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Research Article

An Assessment of the Nutritional, Phytochemical and Antioxidant Properties of *Hibiscus asper* Hook. F. (Malvaceae)

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ABSTRACT

Hibiscus asper is used traditionally as potent sedative, restorative tonic, anti-inflammatory, anti-depressive and anti-anaemic drug, as well as in the management of jaundice. This study evaluated the nutritional, phytochemical and antioxidant properties of the leaf and calyx of the plant with a view to providing more scientific information on its therapeutic potentials. The two plant parts were screened for proximate, mineral and vitamin compositions using standard protocols. The phytochemical analysis of dry samples was done using standard protocols. The antioxidant activity of samples was against 1,1 – diphenyl – 2 picrylhydrazyl (DPPH) radical. All data were subjected to statistical analysis. *H. asper* calyx had significantly higher nutrients than the leaf except carbohydrate content. The calyx was richer in mineral and vitamin contents than the leaf, especially ascorbic acid (22.17±0.21 mg/100g) and carotenoids (1660.00±15.00 mg/100g). Interestingly, the leaf had higher phytochemical contents compared to the calyx. It had alkaloids (1260.00±18.03 mg/100g), flavoniods (471.67±16.07 mg/100g), and cardiac glycosides (4.10±0.10 mg/100g), whereas the calyx (53.33±0.25 %) showed higher inhibition against DPPH radical than the leaf (36.33±0.45 %). Overall, the calyx was richer in proximate, mineral and vitamin contents. It also showed higher antioxidant activity than the leaf. However the leaf contained significantly higher phytochemicals than the calyx. *H. asper* is very rich in nutrients and phytochemicals with valuable antioxidant property. The calyx could be an important source of nutrients and the leaf had strong therapeutic potentials in the management of diseases. This study justifies the traditional uses of the plant as food and medicine.

Keywords: *Hibiscus asper*, secondary metabolites, nutrients, vitamins, natural antioxidant

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INTRODUCTION

The genus *Hibiscus* Linnaeus belongs to the family Malvaceae. It comprises about 250 species distributed in tropical and subtropical areas. The plants are annual erect, bushy herbs and sub - shrubs (Amin *et al*., 2008). The genus consist of some very economical and useful species such as *Hibiscus cannabinus* L., *Hibiscus asper* F., *Hibiscus tiliaceus* L., *Hibiscus acetosella* Welw. ex Hiern and *Hibiscus sabdariffa* L. whose importance cannot be overemphasized. These plants serve as source of fibers, ornamentals, medicine, tea, oil and cosmetic products (Fasoyiro *et al*., 2005; Ayanbadejo *et al*., 2012).

H. asper (Plates 1a & 1b) was first described in 1849 from the specimen collected in Sierra Leone (Sprague, 1913). It is widely distributed throughout tropical Africa, grows in fallow fields, grassland and edges of gallery forest. It is a perennial herb whose stems are with five prickles, simple or stellate hairs up to 2 m tall. Its leaves are alternate, simple and stipules up to 6 mm long. *H. asper* belongs to section *Furcaria*, having in common a pergamentaceous calyx (rarely fleshy) with 10 strongly prominent veins, 5 running to the apices of the segments and 5 to the sinuses but the leaf of several other species of section *Furcaria* are more well-known vegetables (Wilson, 1999). *H. asper* can be distinguished from related *Hibiscus spp*. by its stems with fine prickles, poorly developed vegetative branches, narrow epicalyx lobes which are not bifurcate having a calyx with nectary, white woolly hairs and curved prickles or bristles (Burkill, 1997). *H. asper* was and sometimes still is considered conspecific with *H. cannabinus* and it is probably mainly self-pollinating which is favoured by the flower structure having style

branches included in the stamina column or hardly exerted. The study of the material at the Kew Herbarium showed that *H. asper* can be distinguished from *H. cannabinus* by several characteristics such as the repand lobing of the leaf segments, the small subglobose capsule and the smaller but more rounded minutely and densely tubercle seeds with a setose sinus, all of which warrant its restoration to its own rank (Sprague 1913).

