

Full Length Research Paper

## Standardization of Roselle (*Hibiscus sabdariffa* L.) Calyx cultivated in Sudan

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Herbal products have become increasingly popular throughout the world; one of the impediments in its acceptance is the lack of standard quality control profile. This research was conducted to standardize Roselle (*Hibiscus sabdariffa*) cultivated in Sudan. For this purpose, world health organization (WHO) monograph for medicinal plants was used to assess certain particulars of the plant including macro and microscopical characters, physical and chemical characteristics. Results obtained revealed that the macro and microscopical characters of *H. sabdariffa* were in accordance to those specified in WHO monographs. Results of purity tests were obtained as (16.3) water soluble extractive value (21.4%), 70% ethanol soluble extractive, (11.7%) the total ash, (3.8%) acid insoluble ash values and (8.5%) moisture content, respectively whereas value of foreign materials were calculated to be 2%. The heavy metal lead in the plant sample tested was found to be below the permissible limit (10 mg/kg) and the plant was found to be free from bacterial contamination. The high performance liquid chromatography (HPLC) analysis of *H. sabdariffa* revealed the presence of quercetin as a major phytoconstituent in a total content of 12.96%. It can be concluded that the obtained data of *H. sabdariffa* could be taken as guidelines in its quality assessment and will contribute positively to establish standards for the quality assessment of herbal products in Sudan.

**Key words:** Standardization, herbal medicine, *Hibiscus sabdariffa*.

### INTRODUCTION

In recent years, plant derived products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics and are available in health food shops and pharmacies over the counter as self-medication or also as drugs prescribed in the non-allopathic systems (Neeraj and Bhupinder, 2011). According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs (Amit et al., 2007). Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics,

liver diseases, cough remedies, memory enhancers and adoptogens (Patel et al., 2006). Herbals are traditionally considered harmless and increasingly being consumed by people without prescription. However, some can cause health problems, some are not effective and some may interact with other drugs. Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles (Yadav and Dixit, 2008). Quality evaluation of herbal preparation is a fundamental requirement of industry and other organization dealing with herbal products.

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The growing use of botanicals by the public is forcing moves to assess the health claims of these agents and to develop standards of quality and manufacture (Neeraj and Bhupinder, 2011). Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carries an assurance of quality, efficacy, safety and reproducibility (Kunle et al., 2012).

Roselle (*H. sabdariffa*) is an edible plant used in various applications including foods. The fleshy red calyces are used for making wine, juice, jam, syrup, pudding, cakes, ice cream or herbal tea. Roselle flowers and calyces are also known for their antiseptic, diuretic, antioxidant and antimutagenic properties (Ali-Bradeldin et al., 2005). Roselle is an important source of vitamins, minerals, and bioactive compounds, such as organic acids, phytosterols and polyphenols, some of them with antioxidant properties. The phenolic content in the plant consists mainly of anthocyanins like delphinidin-3-glucoside, sambubioside and cyanidin-3-sambubioside; other flavonoids like gossypetin, hibiscetin, and their respective glycosides; protocatechuic acid, eugenol, and sterols like  $\beta$ -sitosterol and ergosterol (Azza et al., 2011). Hence, this study was undertaken to standardize the popular and widely used plant *H. sabdariffa*.

## MATERIALS AND METHODS

*H. sabdariffa* calyces were purchased from the suppliers of natural remedies in Khartoum North Province during November, 2005.

### Macroscopical characters of the plant

The authentication and macroscopical characters of the plant had been performed at the Medicinal and Aromatic Plant Research Institute (MAPRI), National Centre for Research (NCR), Khartoum, Sudan and Department of Botany, Faculty of Science, University of Khartoum.

