sepals was performed to determine the characteristic colour when dissolved in specific solvents according to methods described by Ranjith, 2018 [23]. Observations were made under visible day light and UV light of short wavelength (λ 254 nm) and UV light of long wavelength (365 nm) [23]. The solvents employed were distilled water, 1N hydrochloric acid, 1N NaOH, 10N sulphuric acid, methanol, glacial acetic acid, nitric acid, chloroform, 50% FeCl₃ and 96 % ethanol.

HPLC analysis

Five g of the pulverized sepals of *H. sabdariffa* was extracted with 50 mL of 50% ethanol by ultra-sonication for 15 min and was then centrifuged. The supernatant was collected and the residue was again extracted with another 50 mL of solvent. The combined extracts were concentrated at 40 °C and freeze-dried. The resulting crude extract was dissolved in methanol to prepare a solution of 1 mg/mL and filtered. The samples were then analyzed using KNAUER HPLC (KNAUER Products, Germany) and the ClarityChromVR Software (Vertex Plus C18-KNAUER, Germany). HPLC fingerprint was developed under the chromatographic conditions: injection volume: 10 μ L; Mobile phase (v/v): Methanol: Water (5: 95); Flow rate: 1 mL/min; detection wavelength: λ 254 nm; Stationary phase: Luna C18 reverse-phase column (250 \times 4.6 mm, particle size 5 μ m).

Heavy metal analysis

The heavy metal content of *H. sabdariffa* was analyzed with Olympus Vanta M Portable ED-XRF (VMR) analyzer (USA) equipped with 4-Watt x-ray tube with application optimized anode material rhodium (Rh) and tungsten (W), 50kV x-ray tube and large area silicon drift detector was used to analyze the sample for heavy metals. Calibration of the XRF was done using the SARM 2711A, certified reference material from the manufacturer. Twelve grams of powdered petals were sieved through a 180 μ m mesh size sieve. The finely powdered plant material was kept for use during the analysis. The loose sample was irradiated following the manufacturer's protocol. Triplicate measurement of the samples was made [24].

Microbiological evaluation

Aerobic bacterial and fungal counts were

performed on one gram of the powdered sepals of *H. sabdariffa* as per the method recommended by WHO, 2011[19]. *Bacillus subtilis* ATCC 6538-P was used as growth controls for bacteria in the nutrient agar; *Candida albicans* ATCC 2091 was used as the fungal growth control for potato dextrose agar. All experiments were performed in triplicates.

Results and Discussion

The pharmacognostic parameters of authentic plant samples are of the utmost importance because they can be employed to ensure or control the quality of market samples. Ensuring the quality of plant samples that are highly patronized will reduce the incidence of adulteration and hence improve the efficacy and safety of such medicines. In developing countries especially, there is a need to use simple, available and rapid means to control the quality of such widely used herbs. Hence the evaluation of *H. sabdariffa* was focused on organoleptic, macroscopic, microscopic, physico-chemical, fluorescence and phytochemical characteristics of the crude drug [25]. These preliminary evaluations employed the human senses and simple experimental procedures to establish parameters that can be subsequently used to determine the authenticity, the quality and the purity of the plant samples. If the herb varies significantly in terms of its odour, colour, texture or taste, it is considered of poor quality [20]. Figure 1 depicts the leaves and sepals of *H. sabdariffa*. *Hibiscus sabdariffa* has dark red highly united sepals with valvate aestivation in the bud stage. A summary of the respective macroscopic characteristics and organoleptic properties have been given in tables 1 and 2, respectively.

Other studies have focused on the description of the various parts of the plant, including stems, leaves, flowers, sepals and seeds [11,26]. However this study focused on the leaves and calyx. Description of the leaves as alternate and greenish in colour and the typical red calyx is in agreement with published works by Mahevan et al., 2009 [27].

The leaf constants of *H. sabdriffa* were determined in terms of epidermal cell number, stomatal number, stomatal index, vein islet number and veinlet termination number which have been provided in table 3.



