

Original Review Article

Hibiscus sabdariffa: An Underutilised Powerhouse of Plant Bioactives

Idolo Ifie

Department of Food Science and Technology
Delta State University, PMB 1, Abraka
Delta State, Nigeria

Email: idifie@yahoo.com

Received 20th April, 2018; Accepted 5th June, 2018

Abstract

Hibiscus sabdariffa (H. sabdariffa) is a plant containing a plethora of health-promoting phytochemicals such as anthocyanins, organic acids, flavonoids and polysaccharides with potential biological properties. Furthermore, its reported use in folk medicine for the treatment of several non-communicable diseases is well documented. Indeed, a growing body of evidence give credence to its anti-diabetic, anti-hypertensive, cholesterol-lowering and anti-obesity properties. Other pharmacological benefits ascribed to H. sabdariffa include immunomodulatory, diuretic and renal protection properties. Owing to its perceived health benefits, the manufacture of diverse functional foods from H. sabdariffa have been seen in recent years, showing a growing interest in novel technological applications of potential products from the plant. A highlight of some of the patented products alongside possible future application in the food and nutraceutical industries is presented in this review

Keywords: *Hibiscus sabdariffa*, anthocyanins, phenolic acids, flavonoids, health benefits, food products.

Introduction

Hibiscus sabdariffa L. (*H. sabdariffa*) is a short-day annual shrub of the *Malvaceae* family grown in Sub Saharan Africa, Asia and Caribbean (Aurelio *et al.*, 2008). Although it is not clear where *H. sabdariffa* was first discovered, there is some evidence that it was first domesticated by the black populations of western Sudan, and later transported to other parts of the world. In addition, its use for food and curative purposes dates back to 5000 BC, spanning from Egypt, the West Indies, China, India to Latin America (Mohamed *et al.*, 2012). Today, the leading producers of *H. sabdariffa* include Sudan, Jamaica, Thailand and China, with the quality of Sudan rated the best in the world. *H. sabdariffa* is known by different names across the world as shown in Table 1.

Botanical description and morphology

The shrub, *H. sabdariffa*, grows up to 2.4 m, and the leaves, which are 7.5 -12.5 cm, are green, having reddish veins and long or short petioles. The flowers, borne singly in the leaf axils, are up

Table 1: Vernacular names of *H. sabdariffa* in different countries

Country	Vernacular name
England	Roselle
U.S	Roselle
France	Oseille
Jamaica	Flor de Jamaica
Switzerland, Sudan and Arabia	Karkade
Nigeria	Zobo
Senegal	Byssap

Source: Ismail *et al.*, 2008

to 12.5 cm wide, yellow or buff with a rose (maroon eye), which turn pink upon withering at the end of the day. Thereafter, the typically red calyx (3.2 - 5.7 cm long), which consists of 5 large sepals with a collar (epicalyx) of 8 to 12 slim, pointed bracts (or bracteoles) around the base, begins to enlarge, becomes fleshy, crisp and juicy, and fully encompasses the velvety capsule that turns brown and splits open when mature and dry (Morton, 1987). The seeds which are covered with minute, stout and stellate hairs, are light brown, 3 mm long and kidney-shaped (Morton, 1987). There is another less popular variety of *H. sabdariffa* referred to as white roselle with its distinct green/whitish calyces which is commonly consumed as a vegetable.

Cultivation and harvesting of H. sabdariffa

The plant thrives in tropical and subtropical regions, and is very susceptible to damage from fog and frost. *H. sabdariffa* requires an average rainfall of about 182 cm (72 inches), and propagation is by seeds, although it can be cultivated by shoot cuttings. The seedlings may be raised in the nursery and later transplanted to the field when they are 7.5 -10 cm (3 to 4 inches) high. It is essential to weed in the first month, while the application of fertilizer varies amongst regions, although *H. sabdariffa* may benefit from nitrogen application. The fruit consisting of the large reddish calyces is harvested 2-3 weeks after the onset of flowering, while the rest of the crop remains in the field until the seeds are ready for threshing.

Uses of H. sabdariffa

(i) Food uses

The calyces, fresh or sometimes dried, are employed in the production of teas, ice cream, jams, marmalade, sherbets and sauces. In addition, wines, jellies, beverages and other desserts can also be made from the calyces (Tsai *et al.* 2002; Herrera-Arellano *et al.*, 2004). In Nigeria, a refreshing drink known as “Soborodo” is prepared by boiling the calyces along with sugar and spices, while in Mexico a drink processed in similar manner is referred to as “Agua de Jamaica”. The seeds can be taken as a coffee substitute or fermented to produce a condiment known as “Mungza ntusa” in

the northern parts of Nigeria (Omobuwajo *et al.*, 2000), while in China and certain parts of western Africa, the seeds are used for oil production (Atta and Imaizumi, 2002). Furthermore, the seeds are used in the production of feed for both poultry and sheep. The leaves are also edible and are eaten raw, dried or cooked in Sudan and Malaysia (Ismail *et al.*, 2008). They also serve as a rich source for animal fodder and fibre.

(ii) Traditional and folk medicine

The calyces of *H. sabdariffa* have been used in traditional medicine to treat various health conditions such as hypertension, diabetes, nerve disorders and heart ailments. Furthermore, several ethnomedicinal studies have reported the use of infusions of the calyces for treatment of hyperlipidaemia, obesity, and urinary tract infections (Hopkins *et al.*, 2013; Patel, 2014). In Guinea, the leaves are deemed to be diuretic, refrigerant and sedative, while the seeds are regarded as antiscorbutic and laxative. In addition, the leaves are employed in the treatment of cough and abscesses in parts of Central Africa and Angola (Duke, 1983).