### Microscopical characters of the plant

The Permanent slides (Transverse sections (TS), Horizontal section (HS), were prepared according to Alexander (1940). Slides sample taken were prepared from soft tissue of *H. sabdariffa* and subjected to the following treatments. Preliminary treatment of the plant material was by using the standard fixative (FAA), formaldehyde was glacial acetic acid: 70% ethyl alcohol (5:5: 90 v/v). A mixture of 1:1 (cedar wood oil: absolute alcohol), pure cedar wood oil, a mixture of cedar wood oil and xylene, pure xylene. Sectioning: Soft tissues were sectioned using a rotary microtome (Leitz 1512 Germany), adjusted at 12 microns using a brush. Staining: by using pure xylene, safranin stain dissolved in 50% ethyl alcohol, fast green stain dissolved in absolute ethyl alcohol, clove oil, *Canada balsam*. Drying: carried in an oven adjusted at 60°C. Microscopical examination: by using Olympus CH2O microscope. The lens used was ( $\times 20$ ,  $\times 40$ ), and photographing by using (LeitzDialux 20) microscope fitted with (Wild PMPS II) camera, using Kodak coloured films 36 ExP. 24  $\times$  36 mm ISO

100/210.

### Purity tests

Determination of total ash, acid insoluble ash, water soluble extractive, and determination of ethyl alcohol (70%) soluble extractive were carried out by following the methods described in (IHP, 2002). The determination of petroleum ether soluble extractive and determination of moisture content were carried out by using the methods described in Pulok (2002). The foreign organic matter determination was carried out by following the method described in (WHO, 1998).

### Determination of heavy metals

Analysis was made on a known 0.5 g of the dried plant sample. Digestion was conducted using a microwave oven (milestone mps1200 mega). The conditions for wet ashing were carried as described by Shaole et al. (1997). Total content of cadmium and lead were determined in the digested solution using inductively coupled plasma (400) emission spectrometry (Perkin elmer emission spectrometer) according to Allen et al. (1997).

### Determination of pesticide residues

Determination of pesticide residues was carried out by gas chromatographic multi residue quantitative determination of organohalogen, organonitrogen, organophosphorous and some pyrethroids pesticide residues, an official method for analysis (Association of Official Agricultural Chemists, 1995), adopted by Agricultural Research Center; Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food, Egypt.

### Determination of microbial contaminants

**Pretreatment of the plant material:** Ten grams of the grinded plant material was dissolved in 100 ml lactose broth enrichment medium, mixed well for homogenization and incubated at 37°C for 3 h so that *Esherichia coli* and *Salmonella typhimurium* can revive if present, to enable their detection (WHO, 1998).

**Isolation and identification of *E. coli*:** One ml of the homogenized material from the enriched plant material was transferred to a bottle containing 100 ml of MacConkey broth and incubated at 43 to 44°C for 20 h. A drop from the bottle was plated separately on MacConkey agar. Another drop was transferred to a tube of peptone water (for indole test). Plate and tube was incubated at 43 to 44°C for 20 h. Growth of red, generally non-mucoid colonies, sometimes surrounded by a reddish zone of precipitation, indicates the possible presence of *E. coli*, which may further be confirmed by indole reaction. The material passes the test if no such colonies are detected or if the confirmatory biochemical reaction is negative.

### Isolation and identification of *Salmonella typhimurium*:

**Primary test:** The test was carried out by following the method described in WHO (1998). The appearing colonies should be compared with the description included in the same method.

**Secondary test:** The following biochemical reactions were performed for the plant material preparation: oxidase, urease, indole and Kligler iron agar (deep inoculation technique); all incubated at



**Figure 1.** Plant material of *Hibiscus sabdariffa* calyx.

incubated at 37°C for 24 h and confirmed by api 20E identification system (Eiman et al., 2008). The material being examined passes the test if colonies of the type described in the WHO (1998) do not appear in the primary test, or if the confirmatory biochemical reaction is negative.

#### **HPLC determination of flavonoids in *Hibiscus sabdariffa***

One gram accurately weighed to prepare test solution of *Hibiscus* powder, 5 ml of methanol was added and 20 ml distilled water, the mixture was sonicated for 10 min and cooled. The amount transferred to 50 ml volumetric flask, 10 ml of dilute HCl acid was added and shaken well, completed to the volume using methanol and the solution was then filtered. 12 mg of quercetin standard accurately weighed to prepare standard solution, transferred quantitatively into 50 ml volumetric flask, dissolved and completed to volume with methanol. The test solution and quercetin standard sample were co-chromatographed on C<sub>18</sub>-HPLC Column = Hypersil ODS C<sub>18</sub>250×4.6 mm, HPLC (Hewlett Packard) series 1050 (Germany). A mixture of citric acid solution: acetonitrile: isopropyl alcohol (100:47:5) was used as mobile phase at flow rate 1.5 ml/min at room temperature. 20 µl from test and standard sample was injected separately into the HPLC system and the peaks monitored by UV absorbance at  $\lambda_{max}$  370 nm (Mattila et al., 2000).