In Nigeria, the calyx and leaf of *H. asper* are commonly used in soups as vegetables in the middle belt and north eastern regions while it is largely used in the tropical regions of Africa as potent sedative, restorative tonic, anti-inflammatory, anti-depressive, anti-anaemic drug as well as in the management of jaundice (Lucian *et al*., 2014,). It was reported by Burkill (1997) that the leaf is highly recommended by traditional practitioners for the treatment of abscesses, urethritis, joint pain, male infertility and skin infections in western region of Cameroon. Also, Schippers and Bosch (2004) reported the use of the plant in veterinary medicine in the management of cutaneous infections of the domestic animals and as an anti-parasitic drug. Although the plant is used as food and herbs by indigenous people but there is scarcity of scientific information on its nutritional and chemical components. This study therefore screened the leaf and calyx of *H. asper* for their mineral, proximate and phytochemical components as well as antioxidant activity with a view to providing more scientific information on its nutritional and therapeutic potentials.

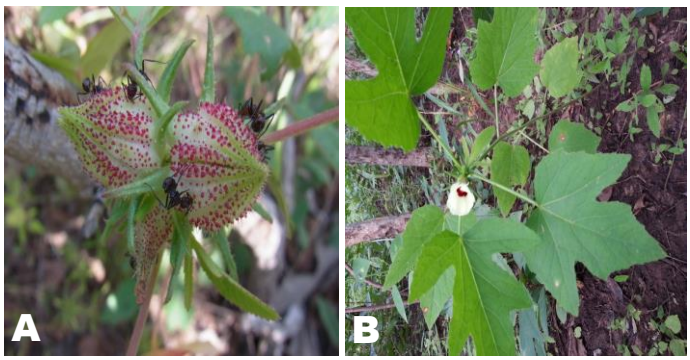

Plate 1
A: Calyses of *Hibiscus asper; B. Leaves of Hibiscus asper*

MATERIALS AND METHODS

Collection and identification of plant material

The whole plant of *H.asper* was collected from a garden at Eyenkorin, Asa Local Government Area, Kwara State, Nigeria and identified at the University of Ibadan Herbarium (UIH). The leaf and calyx were separated, washed thoroughly, cut into small pieces, air dried at room temperature for two weeks. The dried samples were ground into fine powder using a milling machine (Miller's blender). The powdered samples were stored in an air tight container at 4^{o}C prior to use.

Proximate analysis

The proximate analysis of *H. asper* leaf and calyx was screened for the presence of protein, ash, moisture, crude fibre, fats and carbohydrate using standard procedures (Greenfield and Southgate, 1992; Horwitz, 2000; AOAC, 2006).

Mineral analysis

The samples were screened for the presence of mineral contents. The method of Walsh (1971) was used for digestion of all plant samples thereafter Calcium (Ca), Copper (Cu), Zinc (Zn), Iron (Fe), Sodium (Na), Potassium (K), were analysed using Atomic Absorption Spectrophotometer while Phosphorus was determined using Vanadomolybdate (Yellow method), (AOAC, 2006; ASEAN, 2011).

Vitamins analyses

The samples were screened for the presence of thiamin, riboflavin, niacin, ascorbic acid and carotenoids in dry samples. Riboflavin and thiamin were analysed using HPLC method as described by Wehling and Wetzel, (1984); while niacin and ascorbic acid was analysed using HPLC method described by Wills *et al*., (1977).

Phytochemical Screening

The powdered dried samples of plant were screened for the presence of active components. The alkaloids, anthraquinones, flavonoids, cardiac glycosides, saponins, tannins and proanthocyanidins in samples were determined using standard procedures (Sofowora, 1993; Harborne, 2005; Tease and Evans, 2005).

DPPH free radical scavenging assay

The method of Brand-Williams *et al*.,1995 was used to determine antioxidant activity through DPPH (1, 1–diphenyl–2–picrylhydrazyl) assay. DPPH (20 mg/dm) was prepared in 80% methanol then 0.2 mg of extract of sample was added to 2.8 ml of DPPH in methanol solution. The mixture was incubated for 20min in dark room at room temperature (25-30 ^{o}C) and the absorbance was read at 517 nm using a spectrophotometer. Methanol only was used as a blank to adjust the spectrophotometer to zero absorbance and DPPH solution was used as the control. The scavenging activity of each extract was calculated as follows:

Sample control % Inhibition = [1 – (A^{sample} /$A^{control}$)] x 100
control

Where $A^{control}$ is the absorbance for control and A^{sample} sample is absorbance for test sample.