Phytochemistry

H. sabdariffa is a rich source of bioactive phytochemicals (Tables 2 & 3) which may be responsible for the reported health benefits. The phytochemical profile of *H. sabdariffa* calyces reported differs between studies, probably due to factors such as variations in geographical locations, varieties, genetics, ecology and harvesting conditions. The main bioactive constituents in the calyces related to its pharmacological activities are the anthocyanins, organic acids, flavonoids and polysaccharides (Borrás-Linares *et al.*, 2015). Moreover, the seeds is documented to be rich in polyunsaturated fatty acids and lipids soluble antioxidants (Mahadevan *et al.*, 2009).

Anthocyanins

These are a group of flavonoid derivatives responsible for the red, blue and purple pigments found in *H. sabdariffa*, and they account for about 1.7 to 2.5 % dry weight of the calyces. The first anthocyanin to be characterised from the calyces of *H. sabdariffa* was “hibiscin” which was later renamed delphinidin 3-*O*-sambubioside (DS) (Yamamoto and Osima, 1932). Thereafter, three different anthocyanins were identified in the calyces namely cyanidin 3-*O*-sambubioside (CS), delphinidin 3-*O*-glucoside and cyanidin 3-*O*-glucoside (Du and Francis, 1973). In most published studies, delphinidin 3-*O*-sambubioside and cyanidin 3-*O*-sambubioside have been reported as being the main anthocyanins present within the calyces, accounting for over 75 % of the total anthocyanins (Ramirez-Rodrigues *et al.*, 2011; Sindi *et al.*, 2014).

However, a study also reported the absence of delphinidin-3-*O*-glucoside in the extracts of some strains (Da-Costa-Rocha *et al.*, 2014). The chemical structure of the main anthocyanins and some phytochemicals found in *H. sabdariffa* are presented in Figure 1.

Organic acids

The calyces of *H. sabdariffa* contain a myriad of organic acids which include malic, citric, ascorbic, hibiscus and hydroxycitric acids (Wong *et al.*, 2002). In addition, tartaric acid, oxalic

Table 1: Phytochemicals present in *H. sabdariffa*

Class	Compound	Reference(s)
Anthocyanins	Delphinidin 3- <i>O</i> -sambubioside (DS)	(Du and Francis, 1973; Ramirez-Rodrigues <i>et al.</i> , 2011)
	Cyanidin 3- <i>O</i> -sambubioside (CS)	(Borrás-Linares <i>et al.</i> , 2015; Wong <i>et al.</i> , 2002)
	Delphinidin 3- <i>O</i> -glucoside	(Sindi <i>et al.</i> , 2014)
	Cyanidin 3- <i>O</i> -glucoside	(Rodriguez-Medina <i>et al.</i> , 2009)
	Cyanidin-3,5- <i>O</i> -diglucoside	(Segura-Carretero <i>et al.</i> , 2008)
Organic acids	Hibiscus acid	(Ramirez-Rodrigues <i>et al.</i> , 2012; Rodriguez-Medina <i>et al.</i> , 2009)
	Hibiscus acid glucoside	(Ramirez-Rodrigues <i>et al.</i> , 2011; Ramirez-Rodrigues <i>et al.</i> , 2012)
	Hibiscus acid 6-methyl ester	(Borrás-Linares <i>et al.</i> , 2015; Ramirez-Rodrigues <i>et al.</i> , 2011)
	Hydroxycitric acid	(Borrás-Linares <i>et al.</i> , 2015; Mahadevan <i>et al.</i> , 2009)
	Malic acid	(Jung <i>et al.</i> , 2013; Wong <i>et al.</i> , 2002)
	Oxalic acid	(Ali <i>et al.</i> , 2005; Wong <i>et al.</i> , 2002)
	Citric acid	(Ali <i>et al.</i> , 2005; Wong <i>et al.</i> , 2002)
	Succinic acid	(Wong <i>et al.</i> , 2002)
Phenolic acids	Tartaric acid	(Mahadevan <i>et al.</i> , 2009)
	3- <i>O</i> -caffeoylquinic acid	(Borrás-Linares <i>et al.</i> , 2015; Rodriguez-Medina <i>et al.</i> , 2009)
	4- <i>O</i> -caffeoylquinic acid	(Ramirez-Rodrigues <i>et al.</i> , 2011)
	Chlorogenic acid	(Borrás-Linares <i>et al.</i> , 2015; Jiménez-Ferrer <i>et al.</i> , 2012)
	5- <i>O</i> -caffeoylshikimic acid	(Borrás-Linares <i>et al.</i> , 2015; Rodriguez-Medina <i>et al.</i> , 2009)
	N-Feruloyltyramine	(Borrás-Linares <i>et al.</i> , 2015; Rodriguez-Medina <i>et al.</i> , 2009)
	Caffeic acid	(Ramirez-Rodrigues <i>et al.</i> , 2011; Ramirez-Rodrigues <i>et al.</i> , 2012)
	Coumaroylquinic acid	(Borrás-Linares <i>et al.</i> , 2015)
	Gallic acid	(Ramirez-Rodrigues <i>et al.</i> , 2012)
Flavonols	Protocatechuic acid	(Mahadevan <i>et al.</i> , 2009; Ramirez-Rodrigues <i>et al.</i> , 2011)
	Quercetin	(Fernandez-Arroyo <i>et al.</i> , 2012; Fernandez-Arroyo <i>et al.</i> , 2011)
	Quercetin 3- <i>O</i> -glucoside	(Borrás-Linares <i>et al.</i> , 2015; Ramirez-Rodrigues <i>et al.</i> , 2011)
	Quercetin 3-sambubioside (Q3A)	(Rodriguez-Medina <i>et al.</i> , 2009)
	Myricetin	(Borrás-Linares <i>et al.</i> , 2015)
	Myricetin-3-arabinogalactoside (M3A)	(Rodriguez-Medina <i>et al.</i> , 2009)
	Rutin	(Rodriguez-Medina <i>et al.</i> , 2009)
Sterols	Kaempferol-3- <i>O</i> -glucoside	(Fernandez-Arroyo <i>et al.</i> , 2011)
	Ergosterol	(Ali <i>et al.</i> , 2005; Mahadevan <i>et al.</i> , 2009)
	Stigmasterol	(Ali <i>et al.</i> , 2005)

