



## COMPARATIVE STUDIES OF PHYTOCHEMICAL CONSTITUENTS OF THREE SPECIES IN CALYCES OF HIBISCUS SABDARIFFA LINN

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**ABSTRACT:** *Hibiscus Sabdarifa Linn belong to the family malvaceae. It is an annual herbaceous sub- shrub with more than 300 species which are distributed in tropical and sub-tropical regions around the world. Most hibiscus species are used as ornamental plants, medicinal values; among them is Hibiscus sabdariffa commonly named as 'Red sorrel' and 'Roselle'. Hibiscus sabdarifa (calyces) have been found to be rich in vitamin, antioxidant and minerals. Therefore, the aim of this work is to Compared the aqueous extract and methanolic extract of Phytochemical Constituents of three Species in Calyces of Hibiscus sabdariffa Linn. The Phytochemical Constituents of aqueous extract and methanolic extract of calyces (Hibiscus Sabdariffa L) include Alkaloids, Tannin, Glycoside, Flavonoids, Saponins and Hydroxyl anthraquines. the present of saponine and flavonoids in this plant may give support to their therapeutic effects especially in the treatment of hypertension and diseases.*

**KEYWORD:** Phytochemical Constituents, Hibiscus Sabdarifa Linn, Distillation, Filtrate, Aqueous Extract, Medicinal Plant

### INTRODUCTION

The contribution of medicinal plants to sciences in the traditional system of medicine for curing diseases has been interestingly on the increased and consumer demand have promoted the development of herbal products as dietary supplements. In view of renewed interest, herbal medicines have a prominent role to play in the pharmaceutical and health markets of the 21<sup>st</sup> century. It has been reported that whatever is taken as food could cause metabolic disturbance subject to the allowed upper and lower limits of trace metals. Both the deficiency and excess of essential micronutrients and trace toxic metals may cause serious effects on human health (Singh et al., 2010). The use of medicinal plants as therapeutics and dietary supplements goes back beyond history, but has increased substantially in the last decade. However, the safety of their use has recently been questioned due to the reports of illness and fatalities (Singh et al., 2010). WHO (1998) reported that medicinal plants which form the raw materials from the finished products may be checked for the presence of heavy metals and further, regulates maximum permissible limits of toxic metals like lead and arsenic which account to about 1.0 - 10 mg/L. Medicinal herbs are easily contaminated during growth, development and processes. After collection and transformation into dosage form, the heavy metals confined in plants finally enter the human body and may disturb the normal functions



of central nervous system, liver, lungs, heart, kidney and brain, leading to hypertension, abdominal pains, skin eruptions, intestinal ulcer and different types of cancers (Singh et al., 2010).