Statistical Analysis

All data were subjected to statistical analysis using Statistical Analysis System (SAS) and the difference between means was separated using Fishers Pairwise Comparison (FPC).

RESULTS

The nutritional composition of *H. asper* is presented in Table 1. The calyx had a significantly higher moisture content (9.03±0.15 %), crude protein (22.93±0.25 %), fat (1.77±0.15 %), ash (3.67±0.15 %) and crude fibre (3.43±0.21 %) while the leaf had a higher amount of carbohydrate (65.57±0.29 %). Table 2 shows that the calyx had significant higher calcium (718.33±7.64 mg/100g), sodium (921.67±5.77 mg/100g),

phosphate (683.33±12.58 mg/100g), iron (17.43±0.12 mg/100g), zinc (0.77±0.06 mg/100g), copper (0.57±0.06 mg/100g) and potassium (78.33±2.89 mg/100g) than the leaf.

Table 1:
Proximate composition of the leaf and calyx of *Hibiscus asper*

Parameters	Contents (%)	
	Leaf	Calyx
Ash	3.27±0.06[b]	3.67±0.15[a]
Crude fibre	2.67±0.15[b]	3.43±0.21[a]
Carbohydrates	65.57±0.29[a]	59.17±0.32[b]
Energy (calculated) Kcal	2343.97±58.30[a]	2285.03±22.39[a]
Ether extract (fat)	1.33±0.06[b]	1.77±0.15[a]
Moisture content	8.57±0.21[b]	9.03±0.15[a]
Crude protein	18.60±0.26[b]	22.93±0.25[a]

Values are expressed as means ± SD. Means with same alphabet across column are not significantly different at p<0.05.

Table 2:
Mineral composition of leaf and calyx of *Hibiscus asper*

Parameters	Composition (mg/100g)	
	Leaf	Calyx
Calcium	570.00±13.23[b]	718.33±7.64[a]
Copper	0.33±0.06[b]	0.57±0.06[a]
Iron	13.33±0.21[b]	17.43±0.12[a]
Phosphate	476.67±2.89[b]	683.33±12.58[a]
Potassium	61.67±5.77[b]	78.33±2.89[a]
Sodium	878.33±2.87[b]	921.67±5.77[a]
Zinc	0.53±0.06[b]	0.77±0.06[a]

Values are expressed as means ± SD. Means with same alphabet across column are not significantly different at p<0.05.

Table 3:
The Vitamin Composition of the Leaf and Calyx of *Hibiscus asper*

Parameters	Composition (mg/100g)	
	Leaf	Calyx
Ascorbic acid	15.03±0.21[b]	22.17±0.21[a]
Carotenoids	1255.00±21.79[b]	1660.00±15.00[a]
Niacin	0.45±0.21[b]	0.52±0.02[a]
Riboflavin	0.01±0.02[b]	0.16±0.02[a]
Thiamin	0.13±0.02[b]	0.18±0.01[a]

Values are expressed as means ± SD. Means with same alphabet across column are not significantly different at p<0.05.

The calyx of the plant was richer in vitamins than the leaf (Table 3). It contained significantly higher ascorbic acid (22.17±0.21 mg/100g), carotenoids (1660.00±15.00 mg/100g), riboflavin (0.16±0.02 mg/100g) and thiamin (0.18±0.01 mg/100g) than the leaf. The leaf had significant higher phytochemical contents than the calyx (Table 4). It had higher alkaloids (1260.00±18.03 mg/100g), cardiac glycosides (4.10±0.10 mg/100g), flavoniods (471.67±16.07 mg/100g), saponins (471.67±12.58 mg/100g), and proanthocyaanidins (3.57±0.12 CE/g) than the calyx.

H. asper showed antioxidant activity against DPPH radical (Table 5). The antioxidant activity of the calyx (53.33±0.25 %) was significantly higher than that in the leaf (36.33±0.45 %) whereas the polyphenols of the leaf (72.50±0.20 mgGAE/g) was higher than that of the calyx (59.27±0.31 GAE/g).