Table 2: Volatiles present in *H. sabdariffa*

Class	Compound	Reference(s)
Volatiles	1-octen-3-ol	(Ramírez-Rodrigues <i>et al.</i> , 2011)
	1-Hexanol	(Ramírez-Rodrigues <i>et al.</i> , 2011; Ramírez-Rodrigues <i>et al.</i> , 2012; Ramírez-Rodrigues <i>et al.</i> , 2011)
	Hexanal	(Ramírez-Rodrigues <i>et al.</i> , 2011)
	Heptanal	(Ramírez-Rodrigues <i>et al.</i> , 2011)
	Eugenol	(Ramírez-Rodrigues <i>et al.</i> , 2011)
	Linalool	(Ramírez-Rodrigues <i>et al.</i> , 2011)
	2-Hexenol	(Chen <i>et al.</i> , 1998)
	Limonene	(Chen <i>et al.</i> , 1998)
	4-Methyl-2,6-di- <i>tert</i> -butylphenol	(Jung <i>et al.</i> , 2013)
	2-Pentylfuran	(Jung <i>et al.</i> , 2013)

acid, succinic acid and malic acid have also been identified in the calyces (Ali *et al.*, 2005). Citric acid and malic acid were the first acids to be isolated in the calyces, and their contents vary widely between studies (Buogo and Picchinenna, 1937). The reported concentrations of ascorbic and malic acids in *H. sabdariffa* varied between 80 and 120 mg/100 g, while succinic, oxalic and tartaric acid contents ranged from 170 – 510 mg/100 g (Wong *et al.*, 2002; Mohamed *et al.*, 2012). Hydroxycitric acid and its lactone form (+) -allo-hydroxycitric acid (hibiscus acid) are the main organic acids found in the calyces of *H. sabdariffa*. Hydroxycitric acid - (2S, 3R) is different from the (2S, 3S) - hydroxycitric acid isolated from the leaves of *Garcinia spp.* (Da-Costa-Rocha *et al.*, 2014).

Phenolic acids

Chlorogenic acid is the main phenolic acid present in *H. sabdariffa* which belongs to the family of esters formed between certain trans-cinnamic acids (caffeic acid, ferulic acid and p-coumaric acid) and quinic acid (Clifford *et al.*, 2003; Borrás-Linares *et al.*, 2015). Chlorogenic acid and its derivatives have been identified in many studies involving extracts of *H. sabdariffa* (Salah *et al.*, 2002; Rodriguez-Medina *et al.*, 2009; Ramirez-Rodrigues *et al.*, 2011). Another phenolic acid characterised from the dried flowers of *H. sabdariffa* and assigned the structure of 3, 4-dihydrobenzoic acid is protocatechuic acid, while gallic and ellagic acids have also been reported in *H. sabdariffa* (Lin *et al.*, 2011; Peng *et al.*, 2011; Lin *et al.*, 2012).

Flavonoids

H. sabdariffa calyces contain different classes of the flavonoids namely flavonols, flavones and flavan-3-ols. These include quercetin and its glucosides, alongside myricetin, rutin, kaempferol, luteolin and its glucoside all being present in *H. sabdariffa* (Salah *et al.*, 2002; Rodriguez-Medina *et al.*, 2009; Fernandez-Arroyo *et al.*, 2012). The flavan 3-ols present in *H. sabdariffa* include catechin and gallo catechins gallates (Yang *et al.*, 2009).