Roselle is a *Hibiscus sabdariffa* L. family “Malvaceae”, tribe “Hibiceae”. The most important species is *Hibiscus sabdariffa* var. *altissima* Wester, an erect, sparsely-branched annual plant that grows to about 16 ft (4.8 m) high, which is cultivated for its juice - like fiber in India, the East Indies, Nigeria and Tropical America. The stems of this variety are green or red and the leaves are green, sometimes spiny and not used for food. This type at times have been confused with kenaf, *Hibiscus cannabinus* L. a similar but more widely exploited fiber source. The other type of Roselle, *Hibiscus sabdariffa* var. embraces shorter, fleshy with green, red - steam, inedible calyces (Morton, 1987). The last one is commonly known as hibiscus and grows in many tropical and subtropical countries as well as one of highest volume especially botanical products in international commerce. Hibiscus is a fiber, with swollen calyces that are the plant part of commercial interest. As flowers fall off, the bright red calyces swell. These are harvested dried and sold as the herbal tea and beverage industries. The flavour is a combination of sweet and tart, similar to berry. In addition to international markets, there are extensive local and regional markets, where it is processed into hot and cold herbal beverages, jellies, confectionaries and other products. In some African countries, it is called *beri* in the Dagari language, *isapa* in Yoruba, *yakuwa* in Hausa, *mnyanya oji* in Igbo and it is *sour-sour* in Sierra Leone. In the Sudan the plant is called *karkade*, Sahelian countries it is called *dah*, while in Senegal and surrounding countries it is referred to as *bissap*. The French name is *oseille de Guinee* or *roselle*. In the Caribbean area the crop is known under the name of ‘Jamaican sorrel’ or ‘Florida cranberry’. It is used in urban areas; it is the calyces of the flower that is the article of trade and the most important part of the plant (Schipper, 2002). The water extract in calyces is rich in carotenoids (especially beta-carotene) and ascorbic acid. The calyces of *Hibiscus sabdariffa* have also been found to be rich in vitamin and other antioxidant and also minerals (Craig, 1999). The physico-chemical characteristics of Roselle was studied and it was characterized as a highly acidic fruit with low sugar content. Succinic acid and oxalic acid were quantified as a predominant organic acid in Halics. Halics was found to contain higher amount of ascorbic acid compared to orange and mango (Odigie *et al.*, 2003). It was found to be fair source of vitamin A. It is also rich in riboflavin, niacin, calcium and iron. It also contains antioxidants including, flavonoids, gossypetine, hibiscetine and saddertine. Some of the anthocyanins of Roselle indentified by chromatographic process include delphinidin-3-sambubioside, cyaniding-3- sambuboiside and delphinidin -3- glucose (Hung, *et al.*, 2004) They are also known for their unique flavour characteristic that makes them appealing to taste. Roselle drink had been improved nutritionally by producing fruit-flavoured Roselle drink which are richer in vitamin and minerals by addition of different fruits (Abdulazeez A *et al.*, 2016)

## METHODOLOGY

### Sample Collection and Preparation.

The plant sample was collected from Ibrahim Badamasi Babangida University, Lapai, farm, Niger State, Nigeria and was identified at the Herbarium section of the Department of Biological Science of Ibrahim Badamasi Babangida University, Lapai, as *Hibiscus*



*sabdariffa*. The calyces were air dried for two weeks and ground to uniform powder using wood type of mortar and pestle. The aqueous extract of sample was prepared by soaking 100grams of dried powder of each sample in 500 cm<sup>3</sup> of distilled water for 12 hours. The filtrate was used for phytochemical screening. The methanolic extract of the calyces of each *Hibiscus sabdariffa* species was prepared by using the same method for the aqueous extract, but methanol was the solvent used instead of distilled water. (AOAC, 1990).

### **Reagents**

All reagents used were of analytical reagent grade. Distilled water was used in the preparation of all aqueous solutions. All solutions were stored in amber colour bottles.

#### **10% Sodium Hydroxide Solution**

The solution was prepared by dissolving 10g of sodium hydroxide pellet in 100cm<sup>3</sup> volumetric flask and made up to the mark with distilled water.

#### **5% Ferric Chloride Solution**

The solution was prepared by dissolving 5g of ferric chloride in 100cm<sup>3</sup> of volumetric flask and made up to the mark with distilled water.

#### **50% H<sub>2</sub> SO<sub>4</sub> Solution (V/V)**

The solution was prepared by diluting 50cm<sup>3</sup> of Conc. H<sub>2</sub>SO<sub>4</sub> in 100cm<sup>3</sup> volumetric flask and made up to mark with distilled water.

#### **10% HCl solution (V/V)**

The solution was prepared by diluting 10cm<sup>3</sup> of Concentrated HCl in 100cm<sup>3</sup> volumetric flask and made up to mark with distilled water.

#### **Mayer's Reagent**

13.35g of mercuric chloride was dissolved in 60cm<sup>3</sup> of distilled water to give solution A. 5g of potassium iodide was dissolved in 20 cm<sup>3</sup> of distilled water to make solution B. The two solution (A and B) were mixed and made up to 1000cm<sup>3</sup> with distilled water in a 1000cm<sup>3</sup> volumetric flask (AOAC, 1990).

#### **Wagner's Reagent**

1.27g of sublimed solution of iodine and 2g of potassium iodide was 20cm<sup>3</sup> of distilled water in a beaker. This was transferred into 1000cm<sup>3</sup> volumetric flask and made up to mark with distilled water (AOAC, 1990).

