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Review

# *Hibiscus sabdariffa* L. – A phytochemical and pharmacological review

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ABSTRACT

*Hibiscus sabdariffa* L. (Hs, roselle; Malvaceae) has been used traditionally as a food, in herbal drinks, in hot and cold beverages, as a flavouring agent in the food industry and as a herbal medicine. *In vitro* and *in vivo* studies as well as some clinical trials provide some evidence mostly for phytochemically poorly characterised Hs extracts. Extracts showed antibacterial, anti-oxidant, nephro- and hepato-protective, renal/diuretic effect, effects on lipid metabolism (anti-cholesterol), anti-diabetic and anti-hypertensive effects among others. This might be linked to strong antioxidant activities, inhibition of α-glucosidase and α-amylase, inhibition of angiotensin-converting enzymes (ACE), and direct vaso-relaxant effect or calcium channel modulation. Phenolic acids (esp. protocatechuic acid), organic acid (hydroxycitric acid and hibiscus acid) and anthocyanins (delphinidin-3-sambubioside and cyanidin-3-sambubioside) are likely to contribute to the reported effects.

More well designed controlled clinical trials are needed which use phytochemically characterised preparations. Hs has an excellent safety and tolerability record.



**Contents**



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## 1. Introduction

*Hibiscus sabdariffa* L. (*Hs*), also known as roselle, is an ideal crop for developing countries as it is relatively easy to grow, can be grown as part of multi-cropping systems and can be used as food and fibre. In China the seeds are used for their oil and the plant is used for its medicinal properties, while in West Africa the leaves and powdered seeds are used in meals. Additionally, it is used in the pharmaceutical and food industries.

A limited number of reviews on Hs have been conducted. Only one detailed review on the phytochemical, pharmacological and toxicological properties of *Hs* (Ali, Al Wabel, & Blunden, 2005) and two more focused, later reviews are available: One on the effectiveness of *Hs* in the treatment of hypertension (Wahabi, Alansary, Al-Sabban, & Glasziuo, 2010) and another on the treatment of hypertension and hyperlipidemia (Hopkins, Lamm, Funk, & Ritenbaugh, 2013). This review will focus not only on the phytochemistry and pharmacological properties of Hs in more detail, but also on economic-botanical aspects of *Hs,* its scientific applications and translational research.

## 2. Botanical description

The genus *Hibiscus* (Malvaceae) includes more than 300 species of annual or perennial herbs, shrubs or trees (Wang, Morris, Tonnis, Davis, & Pederson, 2012). *Hs* (syn.: *Abelmoschus cruentus* (Bertol.) Walp., *Furcaria sabdariffa* Ulbr., *Hibiscus cruentus* Bertol., *Hibiscus fraternus* L., *Hibiscus palmatilobus* Baill. and *Sabdariffa rubra* Kostel (The Plant list, 2010) is commonly known as roselle, hibiscus, Jamaica sorrel or red sorrel (English) and in Arabic, karkadeh (Ali et al., 2005; Ross, 2003). Its native distribution is uncertain, some believe that is from India or Saudi Arabia (Ismail, Ikram, & Nazri, 2008), while Murdock (Murdock, 1959) showed evidence that *Hs* was domesticated by the black populations of western Sudan (Africa) sometime before 4000 BC. Nowadays, it is widely cultivated in both tropical and subtropical regions (Morton, 1987; USDA, 2007) including India, Saudi Arabia, China, Malaysia, Indonesia, The Philippines, Vietnam, Sudan, Egypt, Nigeria and México (Chewonarin et al., 1999; Dung et al., 1999; Eslaminejad & Zakaria, 2011; Ismail, Ikram, & Nazri, 2008; Mahran, El-Hossary, & El-Labban, 1979; Rao, 1996; Sharaf, 1962; Yagoub Ael, Mohamed, Ahmed, & El Tinay, 2004).