## **RESULTS AND DISCUSSION**

### **Macroscopical characters of *Hibiscus sabdariffa* calyx**

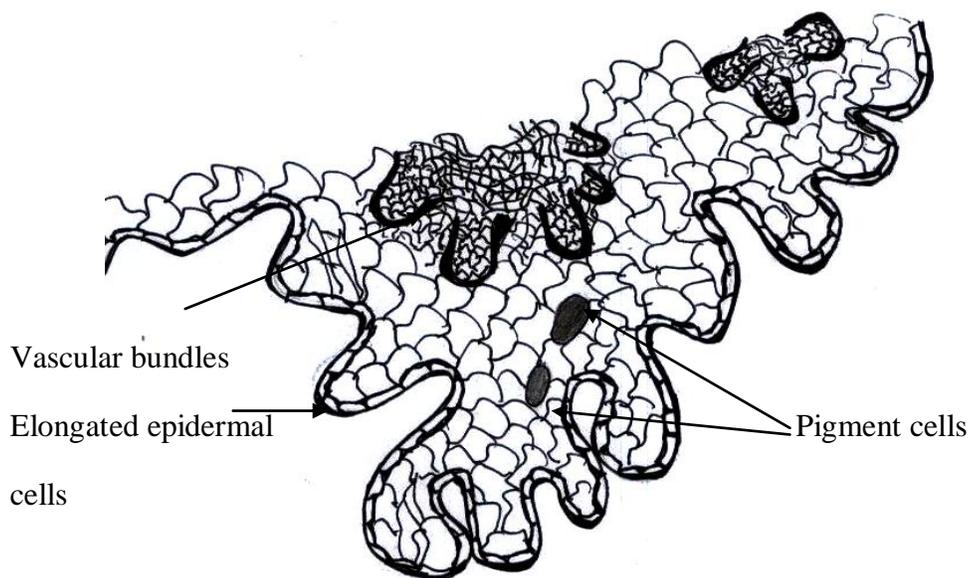
The macroscopical characters of *H. sabdariffa* calyxes are bright red calyxes 1.5 to 2 cm long (Figure 1). These morphological features are identical to WHO (1999) and Morton (1987).

### **Microscopical characters of *Hibiscus sabdariffa***

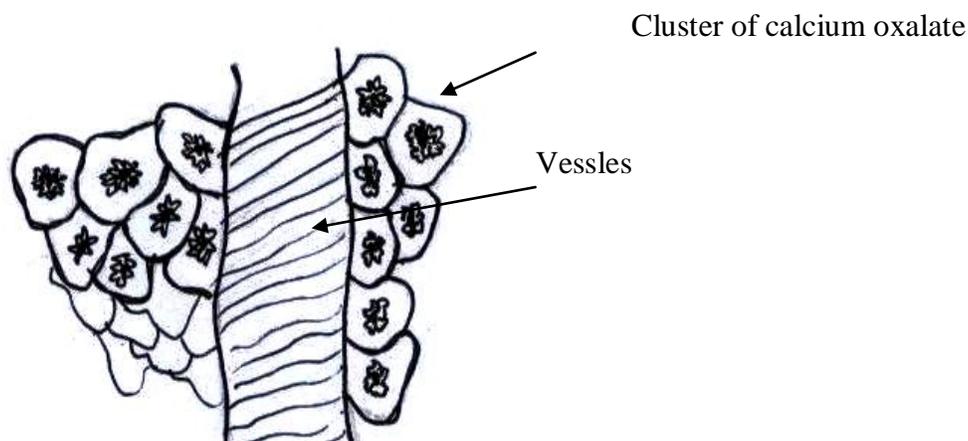
Transverse section showed elongated epidermal cells, vascular bundles and pigments cells (Figure 2) and the horizontal section showed clusters of calcium oxalate concentrated around the vessels in rows enclosed in parenchyma cells (Figure 3). These findings coincide with those stated in WHO (1999).