Table 4:
Phytochemical components of leaf and calyx of *Hibiscus asper*

Parameters	Components (mg/100g)	
	Leaf	Calyx
Alkaloids	1260.00±18.03[a]	748.33±16.07[b]
Flavanoids	471.67±16.07[a]	260.00±15.00[b]
Tannins	283.33±16.07[b]	1366.67±20.21[a]
Saponins	471.67±12.58[a]	215.00±0.00[b]
Cardiac glycosides	4.10±0.10[a]	2.60±0.17[b]
Anthraquinones	135.00±10.00[b]	418.33±10.41[a]
Proanthocyanidins	3.57±0.12[a]	3.10±0.10[b]

Values are expressed as means ± SD. Means with same alphabet across column are not significantly different at p<0.05.

Table 5:
Antioxidant property and polyphenolic composition of *Hibiscus asper*

Parameters	Leaf	Calyx
Antioxidants (% Inhibition)	36.33±0.45[b]	53.33±0.25[a]
Polyphenols (mgGAE/g)	72.50±0.20[a]	59.27±0.31[b]

Values are expressed as means ± SD. Means with same alphabet across row are not significantly different at p<0.05.

DISCUSSION

H. asper contained appreciable proximate content. In the present study, the leaf and calyx had significant amounts of protein, carbohydrate, fibre and ash with low values of fat. The calyx was richer in nutrients than the leaf. The proximate composition of the plant recorded in this study is in agreement with the report of Ayanbadejo *et al*., (2012). Nutritional compositions of plants have been reported to have numerous health benefits to man. The significantly high amount of crude protein, crude fiber and ash recorded in the calyx could be of immense nutritional benefit since the plant is consumed as vegetable and the decoction of its dried calyx is prepared as tonic or juice. Diet rich in fiber content is known to lower cholesterol level, aid smooth intestinal functioning, stimulating the proliferation of the intestinal flora and decreased incidence of several types of diseases such as diverticular disease and constipation in patients (Naeem *et al*., 2013). Proteins are needed for growth, body building and repair of worn-out tissue. Plant protein has been reported to have beneficial effect in the management of blood pressure therefore blood pressure lowering effect of protein may have important public health implication (Altorf–van der Kuil, 2010; Gbadamosi and Kalejaye, 2017). The presence of ash

content in the leaf and calyx indicates that the plant is rich in minerals.

The calcium, sodium, phosphate, iron, zinc and potassium contents were significantly higher in the calyx than the leaf. Ahmed and Chaudhary, (2009) reported that calcium plays a significant role in muscle contraction, bone and teeth formation and blood clotting. Magnesium is needed as cofactor in enzyme catalysis in the body. Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and needed in the transport of oxygen, carbon dioxide during respiration or cellular metabolism (Heaney, 2009). Klasco, (2011) reported that zinc stabilizes the structure of proteins, cell membranes and regulates gene expression and DNA function. Based on findings of this study regular consumption of the plant as vegetable or juice could serve as cheap source of the beneficial nutrients and minerals required for healthy immune and body function because deficiency in any of these basic nutrients, micro and macro mineral constituents could lead to a number of health problems.

The findings on the vitamin contents of leaf and calyx of the plant indicated that they are good sources of vitamins particularly ascorbic acid and carotenoids. Fruits and vegetables are known to be very good sources of vitamins which functions as immune booster, support normal growth and development (Jaswir *et al*., 2011). Ascorbic acid (vitamin C) is an antioxidant which helps to protect the body against cancer and other degenerative diseases such as arthritis and type II diabetes mellitus and strengthens the immune system. According to Okwu (2004) natural ascorbic acid is vital for body performance and used in herbal medicine for the management of common cold and other diseases such as prostate cancer. The calyx in this study had significantly higher carotenoids than the leaf. Carotenoids are known to be the basic source of yellow, orange and red pigments found in plants and animals (Basu *et al*., 2001; Sugawara *et al*., 2009). Animals are incapable of producing carotenoids therefore must obtain them from leaves, fruits, flowers of higher plants and microorganisms such as algae, fungi and bacteria (Mattea *et al*., 2009, Hosokawa *et al*.,2008). Carotenoids are known to be efficient physical and chemical quenchers of singlet oxygen ($^1O_2$) as well as potent scavengers of other reactive oxygen species (ROS) (Edge and Touscott, 2010; Cvetkovic *et al*., 2013). Carotenoids posses therapeutic properties as it serves as source of pro-vitamin A or retinol in the body capable of preventing serious eye diseases such as night blindness (Takahashi *et al*.,2006), they act as antioxidants and promote oxidative stress resistance (Yeum *et al*., 2009), anti-cancer (Nishino *et al*., 2002) and anti-obesity by reducing white adipose tissue (Maeda *et al*. 2007). Overall, the higher antioxidant activity of the calyx could be attributed to its carotinoids and vitamin C contents, regular consumption of the calyx may boost body immune, protect cells and prevent oxidative stress.