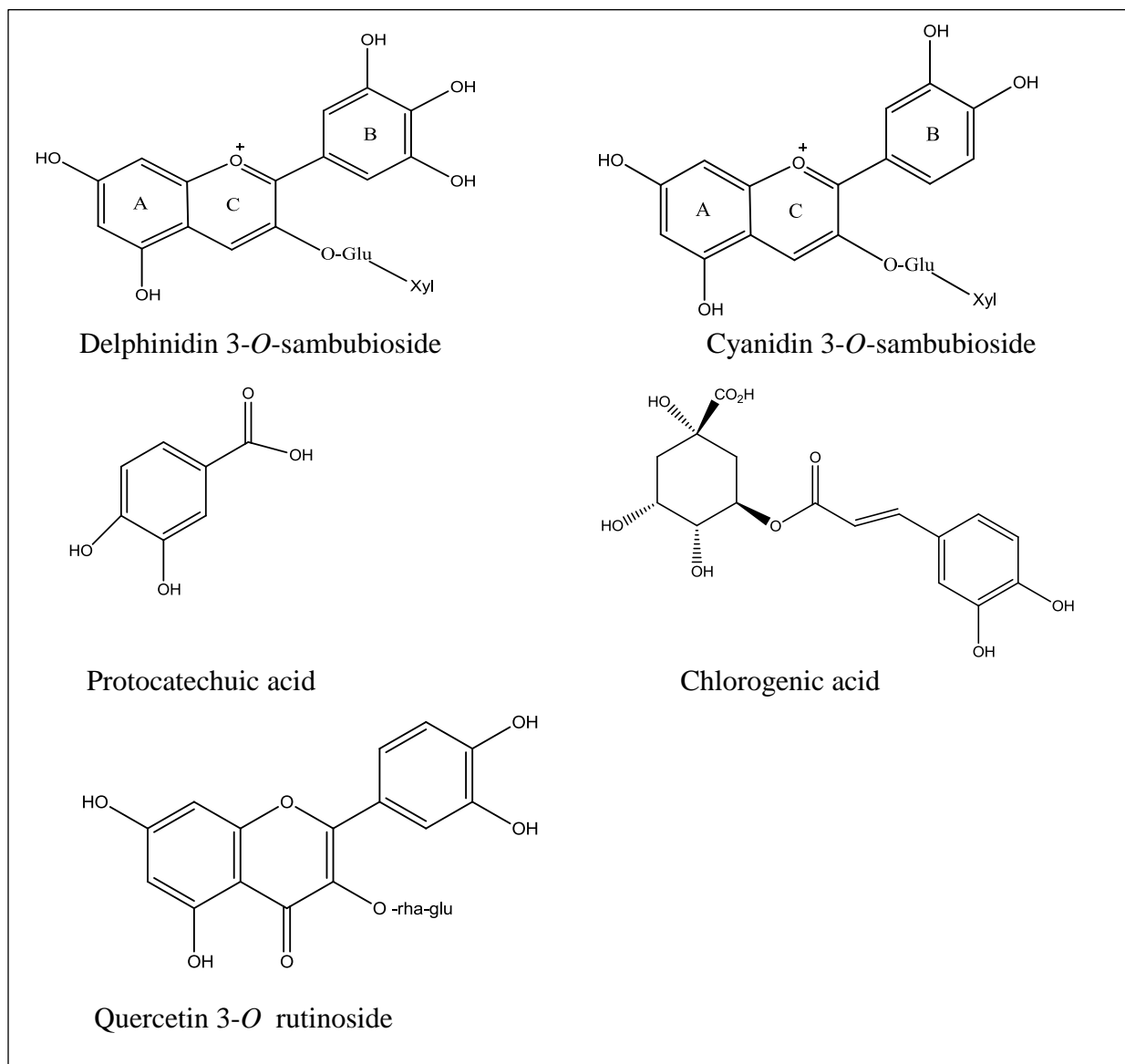


Figure 1: Chemical structures of some phytochemicals found in *H. sabdariffa*

Polysaccharides

The petals of *H. sabdariffa* yield 65 % (dry weight) of mucilage which, on hydrolysis, produces galacturonic acid, glucose and rhamnose (El-Hamidi *et al.*, 1967). Furthermore, three water-soluble polysaccharides have also been isolated from the flower buds, with the neutral fractions composed of arabinans and arabinogalactans. The major fraction was shown to be a pectin-like molecule ($M_r = 105$ Da) linked together by α -1,4-galacturonic acid and 1,2-rhamnose bonds.

Furthermore, side chains of galactose and arabinose are connected to the main chain through C- 4 bond of every third rhamnose (Ali *et al.*, 2005).

Volatile composition

The volatile composition in *H. sabdariffa* calyces was analysed by Gonzalez-Palomares *et al.* (2009). Twenty volatile compounds were identified in the ethanol extract, which include terpenoids, esters, hydrocarbons and aldehydes. However after drying, some of the volatiles were lost as only fourteen volatile compounds were identified. In another study, a total of 28, 25, 17, and 16 volatiles were identified in the dried hot extract (DHE), dried cold extract (DCE), fresh hot extract (FHE), and fresh cold extract (FCE) samples, respectively (Ramírez-Rodrigues *et al.*, 2011). The most potent aroma compounds common to all four extracts were 1-octen-3-ol and nonanal. Furthermore, linalool and 2-ethyl-1-hexanol were detected only in beverages from fresh hibiscus, while furfural and 5-methyl furfural derived from the drying process were found only in dried hibiscus extracts. The same observation regarding furfural and 5-methyl furfural production after drying of *H. sabdariffa* calyces was reported by Chen *et al.* (1998). Furthermore, the analysis of four differently treated *H. sabdariffa* samples: (untreated, frozen, hot-air-dried at 50°C, and hot-air-dried at 75°C), revealed more than 37 volatile compounds characterized by GC-MS. These were the fatty acid derivatives, sugar derivatives, phenolic derivatives, and terpenes. The results also showed higher amounts of the aliphatic C6 lipid derivative (responsible for green aroma notes) in the fresh calyces, while only trace amounts were found in the frozen and air-dried samples.

Pharmacological and biological properties of H. sabdariffa

H. sabdariffa has been proven to exhibit some pharmacological properties, resulting in its being a suitable candidate for therapeutic application. A growing body of evidence give credence to its anti-diabetic properties (Peng *et al.*, 2011; Adisakwattana *et al.*, 2012; Ademiluyi and Oboh, 2013), anti-hypertensive properties (Ajay *et al.*, 2007; Saltzman and Blumberg, 2009; McKay *et al.*, 2009; Inuwa *et al.*, 2012), cholesterol-lowering and anti-obesity properties (Carvajal-Zarrabal *et al.*, 2009; Hernández-Pérez and Herrera-Arellano, 2011; Sabzghabae *et al.*, 2013). Other pharmacological benefits ascribed to *H. sabdariffa* include immunomodulatory, diuretic and renal protection properties (Patel, 2014).