Figure 1. Leaves and sepals of *Hibiscus sabdariffa* photographed by Gladys Amponsah Agyei

Table 1. Macroscopic parameters of *Hibiscus sabdariffa* sepals

Morphology	Description
Nature	Fresh
Colour	Red
Shape	Conical
Texture	Papery
Odour	Odourless
Surface	Pubescent
Aestivation (arrangement)	Valvate

Table 2. Macroscopic characteristics of *Hibiscus sabdariffa* leaves

Morphology	Description
Type	Simple
Shape	Palmately lobed
Colour	Green
Arrangement	Alternate
Venation	Reticulate
Base	Symmetrical
Apex	Acute
Margin	Serrate
Surface	Glabrous
Texture	Leathery

The stomata present were characteristically pericytic with one subsidiary cell surrounding the guard cell. Other microscopic features identified included rosette shaped calcium oxalate crystals (figure 2) which is in agreement with published literature [9]. The calcium oxalates were distinctly dispersed around the leaf veins (figure 2). Similarly, calcium oxalate crystals have been documented in the parenchyma cells of the *H. sabdariffa* leaves. Both the macroscopic and microscopic evaluations can be rapidly used as quality control parameters of this crude drug. Olotuah (2014) has reported stomata and epidermal cell number of *H. sabdariffa* to be 8 and 22, respectively [27]. This is in close range to that reported in this article, being 8.1 and 31.6,

respectively. Lavanya Vasavi et al. 2019) [9] also provided quantitative surface data of the leaf which is however in variance to that of this article. This is likely due to variations in the method employed. The variations in such parameters may arise as a result of the differences in methods employed and also environmental factors to which the plants were exposed.

The physico-chemical parameters in terms of solvent extractive values, ash values, foreign matter, swelling index, foaming index and moisture content of *H. sabdariffa* samples have been recorded in table 4. The total ash content was 6.71 % w/w, followed by the water-soluble ash value of 5.72 % w/w and acid insoluble ash value of 4.47 % w/w. Lavanya Vasavi et al. also reported the total ash to be approximately 6% [9]. There were however variations in other physicochemical parameters. The total ash indicates the amount of physiological ash, which includes mineral components such as calcium, magnesium and potassium and non-physiological ash mainly due to sand and soil that may be present in the sample [28]. The water soluble ash value on the other hand could give an indication of previously extracted plant materials, while acid insoluble ash also gives the amount of inorganic material such as silica or metals being present. This is important in detecting the presence of excess sand and silica that could adulterate the sample. These total ash values aids in the identification of low-grade products and the establishment of purity characteristics.

The foaming index was determined as 111.11. A similar study conducted by Rao et al., recorded a foaming index of 166.67 [29]. This suggests the presence of saponins in the decoction of the sepals as indicated by the phytochemical

screening [19]. No swelling index was observed for the powdered sepals of *H. sabdariffa*. This also suggests the absence of appreciable amounts of gums and mucilage [19].

Table 3. Leaf constants *Hibiscus sabdariffa*

Parameter	<i>Hibiscus sabdariffa</i>
Stomatal Number	8.1
Epidermal Cell Number	31.6
Vein islet Number	12.7
Veinlet termination number	17.7
The stomatal index [stomata per square mm of epidermis]	20.40

Table 4. Physico-chemical parameters Physico-chemical parameters of *Hibiscus sabdariffa* sepals

Parameters	<i>Hibiscus sabdariffa</i> (% w/w)
Total Ash	6.71 ± 0.56
Acid insoluble ash	4.47 ± 0.25
Water soluble ash	5.72 ± 0.77
Moisture content	13.50 ± 0.50
Foreign organic matter	0.0
Foaming index	111.11 ± 3.5
Swelling index	0.0

However, mucilage cavities and secretory glands have been reported in the young stem bark of *H. sabdariffa* in cortex and pith [30]. Moisture content is used to determine the actual weight of herbal materials [3] and also estimate the presence of water. This should be defined for every herbal material since high moisture content can speed up microbial growth and facilitate decay. The moisture content was determined to be 13.50 % w/w, similar to that recommended by Ghana Herbal Pharmacopoeia, 2013 which states that the moisture content should not exceed 12.00 %. Lavanya Vasavi et al., 2019 has also reported a moisture content of 16.00 % [8]. *Hibiscus sabdariffa* sepals have shorter shelf life due to their high moisture content hence there is a need to control the moisture content [31].