### **Purity tests of *Hibiscus sabdariffa***

Results show high numerical value obtained as water soluble extractive value (16.3), with considerable value for the 70% ethanol soluble extractive (21.4%), which indicates that the drug contains large amounts of somewhat polar constituents such as phenolic compounds, organic acids and minerals. This is in agreement with the conclusion obtained by German commission (1990). The total ash and acid insoluble ash values of *H. sabdariffa* were calculated to be 11.7 and 3.8%, respectively. The values of ash and acid insoluble ash (not exceeding 2%) may not be constantly the same for the plant sample which indicate contamination with siliceous material (earth and sand). Also these differences may result from the change in climate, soil and age of the plant and/or change in care taken when preparing drugs (WHO, 1999, 2002; Pulok, 2002). The value of the total ash for *Hibiscus* has been set at 10% by Plotto (1999), which showed reasonable results when compared with the



**Figure 2.** Transverse section of *Hibiscus sabdariffa* showing elongated epidermal cells, vascular bundles and pigments cells.



**Figure 3.** Horizontal section through the sepals of *Hibiscus sabdariffa*, showing clusters of calcium oxalate concentrated around the vessels in rows enclosed in parenchyma cells.

results obtained in this monograph. The suggested results by (Juliani et al., 2009) to obtain higher quality of *Hibiscus calyx*, a maximum of 7.5 and 1% are recommended for total and acid insoluble ashes, respectively. These results are not in agreement with the results obtained in this study. This may be attributed to variation in soil, good agricultural and collection practice (GACP). Value of moisture content was calculated to be 8.5%, as shown in Table 1. This value no doubt affects quality and safety of the medicinal plants as the higher

moisture content leads to the deterioration of the drug. Most drugs may be stored safe if the moisture content is reduced to 6 percent or less (Pulok, 2002). For pesticide residues and as mentioned in Table 1, *Hibiscus* is not contaminated with profenofos, chlorpyrifos and malathion. Value of foreign materials was calculated to be 2%, as shown in Table 1. Low foreign materials and low microbial load reported by Juliani et al. (2009) showed important parameter in quality, and health is the production and assurance of clean and hygienic products

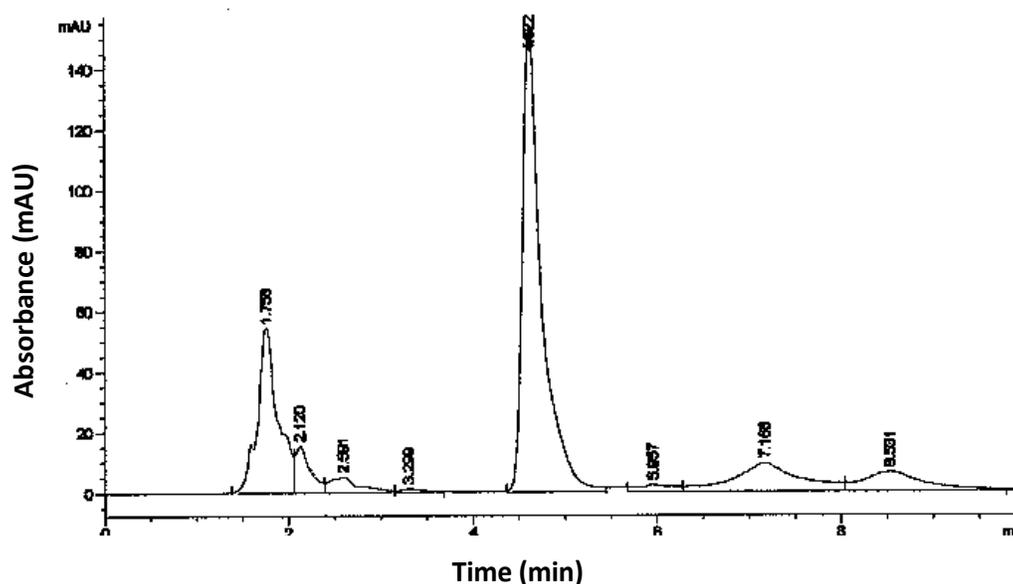


Figure 4. HPLC Chromatogram of quercetin in *Hibiscus sabdariffa* test solution.