#### **Phytochemical Screening of Extract**

Chemical tests were carried out on the aqueous extract and on the powdered samples using standard procedures to identify the constituents as described (Sofoware, 1993; Trease and Evans, 1989); Harborne, 1973).



### **Test for Flavonoids**

3cm<sup>3</sup> aliquot of the filtrate was added to 1cm<sup>3</sup> of 10% NaOH sodium hydroxide, if a yellow color was developed this indicates the possible presence of flavonoids compounds (El-Oleyi *et al.*, 1994; Harbone, 1973).

### **Test for Tannins**

Ferric chloride solution 5% was added drop by drop to 2-3cm<sup>3</sup> of the extract and the colored changed to blue-black, indicating the presence of tannins (Harbone, 1973; Trease and Evans, 1978).

### **Test for Saponins**

10cm<sup>3</sup> of the extract was placed in a test tube and mixed with 5cm<sup>3</sup> of distilled water and shaken vigorously for stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion (Harbone, 1973).

### **Test for Glycosides**

2.5cm<sup>3</sup> of 50% H<sub>2</sub>SO<sub>4</sub> was added to 5cm<sup>3</sup> of the extracts in a test tube. The mixture was heated in boiling water for 15 minutes. Cooled and neutralized with 10% NaOH, and 5cm<sup>3</sup> of Fehling's solution was added and the mixture allow boiling. A brick-red precipitate was observed which indicate the presence of glycosides (Harbone, 1973).

### **Test for Alkaloids**

About 2cm<sup>3</sup> of each extract was stirred with 2cm<sup>3</sup> of 10% aqueous hydrochloric acid. 1cm<sup>3</sup> was treated with a few drops of Wagner's reagent and a second 1cm<sup>3</sup> was treated similarly with Mayer's reagent. Turbidity or precipitation with either of these reagents will be taken as preliminary evidence for the presence of alkaloids (Harbone, 1973).

### **Test for Steroids (Salkowski)**

5cm<sup>3</sup> of the sample extract was dissolved in 2cm<sup>3</sup> of chloroform and 2cm<sup>3</sup> of tetraoxosulphate (VI) acid was carefully added to form a lower layer. A reddish-brown color at the interface indicates the presence of a steroidal ring (Harbone, 1973).

### **Test for Hydroxyl-Anthroquinones**

5cm<sup>3</sup> of each plant extract was shaken with 10cm<sup>3</sup> benzene, and 5cm<sup>3</sup> of 10% ammonia solution was added. The mixture was shaken and the presence of a pink, red or violet color in the Ammoniacal (lower) phase indicates the presence of anthroquinones (Harbone, 1973).

### **Test for Phyto-Tannins**

Deposition of red precipitate when an aqueous extract of plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phytotannins (Harbone, 1973).



## RESULT OF PHYTOCHEMICAL CONSTITUENTS

**Table 1: Phytochemical Constituents of Aqueous Extract of *Hibiscus Sabdariffa* Calyces (Light Green, Bright Red and Dark Red)**

Metabolite	Light green	Bright Red	Dark Red
Alkaloids	+	+	+
Tannins	+	+	+
Glycosides	+	+	+
Flavonoids	+	+	+
Steroids	–	–	–
Phyto-tannins	–	–	–
Saponins	+	+	+
Hydroxyl anthroquinones	+	+	+

**Table 2: Phytochemical Constituents of Methanolic Extract of *Hibiscus Sabdariffa* Calyces (Light green, Bright Red and Dark Red).**

Metabolite	Light Green	Bright Red	Dark Red
Alkaloids	+	+	+
Tannins	+	+	+
Glycosides	+	+	+
Flavonoids	+	+	+
Steroids	–	–	–
Phyto-tannins	–	–	–
Saponins	+	+	+
Hydroxyl-Anthroquinones	+	+	+