Phytochemicals are known to exert their beneficial effects in the management of many chronic diseases such as hypertension by reducing the circulating levels of cholesterol or by inhibiting anti-inflammatory and antiplatelet activities (Upadhyay and Dixit, 2015). *H. asper* leaf contained significant higher amount of alkaloids, flavanoids, saponins, cardiac glycoscides and proanthocyanidins than the calyx which justifies the efficacy of methanolic extract of the leaf in inflammatory disorders like rheumatoid arthritis and as an antioxidant agent in the fight against brain oxidative stress (Lucian *et al*., 2014; Foyet *et al*., 2011). Alkaloids have been used as an antidote in organophosphate poisoning, restoration of mental alertness, treatment of acute pain, management of type II diabetes, treatment of malaria and as antibiotics and analgesic (Zhang *et al.,* 2008; Udochukwu et al., 2015). The high amount of alkaloids in the leaf of *H. asper* may be responsible for its continuous traditional use in the management of menstrual pain. The calyx contained higher amount of anthraquinones than the leaf which suggests that it may be a potent anti-fungal, anti-bacteria and laxative agent. These anthraquinones which are a sub group of plant compounds quinones and anthraquinone glycoscides have been reported to inhibit bacteria, fungi, posses potent laxative properties and as a good source of natural dye, used for comestics, food and pharmaceuticals (Alves *et al.,* 2004; Kumar *et al*., 2006). Flavonoids are group of bioactive compounds that are extensively found in foodstuff of plant origin and its regular consumption is associated with reduced risk of some chronic disease such as cancer, cardiovascular disease, inflammation and neurodegenerative disorders. Therefore the appreciable amount of flavoniod present in the leaf and calyx could be beneficial to the body as an antioxidant, hepatoprotective and antiviral agent (Zhu *et al*., 2012). Its activity in right amount and proportion will promote normal body metabolism and prevent degenerative diseases.

H. asper exhibited inhibitory activity against DPPH radical. The calyx exhibited higher activity than the leaf indicating that the calyx possessing stronger free radical scavenging activity could be useful in the management of metabolic diseases. The high free ravaging scavenging activity exhibited by the plant parts may be due to the scavenging process of flavoniods as reported by previous authors (Kessler *et al*., 2003). It has been reported that phenols and polyphenolic compounds, such as flavonoids show antioxidant activity and their effect on human nutrition and health cannot be overemphasized. These free radicals cause decrease in membrane fluidity, loss of enzyme receptor activity, damage to membrane protein leading to different disorders like ageing, cancer, cardiovascular disease, diabetes, rheumatoid arthritis, epilepsy, degradation of essential fatty acids and death (Barros *et al*., 2007, Li *et al*., 2007). Therefore regular consumption of plants with appreciable antioxidant activity or potentials such as *H. asper* in our diets could reduce the reactive oxygen species (ROS) which have been implicated in pathophysiology of major degenerative disorders. Synthetic antioxidants have been reported to be carcinogenic whereas plant antioxidants have little or no side effects. The consumption of *H. asper* as natural antioxidant could be safe, cheap and effective.

In conclusion, *H.asper* contained appreciable amounts of nutrients, minerals, vitamins and phytochemicals. The calyx could be used as food supplement due to its nutritional contents and antioxidant activity whereas the leaf could be source of herbal remedy for treatment of diseases based on the phytochemical contents. Overall, this study justifies the traditional use of *H.asper* as food and medicine

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