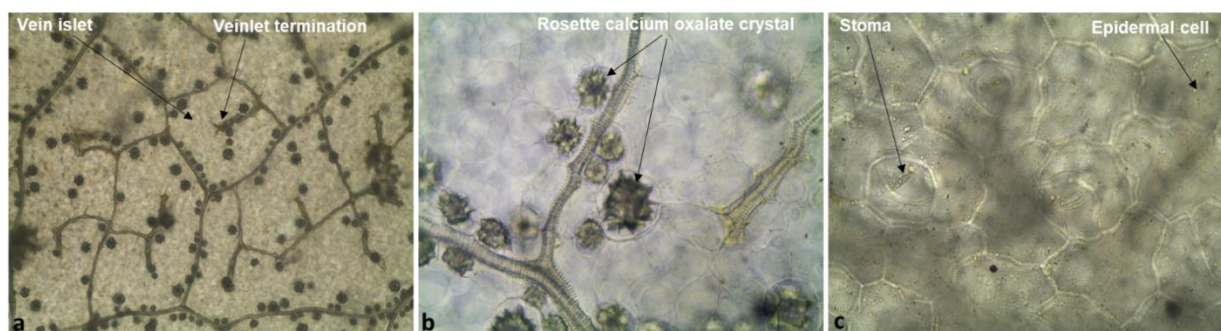


Figure 2. Surface characteristics of *Hibiscus sabdariffa* leaf section; (a) vein islets (regular polygonal reticulate with moderately developed 5-sided areoles) and veinlet terminations (mostly unbranched); (b) rosette calcium oxalate crystals; (c) irregularly shaped epidermal cells and pericytic stomata

The 50 % ethanol extractive value was the highest with a value of 45.00 % w/w of dried plant material, followed by water-soluble and petroleum ether extractive values (table 5). This indicates that most of the constituents of the sepals are middle polar in nature. Less extractive values could indicate the addition of exhausted plant material.

Table 5. Extractive values *Hibiscus sabdariffa*

Solvents	Extractive Value (% w/w)
Petroleum ether	13.00 ± 1.4
50 % ethanol	45.00 ± 1.9
Water	32.00 ± 1.6

Phytochemical analysis of the extract of *H. sabdariffa* sepals showed the presence phyto-constituents such as tannins, alkaloids, glycosides, phenols, flavonoids and saponins which is similar to other previously reported phytochemical studies [9,32,33].

The fluorescence study is a sensitive spectroscopic technique that can determine the presence of conjugated double bond systems of compounds [34,35]. The fluorescence study is a sensitive spectroscopic technique that can determine the presence of various fluorescing pharmaceuticals with conjugated double bond systems [36]. *Hibiscus sabdariffa* presented pink fluorescence in 50 % sulphuric acid, glacial acetic acid, chloroform and 95 % ethanol solvents under UV light of 254 nm (table 6). In nitric acid, it fluoresced green at 365 nm. This was similarly reported by Lavanya Vasavi et al. [36].

This study however differed in the fact that it used the leafy material instead of the solvent

extracts of the sepals, hence this could account for the differences in the fluorescent colours. HPLC fingerprint of *H. sabdariffa* ethanol extract showed seven unresolved peaks with retention times between 1 and 5 minutes (figure 3).

Some studies have focused on the mineral content of *H. sabdariffa*, showing the presence of iron, phosphorous and calcium [11,25]; however, there was a need to analyze for the presence of heavy metals. When *H. sabdariffa* sepals were analyzed, there were no traces of arsenic (As), lead (Pb), mercury (Hg) and chromium (Cr). Cadmium was however estimated to be at a low concentration of 0.02 parts per million (ppm).