H. sabdariffa and translational research

To determine the level of translational research on *H. sabdariffa* products, a patent search at the Espacenet patent database of the European patent office was performed in 2013 (Da-Costa-Rocha *et al.*, 2014). The results showed a range of patents for the manufacture of diverse functional foods and nutraceuticals from *H. sabdariffa*, which shows a growing interest in novel technological applications of potential products from the plant. A brief highlight of some of the patented products alongside possible future application in the food and nutraceutical industries is discussed below.

(i) Beverage industry

The beverage industry comprises of four main sectors as presented in Figure 3. The soft drinks industry can be sub-divided into five main sectors (bottled water; carbonated soft drinks;

dilutables, also known as squash and including powders, cordials and syrups; 100 % fruit juice, and nectars with 25–99 % juice content; still drinks, including ready-to-drink (RTD) teas, sports drinks and other non-carbonated products with less than 25 % fruit juice). The alcoholic beverage industry is also divided into sub-categories namely, wine, beer, spirits, cider, sake and flavoured alcoholic beverages – sometimes referred to as pre-mixed spirits (Roethenbaugh, 2005).

H. sabdariffa has found a place in the beverage industry (hot drinks and soft drinks sectors) owing to its appealing red colour, refreshing properties and the awareness of its potential health benefit. However, the highlighted portion in Figure 3 shows the sectors yet to be fully exploited. Some of the products of *H. sabdariffa* in the beverage industry currently in the market are described below.

(ii) Hibiscus Lemon Bissap

The product is manufactured by the company Adina for Life founded in 2004 and based in California, U.S.A. The company uses certified organic hibiscus blossoms from Senegal under fair trade agreements as ingredients in its drink. The earnings from this product have enhanced the livelihood of the peasant farmers, and offered them decent incomes to improve their standards of living. The drink marketed as a New Age Beverage, with all natural ingredients and good for you appeal serves retailers throughout the United States including Whole Foods and Wegmans (Ramirez *et al.*, 2010; Rodrigues, 2010).

(iii) Squash Hibiscus Pressé

Produced and marketed in New Zealand, Hibiscus Presse has as ingredients, *H. sabdariffa* extract from water, fructose and ascorbic acid. The label on the 375 mL plastic bottle appeals to consumers, enlightening them about hibiscus' use in folklore and folk medicine in the Caribbean, China, Egypt, Sudan, and Asia. It also informs consumers that “Hibiscus is stimulating scientific inquiry into possible health benefits and its antioxidant properties” (Rodrigues, 2010).

(iv) Simply Hibi

The beverage available in 500 mL glass bottles is produced by Hibi Organics based in the UK and the raw material sourced from a distinct variety of hibiscus grown by farmers in Uganda. The product referred to as Hibiscus Elixir is prepared from infusions of the calyx blended together with sugar to make a concentrate which needs to be diluted before consuming it. In addition, the company in conjunction with Britania Allied Industries in Kampala, one of the biggest names in the East African Juice Market, produces Simply Hibi, a single strength juice as part of the ‘Splash’ range of juices spread abroad by the network of Splash distributors across East Africa. The label informs consumers that the product contains the same high quality, goodness and great taste as the Simply Hibi enjoyed in the UK (Rodrigues, 2010).