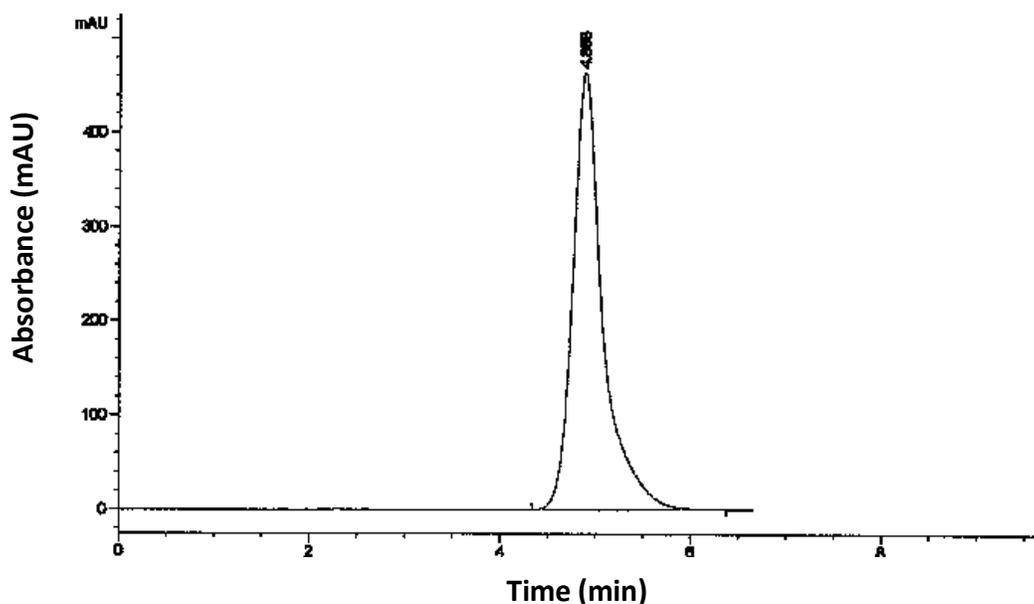


Figure 5. HPLC Chromatogram of standard quercetin solution.

that are safe to consume. The test sample was free from bacterial contamination with *Salmonella* and *E. coli* as mentioned in Table 1. The heavy metal lead in the plant sample tested was found to be below the permissible limit (10 mg/kg), while cadmium was not detected at all as mentioned in Table 1.

#### HPLC determination of flavonoids in *H. sabdariffa*

The HPLC analysis of *H. sabdariffa* revealed the presence of quercetin as a major phytoconstituent in a total content of 12.96% with a retention time 4.62 min (Figure 4) similar to the authentic reference sample (Figure 5). This

**Table 1.** Purity characteristics of *Hibiscus sabdariffa*.

Item	Numerical value
Acid insoluble ash	Not more than 3.8%
Water soluble extractive	Not less than 16.3%
70% ethanol soluble extractive	Not less than 21.4%
Petroleum ether soluble extractive	Not less than 0.02%
Moisture contents	Not more than 8.5%
Foreign organic matter	Not more than 2%
<b>Heavy metals</b>	
Lead	0.0933 ppm
Cadmium	0.00 ppm
Pesticide residues	Not detected
Microbial contaminants	Free of <i>E. coli</i> and <i>S. typhimurium</i>

result was consistent to that reported by Salah et al. (2002).

## Conclusion

It can be concluded that, obtained data of *H. sabdariffa* could be taken as guidelines in its quality assessment and will definitely contribute positively to establish standards for the quality assessment of herbal products in Sudan.

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