There are two main varieties of *Hs*, the first being *Hs* var. *altissima* Wester, cultivated for its jute-like fibre and the second is *Hs* var. *sabdariffa.* The second variety includes shorter bushy forms, which have been described as races: *bhagalpuriensi*, *intermedius*, *albus* and *ruber*. The first variety has green, red-streaked, inedible calyces, while the second and third race have yellow-green edible calyces (var. *ruber*) and also yield fibre (Morton, 1987).

### 2.1. Morphology

*Hs var. sabdariffa* ruber is an annual, erect, bushy, herbaceous subshrub that can grow up to 8 ft (2.4 m) tall, with smooth or nearly smooth, cylindrical, typically red stems. The leaves are alternate, 3 to 5 in (7.5–12.5 cm) long, green with reddish veins and long or short petioles. The leaves of young seedlings and upper leaves of older plants are simple; lower leaves are deeply 3 to 5 or even 7 lobed; the margins are toothed. Flowers, borne singly in the leaf axils, are up to 5 in (12.5 cm) wide, yellow or buff with a rose or maroon eye, and turn pink as they wither at the end of the day. At this time, the typically red calyx, consisting 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 but juicy, 1 1/4 to 2 1/4

in (3.2–5.7 cm) long and fully encloses the velvety capsule, 1/2 to 3/4 in (1.25–2 cm) long, which is green when immature, 5-valved, with each valve containing 3 to 4 kidney-shaped, light-brown seeds, 1/8 to 3/16 in (3–5 mm) long and minutely downy. The capsule turns brown and splits open when mature and dry. The calyx, stems and leaves are acid and closely resemble the cranberry (*Vaccinium* spp.) in flavour (Morton, 1987; Ross, 2003).

### 2.2. Ecology/cultivation

*Hs* is easy to grow in most well drained soils but can tolerate poor soils. It requires 4-8 months growth with night-time temperatures with a minimum of 20 °C, as well as 13 h of sunlight and a monthly rainfall ranging from 5–10″ (130–250 mm) during the first few months to prevent premature flowering. Rain or high humidity during the harvest time and drying process can downgrade the quality of the calyces and reduce the yield. The quality of *Hs* is determined by seed stock, local growing conditions, time of harvest, post-harvest handling and mainly the drying step. Most of the time it grows as a supplement crop and it is susceptible to fungi, viral and bacterial attack and also to insects. A single plant produces about 1.5 kg of fruit, approximately 8 t/ha. Yields of leaves may be about 10 t/ha (EcoCrop., 2007; Plotto, 2004).

### 2.3. Karyotype

$2n = 36$ (Huang, Zhao, Chen, Chen, & Huang, 1989; Menzel & Wilson, 1961) and 72 (Chennaveeraiah & Subbarao, 1965; Rao, 1935; Wilson & Menzel, 1964) were observed. Somatic tissue showing diploid and tetraploid segments were also occasionally noticed (Tjio, 1948). In a karyomorphological study conducted in India, both root and flower segments showed great similarity in the types of chromosomes in the complement. This indicates that the tetraploid tissue must have arisen in an autotetraploid manner (Bhatt & Dasgupta, 1976). Later, this species was reported to be tetraploid ($2n = 72$) (Hiron, Alam, Ahmed, Begum, & Alam, 2006).

## 3. Uses – economic botany

### 3.1. Traditional culinary use

Fresh or dried calyces of *H. sabdariffa* (c*Hs*) are used in the preparation of herbal drinks, hot and cold beverages, fermented drinks, wine, jam, jellied confectionaries, ice cream, chocolates, flavouring agents, puddings and cakes (Bako, Mabrouk, & Abubakar, 2009; Bolade, Oluwalana, & Ojo, 2009; Esselen & Sammy, 1975; Ismail, Ikram, & Nazri, 2008; Okoro, 2007; Plotto, 2004; Rao, 1996; Tsai, McIntosh, Pearce, Camden, & Jordan, 2002; Wilson & Menzel, 1964). In Egypt, the fleshy calyces are used in making "cacody tea" and fermented drinks (Kochhar, 1986), while in Sudan and Nigeria, the calyces are boiled with sugar to produce a drink known as "Karkade" or "Zoborodo" (Gibbon & Pain, 1985). In Mexico this drink is called Jamaica or "*agua de Jamaica*"' or "*té de Jamaica*". In the West Indies the calyces can also be used as colouring and flavouring ingredient in rum (Ismail, Ikram, & Nazri, 2008).