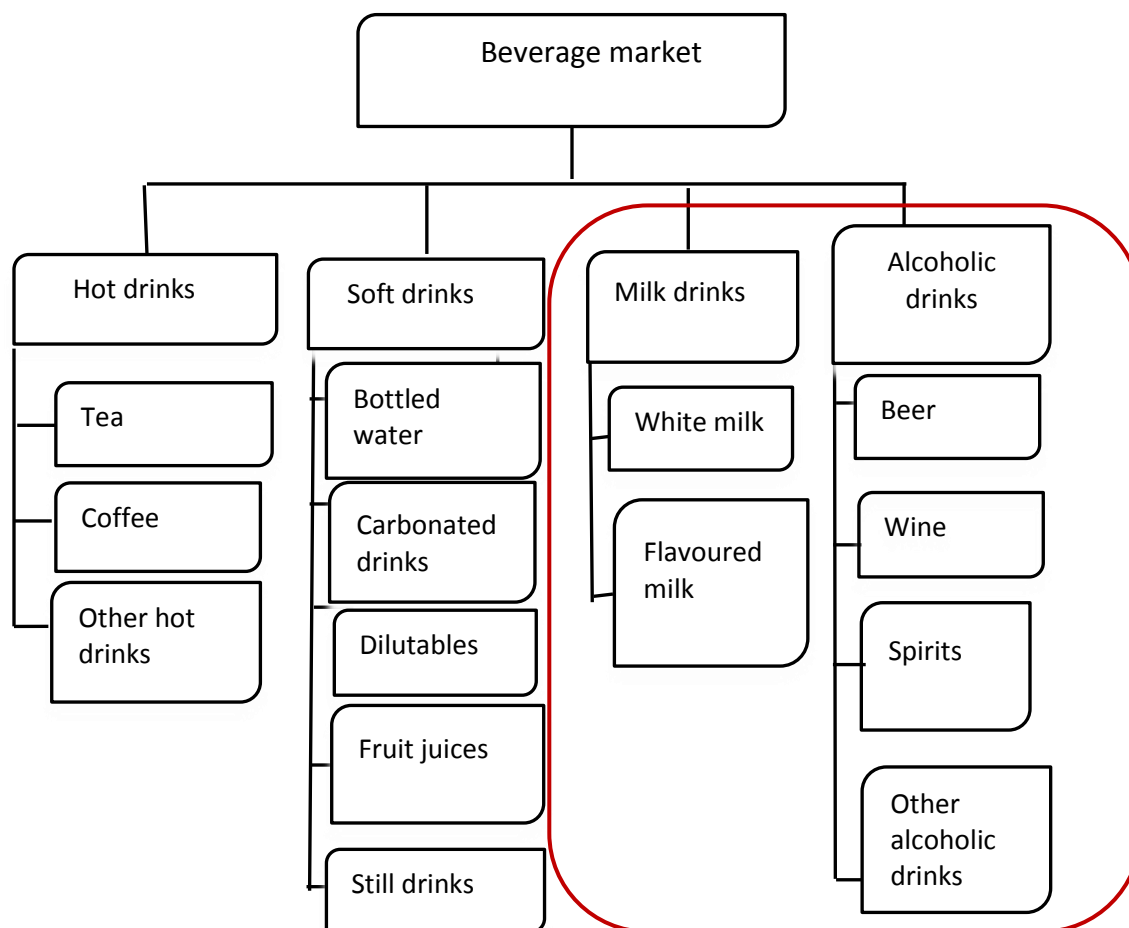


Figure 3: Schematic representation the beverage sector and segments. (Source Rodrigues, 2010)
The highlighted portion shows sectors of the market yet to be fully exploited in the production of hibiscus-based drinks.

(iv) *Yomm Hibiscus drink*

Produced by Yomm Beverage in Canada, the refreshing hibiscus drink comes in four different flavours: original, strawberry, lemon and sugar-free. The ingredients include *H. sabdariffa* extract, water, organic agave syrup, organic cane sugar and natural flavour, and the 335 mL bottle contains 0.270 g and 0.095 g of polyphenols and anthocyanins as stated on the product label.

(v) *Hibiscus Tea*

Known as a caffeine-free herbal drink, hibiscus tea tends to be widely available and produced by different tea companies in tea bags or as loose flower petals. It is known by different brand names and while some contain only hibiscus, some contain it as a major component plus other ingredients to give different flavours.

Conclusion

Although the use of *H. sabdariffa* has gained foray into the food beverage industry, there are other potential application of *H. sabdariffa* in the beverage industry as highlighted in Figure 3. Its high content of anthocyanins can be exploited and used as a natural colourant for beverages like milk- and yogurt-based drinks as well as in the production of hibiscus wine. The application of *H. sabdariffa* in food processing can promote the manufacture of novel functional foods that may ultimately provide health benefit at low cost (Patel, 2014).

References

- Ademiluyi, A.O. and Oboh, G. (2013). Aqueous extracts of Roselle (*Hibiscus sabdariffa* Linn.) varieties inhibit α -amylase and α -glucosidase activities in vitro. *Journal of Medicinal Food*, 16(1): 88-93.
- Adisakwattana, S., Ruengsamran, T., Kampa, P. and Sompong, W. (2012). In vitro inhibitory effects of plant-based foods and their combinations on intestinal α -glucosidase and pancreatic α -amylase. *BMC Complementary and Alternative Medicine* 12(1): 110.
- Ajay, M., Chai, H., Mustafa, A., Gilani, A.H. and Mustafa, M. R. (2007). Mechanisms of the anti-hypertensive effect of *Hibiscus sabdariffa* L. calyces. *Journal of Ethnopharmacology* 109(3): 388-393.
- Ali, B.H., Wabel, N.A. and Blunden, G. (2005). Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: a review. *Phytotherapy Research* 19(5): 369-375.
- Atta, B.M. and Imaizumi, K. (2002). Some Characteristics of Crude Oil Extracted from Roselle (*Hibiscus sabdariffa* L.) Seeds Cultivated in Egypt. *Journal of Oleo Science* 51(7), 457-461. doi:10.5650/jos.51.457
- Aurelio, D.L., Edgardo, R.G. and Navarro-Galindo, S. (2008). Thermal kinetic degradation of anthocyanins in a roselle (*Hibiscus sabdariffa* L. cv. 'Criollo') infusion. *International Journal of Food Science and Technology* 43(2): 322-325.
- Borrás-Linares, I., Fernández-Arroyo, S., Arráez-Roman, D., Palmeros-Suárez, P.A., Del Val-Díaz, R., Andrade-González, I., . . . Segura-Carretero, A. (2015). Characterization of phenolic compounds, anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (*Hibiscus sabdariffa*). *Industrial Crops and Products* 69(0): 385-394. doi:<http://dx.doi.org/10.1016/j.indcrop.2015.02.053>
- Buogo, G. and Picchinenna, D. (1937). Chemical characteristics of Roselle hemp. *Annali Di Chimica Applicata* 27: 577-582.
- Carvajal-Zarrabal, O., Hayward-Jones, P., Orta-Flores, Z., Nolasco-Hipólito, C., Barradas-Dermitz, D., Aguilar-Uscanga, M., and Pedroza-Hernández, M. (2009). Effect of *Hibiscus sabdariffa* L. dried calyx ethanol extract on fat absorption-excretion, and body weight implication in rats. *BioMed Research International* (2009): 1-5.
- Chen, S.H., Huang, T.C., Ho, C.T. and Tsai, P.J. (1998). Extraction, analysis, and study on the volatiles in roselle tea. *Journal of Agricultural and Food Chemistry* 46(3): 1101-1105.
- Clifford, M. N., Johnston, K. L., Knight, S. and Kuhnert, N. (2003). Hierarchical scheme for LC-MS n identification of chlorogenic acids. *Journal of Agricultural and Food Chemistry* 51(10): 2900-2911.

- Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., & Heinrich, M. (2014). Hibiscus sabdariffa L. – A phytochemical and pharmacological review. *Food Chemistry* 165: 424-443. doi:<http://dx.doi.org/10.1016/j.foodchem.2014.05.002>
- Du, C. and Francis, F. (1973). Anthocyanins of roselle (Hibiscus sabdariffa, L.). *Journal of Food Science* 38(5), 810-812.
- Duke, J. (1983). Hibiscus sabdariffa L. *Handbook of Energy Crops*. CRC Press Florida, USA, pp 22
- El-Hamidi, A., Saleh, M. and Ahmed, S. (1967). *Hibiscus sabdariffa*. *Chemical Abstracts* 66: 73243 d.
- Fernandez-Arroyo, S., Herranz-Lopez, M., Beltran-Debon, R., Borrás-Linares, I., Barrajón-Catalán, E., Joven, J., . . . Micol, V. (2012). Bioavailability study of a polyphenol-enriched extract from Hibiscus sabdariffa in rats and associated antioxidant status. *Molecular Nutrition and Food Research* 56(10): 1590-1595. doi:10.1002/mnfr.201200091
- Fernandez-Arroyo, S., Rodríguez-Medina, I.C., Beltran-Debon, R., Pasini, F., Joven, J., Micol, V., . . . Fernandez-Gutierrez, A. (2011). Quantification of the polyphenolic fraction and in vitro antioxidant and in vivo anti-hyperlipemic activities of Hibiscus sabdariffa aqueous extract. *Food Research International* 44(5): 1490-1495. doi:10.1016/j.foodres.2011.03.040
- Gonzalez-Palomares, S., Estarrón-Espinosa, M., Gómez-Leyva, J.F., and Andrade-González, I. (2009). Effect of the temperature on the spray drying of roselle extracts (Hibiscus sabdariffa L.). *Plant Foods for Human Nutrition*, 64(1): 62-67.
- Hernández-Pérez, F. and Herrera-Arellano, A. (2011). Therapeutic use Hibiscus sabdariffa extract in the treatment of hypercholesterolemia. A randomized clinical trial. *Revista Médica del Instituto Mexicano del Seguro Social* 49(5): 469-480.
- Herrera-Arellano, A., Flores-Romero, S., Chávez-Soto, M. A., & Tortoriello, J. (2004). Effectiveness and tolerability of a standardized extract from Hibiscus sabdariffa in patients with mild to moderate hypertension: a controlled and randomized clinical trial. *Phytomedicine*, 11(5), 375-382. doi:<http://dx.doi.org/10.1016/j.phymed.2004.04.001>
- Hopkins, A.L., Lamm, M.G., Funk, J.L. and Ritenbaugh, C. (2013). Hibiscus sabdariffa L. in the treatment of hypertension and hyperlipidemia: A comprehensive review of animal and human studies. *Fitoterapia* 85(0): 84-94. doi:<http://dx.doi.org/10.1016/j.fitote.2013.01.003>
- Inuwa, I., Ali, B.H., Al-Lawati, I., Beegam, S., Ziada, A. and Blunden, G. (2012). Long-term ingestion of Hibiscus sabdariffa calyx extract enhances myocardial capillarization in the spontaneously hypertensive rat. *Experimental Biology and Medicine* 237(5): 563-569.
- Ismail, A., Ikram, E.H.K. and Nazri, H.S.M. (2008). Roselle (Hibiscus sabdariffa L.) Seeds-nutritional composition, protein quality and health benefits. *Food* 2(1): 1-16.
- Jiménez-Ferrer, E., Alarcón-Alonso, J., Aguilar-Rojas, A., Zamilpa, A., Jiménez-Ferrer, C., Tortoriello, J. and Herrera-Ruiz, M. (2012). Diuretic effect of compounds from Hibiscus sabdariffa by modulation of the aldosterone activity. *Planta Medica* 78(18): 1893-1898.
- Jung, E.K., Kim, Y.J. and Joo, N. (2013). Physicochemical properties and antimicrobial activity of Roselle (Hibiscus sabdariffa L.). *Journal of the Science of Food and Agriculture* 93(15): 3769-3776. doi:10.1002/jsfa.6256
- Lin, H.H., Chan, K.C., Sheu, J.Y., Hsuan, S.W., Wang, C.J. and Chen, J.H. (2012). Hibiscus sabdariffa leaf induces apoptosis of human prostate cancer cells in vitro and in vivo. *Food chemistry* 132(2): 880-891.