Crude drugs are easily contaminated with microbes because of poor sanitary conditions during handling, and it is important for microbiological quality control of crude drugs, not only to detect pathogenic bacteria but also to enumerate total and physiologically active bacteria because nonpathogenic bacteria may alter or decompose crude drugs. In addition, contamination by excessive bacteria is an indication of mishandling during preparation. Therefore, for crude drugs, estimation of the total and viable bacterial and fungal counts is important to assure the safety and quality [37]. Few studies have surveyed the microflora present in market samples of these plant materials, and varying numbers and types of organisms were found to be present. However, these were not compared to standard samples. For our authentic sample, the aerobic bacteria count was 10⁶ CFU/g of dried plant material while the fungi count was 10² CFU/g.

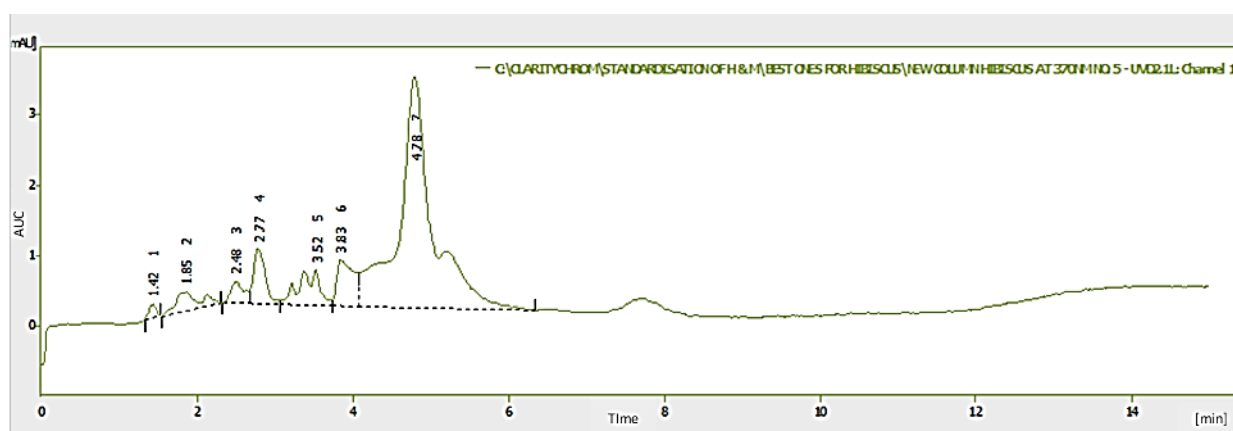


Figure 3. HPLC fingerprint of *Hibiscus sabdariffa* 50 % ethanol extract at 370 nm

Table 6. Fluorescent studies of *Hibiscus sabdariffa* sepals

Powdered plant sample and reagent	Daylight	Short wavelength (λ 254 nm)	Long wavelength (λ 365 nm)
Distilled water	Crimson	Brown	Ash
1 N HCl	Crimson	Black	Ash
1 N NaOH	Orange	Green	Ash
10N Sulphuric acid	Crimson	Pink	Brown
Methanol	Crimson	Ash	Ash
Glacial acetic acid	Crimson	Pink	Ash
Nitric acid	Orange	Green	Ash
Chloroform	Colourless	Pink	Ash
50 % Ferric chloride	Brown	Black	Ash
95 % Ethanol	Crimson	Pink	Ash

For WHO standards, the maximum aerobic bacteria and fungi count should be 10^7 and 10^4 CFU/g, respectively [19]. Therefore, the results were within acceptable limits.

It could be concluded that the botanical, physicochemical, phytochemical and microbiological parameters evaluated in the present study, could be importantly used in rapidly assessing the quality of widely sold *H. sabdariffa* samples on the market.

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Author contributions

Emelia Oppong Bekoe conceptualized the study and coordinated the project; Cindy Kitcher was responsible for the botanical studies (macroscopic and microscopic analysis); Gladys Amponsah Agyei was the research assistant conducting all laboratory analysis; Samuel Frimpong-Manso performed the elemental analysis; all authors read and approved the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

As: arsenic; Cd: cadmium; Pb: lead; Fe: iron; Zn: zinc; UV: ultra-violet