The seeds are eaten roasted or ground in meals, while the leaves and shoots are eaten raw or cooked, or as a sour-flavoured vegetable or condiment (Wilson & Menzel, 1964). In Sudan, the leaves are eaten green or dried, cooked with onions and groundnuts, while in Malaysia the cooked leaves are eaten as vegetables (Ismail, Ikram, & Nazri, 2008). In Africa, the seeds are roasted or ground into powder and used in meals, suh as oily soups and sauces. In China and West Africa, the seeds are also used for their oil (Atta & Imaizumi, 2002). Another use for the seed is as a substitute for coffee (Morton, 1987).

### 3.2. Use in local and traditional food and medicine

*Hs* has been widely used in local medicines. In India, Africa and Mexico, infusions of the leaves or calyces are traditionally used for their diuretic, cholerectic, febrifugal and hypotensive effects, decreasing the viscosity of the blood and stimulating intestinal peristalsis. It is also recommended as a hypotensive in Senegal (Morton, 1987). In Egypt, preparations from the calyces have been used to treat cardiac and nerve diseases and also to increase the production of urine (diuresis). In Egypt and Sudan, an infusion of "Karkade" calyces is also used to help lower body temperature (Leung, 1996). In Guatemala it is used for treating drunkenness (Morton, 1987). In North Africa, calyces preparations are used to treat sore throats and coughs, as well as genital problems, while the emollient leaf pulp is used for treating external wounds and abscesses (Neuwinger, 2000). In India, a decoction from the seeds is used to relieve pain in urination and indigestion. In Brazil, the roots are believed to have stomachic and emollient properties. In Chinese folk medicine, it is used to treat liver disorders and high blood pressure (Morton, 1987). In Iran, sour hibiscus tea is reportedly a traditional treatment for hypertension (Burnham, Wickersham, & Novak, 2002), while in Nigeria the decoction of the seeds is traditionally used to enhance or induce lactation in cases of poor milk production, poor letdown and maternal mortality (Gaya, Mohammad, Suleiman, Maje, & Adekunle, 2009).

## 4. Other uses

### 4.1. Source of fibre

*Hs* is one of the most important species grown commercially as a fibre plant and became increasingly important in India after independence and partition with Pakistan, where the most important jute (*Corchorus capsularis* L. or *Corchorus olitorius* L.) growing areas are. It is used as a jute substitute in making clothing, linen, fishing nets, ropes and similar items (Clydesdale, Main, & Francis, 1979). Despite the fact that this species is slow growing, as it requires about 180 days to produce a satisfactory yield of fibre, there is still interest in the plant as some varieties of *Hs* (not edible but fibre type) have a high degree of genetic resistance to root-knot nematodes. The main disadvantages of growing *Hs* in comparison with other *Hibiscus* species is:

(1) The slow growth rate which increases costs in weed control and land occupation by the crop.
(2) The difficulty of separating the ribboning stalks from the bark when compared to, for example, *H. cannabinus* (Wilson & Menzel, 1964).

While the world's production of Kenaf fibres (*H. cannabinus*) has reached 272,000 tons in 2008, Hs fibres have not gained the same economic importance (http://www.naturalfibersinfo.org/natural-fibers/kenaf/, accessed 09/11/13).

However, Hs fibres are subject to ongoing research showing promising technical properties when used as a substitute for synthetic or mineral fibres in composite materials, as well as a source material for high quality paper production (Dutt, Upadhyaya, & Tyagi, 2010; Kumar, Dutt, & Bharti, 2013; Singha & Kumar, 2008).