- Lin, H.H., Chen, J.H and Wang, C.J. (2011). Chemopreventive Properties and Molecular Mechanisms of the Bioactive Compounds in Hibiscus Sabdariffa Linne. *Current Medicinal Chemistry* 18(8): 1245-1254.
- Mahadevan, N., Shivali, K.P. and Kamboj, P. (2009). Hibiscus sabdariffa Linn: an overview. *Natural Product Radiance* 8(1): 77-83.
- McKay, D.L., Chen, C.O., Saltzman, E. and Blumberg, J.B. (2009). Hibiscus Sabdariffa L. Tea (Tisane) Lowers Blood Pressure in Prehypertensive and Mildly Hypertensive Adults–4. *The Journal of Nutrition* 140(2): 298-303.
- Mohamed, B.B., Sulaiman, A.A. and Dahab, A.A. (2012). Roselle (Hibiscus sabdariffa L.) in Sudan, cultivation and their uses. *Bulletin of Environment, Pharmacology and Life Sciences* 1(6): 48-54.
- Morton, J.F. (1987). *Fruits of Warm Climates*: Published by Julia F. Morton 20534 SW 92 Ct. Miami, Florida, USA. 33189 ISBN: 0-9610184-1-0.
- Omobuwajo, T.O., Sanni, L.A. and Balami, Y.A. (2000). Physical properties of sorrel (Hibiscus sabdariffa) seeds. *Journal of Food Engineering* 45(1): 37-41. doi:[http://dx.doi.org/10.1016/S0260-8774\(00\)00039-X](http://dx.doi.org/10.1016/S0260-8774(00)00039-X)
- Patel, S. (2014). Hibiscus sabdariffa: An ideal yet under-exploited candidate for nutraceutical applications. *Biomedicine and Preventive Nutrition* 4(1): 23-27. doi:<http://dx.doi.org/10.1016/j.bionut.2013.10.004>
- Peng, C.H., Chyau, C.C., Chan, K.C., Chan, T.H., Wang, C.J. and Huang, C.-N. (2011). Hibiscus sabdariffa polyphenolic extract inhibits hyperglycemia, hyperlipidemia, and glycation-oxidative stress while improving insulin resistance. *Journal of Agricultural and Food Chemistry* 59(18): 9901-9909.
- Ramírez-Rodrigues, M.M., Balaban, M., Marshall, M., & Rouseff, R. (2011). Hot and cold water infusion aroma profiles of Hibiscus sabdariffa: fresh compared with dried. *Journal of Food Science* 76(2): C212-C217.
- Ramirez-Rodrigues, M.M., Plaza, M.L., Azeredo, A., Balaban, M.O. and Marshall, M.R. (2011). Physicochemical and Phytochemical Properties of Cold and Hot Water Extraction from Hibiscus sabdariffa. *Journal of Food Science* 76(3): C428-C435. doi:10.1111/j.1750-3841.2011.02091.x
- Ramirez-Rodrigues, M.M., Plaza, M.L., Azeredo, A., Balaban, M.O. and Marshall, M.R. (2012). Phytochemical, sensory attributes and aroma stability of dense phase carbon dioxide processed Hibiscus sabdariffa beverage during storage. *Food Chemistry* 134(3): 1425-1431. doi:10.1016/j.foodchem.2012.03.042
- Ramirez, M.M., Wysocki, A.F., Ramirez, M.A., Sims, C.A. and Balaban, M.O. (2010). Sensory and Marketing Characteristics of a Hibiscus Beverage. *Journal of Food Distribution Research* 41(3): 52-62.
- Rodrigues, M.M.R. (2010). *Processing Hibiscus Beverage Using Dense Phase Carbon Dioxide*. University of Florida, Florida.
- Rodriguez-Medina, I.C., Beltran-Debon, R., Molina, V.M., Alonso-Villaverde, C., Joven, J., Menendez, J. A., . . . Fernandez-Gutierrez, A. (2009). Direct characterization of aqueous extract of Hibiscus sabdariffa using HPLC with diode array detection coupled to ESI and ion trap MS. *Journal of Separation Science* 32(20): 3441-3448. doi:10.1002/jssc.200900298
- Roethenbaugh, G. (2005). Trends in beverage markets. *Chemistry and Technology of Soft Drinks and Fruit Juices*, Second Edition, Blackwell Publishing Ltd, New Jersey, USA Pp. 15-34.

- Sabzghabae, A. M., Ataei, E., Kelishadi, R., Ghannadi, A., Soltani, R., Badri, S., & Shirani, S. (2013). Effect of Hibiscus sabdariffa calices on dyslipidemia in obese adolescents: a triple-masked randomized controlled trial. *Materia Socio-Medica* 25(2): 76.
- Salah, A., Gathumbi, J., & Vierling, W. (2002). Inhibition of intestinal motility by methanol extracts of Hibiscus sabdariffa L.(Malvaceae) in rats. *Phytotherapy Research* 16(3): 283-285.
- Segura-Carretero, A., Puertas-Mejía, M.A., Cortacero-Ramírez, S., Beltrán, R., Alonso-Villaverde, C., Joven, J., . . . Fernández-Gutiérrez, A. (2008). Selective extraction, separation, and identification of anthocyanins from Hibiscus sabdariffa L. using solid phase extraction-capillary electrophoresis-mass spectrometry (time-of-flight/ion trap). *Electrophoresis* 29(13): 2852-2861.
- Sindi, H. A., Marshall, L. J. and Morgan, M.R.A. (2014). Comparative chemical and biochemical analysis of extracts of Hibiscus sabdariffa. *Food Chemistry* 164: 23-29.
doi:<http://dx.doi.org/10.1016/j.foodchem.2014.04.097>
- Tsai, P.J., McIntosh, J., Pearce, P., Camden, B. and Jordan, B.R. (2002). Anthocyanin and antioxidant capacity in Roselle (Hibiscus Sabdariffa L.) extract. *Food Research International* 35(4): 351-356.
doi:[http://dx.doi.org/10.1016/S0963-9969\(01\)00129-6](http://dx.doi.org/10.1016/S0963-9969(01)00129-6)
- Wong, P.K., Yusof, S., Ghazali, H. and Che Man, Y. (2002). Physico-chemical characteristics of roselle (Hibiscus sabdariffa L.). *Nutrition and Food Science* 32(2): 68-73.
- Yamamoto, R. and Osima, Y. (1932). On the Red Colouring Matter of Hibiscus Sabdariffa L: (A New Glycoside Hivistin.). *Journal of the Agricultural Chemical Society of Japan* 8(10-12): 142-149.
- Yang, M.Y., Peng, C.H., Chan, K.C., Yang, Y.S., Huang, C.N. and Wang, C.J. (2009). The hypolipidemic effect of Hibiscus sabdariffa polyphenols via inhibiting lipogenesis and promoting hepatic lipid clearance. *Journal of Agricultural and Food Chemistry* 58(2): 850-859.