## DISCUSSION OF PHYTOCHEMICAL CONSTITUENTS

The phytochemical constituents showed that the aqueous extract and methanolic extract of *Hibiscus sabdariffa* calyces (Light green, bright red and dark red) contain saponins, tannin, hydroxyl anthroquinones, flavonoids, alkaloid and glycosides. These are known to exhibit medicinal activity as well as physiological activity depicted from some of the secondary



metabolites that are present in the extract. The presence of glycosides in the extract serves as clue to its popular use in the treatment of hypertension. Saponins are glycosides of both triterpenes and steroids having hypertensive and cardiac depressant properties. Saponins binds to cholesterol to form insoluble complexes, this prevents cholesterol re-absorption and results in a reduction of serum cholesterol. It has also been found that saponins are potentially useful for the treatment of hypercholesterolemia, which suggests that they might be acting by interfering with intestinal absorption of cholesterol (Malinow, 1977). Alkaloids comprised a large group of nitrogenous compounds which are widely used as cancer chemotherapeutic agents. The presence of alkaloids in sample proves it usefulness in folk medicine. Flavonoids are group of phytochemicals found in varying amounts in foods and medical plants which have been shown to exert potent antioxidant activity against the superoxide radicals. Its consumption has been documented not to be associated with coronary heart disease mortality. Epidemiologic studies indicate an inverse relationship between intake of dietary flavonoids and coronary atherosclerotic disease. Therefore, the present of flavonoids in this plant may give support to their therapeutic effects especially in the treatment of hypertension. Jansen *et al.* (2004) reported *in vivo* cardio-protective activities of *H. sabdariffa* proto catechuic acid (PCA), a phenolic compound, found in the dried flower of the plant. The presence of these metabolites probably explains the various uses of this plant in traditional medicine (Noble, 1990).

## REFERENCES

- Abdulazeez A., Ibrahim I.L., Mohammad Y.S., Gimba A. and Nuhu A. (2016). Comparative Studies of Proximate Composition of Three Species in Calyces of *Hibiscus sabdariffa* Linn. *Lapai Journal of Applied and Natural Sciences LAJANS* Vol 2(1):9-14.
- AOAC (1990). *Official Methods of Analysis*. Association of Official Analytical Chemistry, Washington D.C. 15th Ed.
- Craig, W. J. (1999). Health- promoting properties of common herbs. *America Journal Clin Nutr.* 70 (suppl): 491S-499S.
- Harborne, J.B. (1973). *Phytochemical methods*, London. Chapman and Hall, Ltd. Pp. 49-188.
- Jansen, M.C. Mc Kenna, D., Bueno-de-mesquita, H.B., Feskens, E.J. Streppel, M.T., Kok, F.J., and Kromtrout, D. (2004). Reports: quantity and variety of fruit and vegetable consumption and cancer risk. *Nutr, Cancer* 48: 142.
- Malinow, M. R; McLaughlin P.; Kohler, G. O. and Livingstone A. L. (1997). Saponins: A family of substances potentially useful for treatment of hypercholesterolemia, *Clin. Res.*, 25, 974-979.
- Morton, J.F. (1987). Roselle. In: *Fruits of warm climate*, (CF Dowling ed). Media, Inc. Greensboro, NCP. 281-286.
- Noble, R. L, (1990). The discovery of vinca alkaloids chemotherapeutic agents against cancer, *Biochem. Cell Biol.*, 68(12), 1544-1551.
- Odigie, I. P., Ettarh, R. R., Adigun, S. A. (2003). Chronic administration of aqueous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac hypertrophy in 2K-1 C hypertensive rats. *Journal Ethnopharmacol* 86: 181-185.
- Schipper, R. R. (2002). *African Indigenous Vegetables. An overview of the cultivated species.* 133.
- Singh, G., Sharma, P.K. Dudhe R. and Singh, S. (2010). Biological activities of *Withania somnifera*, *Annals of Biological Research.* 1(3): 56-63.



- Sofoware, A., (1993). Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, PP 223 – 233.
- Trease, G. E. and Evans, W.C. (1989). Pharmacognosy. 11<sup>th</sup> edn. Brailliar Tiridel Can Macmillian publishers.
- WHO (1998). World Health Organization. Quality Control Methods for Medicinal Plant materials, WHO Geneva Switzer Land.