### 4.2. Animal feed

The leaves are used for animal fodder and fibre (Plotto, 2004). The seeds can be used to feed poultry as well as sheep and the residue from the seeds oil extraction can also be used to feed cattle and chicks (Al-Wandawi, Al-Shaikhly, & Abdul-Rahman, 1984;

Elamin, Hassan, Abdalla, Arabi, & Tameem Eldar, 2012; Morton, 1987; Mukhtar, 2007).

### 4.3. Cosmetic

In Malaysia the oil is used to produce scrubs and soaps (Ismail, Ikram, & Nazri, 2008).

### 4.4. The current importance of H. sabdariffa

Besides its importance as a food or traditional medicine in the countries of its geographic origin, hibiscus flower is traded and used worldwide today as an important ingredient in industrially produced teas and beverages. The United States and Germany are the primary markets for dried *cHs*. England satisfies most of its consumers demands by importing herbal teas form Germany (Plotto, 2004). Statistics for the volume and value of dried hibiscus imported into these markets were not available, but the major clients for hibiscus importers are herbal teas manufactures, as this plant is used as base in many herbal/fruit teas, along with apple peel, orange peel and lemon twist. (McCaleb, 2000; Plotto, 2004).

### 4.5. Economical–botanical aspects

Hibiscus is available from China, Thailand, Sudan, Mexico and some other countries with smaller suppliers like Egypt, Senegal, Tanzania, Mali and Jamaica.

Hibiscus quality strongly depends on geographic origin. The most desirable product is from Thailand and Sudan; however the main world suppliers are China and Thailand. The best *Hs* grows in Sudan, however its quality is subsequently often impaired due to poor processing. Thailand has invested greatly in Hibiscus production contrary to China, where the product is less reliable and reputable due to less stringent quality control practices. However, China is the dominant supplier of the United States market (Cooper, 1993; McCaleb, 2000; Mohamed, Sulaiman, & Dahab, 2012; Plotto, 2004).

### 4.6. FairTrade certified and organic certified

Until very recently, the Arab Republic of Egypt was the only source country for certified and organic certified hibiscus flower. In 2011 a number of producers and traders from Burkina Faso achieved certification through the FairTrade Labelling Organisations International FLO-CERT GmbH (http://www.flo-cert.net) (Brinckmann, 2011).

## 5. Phytochemistry

### 5.1. Nutritional value

The nutritional composition of fresh c*Hs* varies between studies, probably due to different varieties, genetic, environmental, ecology and harvest conditions of the plant. Early studies reported that *cHs* contains protein (1.9 g/100 g), fat (0.1 g/100 g), carbohydrates (12.3 g/100 g) and fibre (2.3 g/100 g). They are rich in vitamin C (14 mg/100 g), β-carotene (300 μg/100 g), calcium (1.72 mg/100 g) and iron (57 mg/100 g) (Ismail, Ikram, & Nazri, 2008).

The leaves contain protein (3.3 g/100 g), fat (0.3 g/100 g), carbohydrate (9.2 g/100 g), minerals (phosphorus (214 mg/100 g), iron (4.8 mg/100 g) thiamine (0.45 mg/100 g), β-carotene (4135 μg/100 g), riboflavin (0.45 mg/100 g) and ascorbic acid (54 mg/100 g) (Ismail, Ikram, & Nazri, 2008).

The seeds contained crude fatty oil (21.85%), crude protein (27.78%), carbohydrate (21.25%), crude fibre (16.44%) and ash (6.2%). In terms of minerals, the most prevalent is potassium (1329 ± 1.47 mg/100 g), followed by sodium (659 ± 1.58 mg/100 g), calcium (647 ± 1.21 mg/100 g), phosphorus (510 ± 1.58 mg/100 g) and magnesium (442.8 ± 1.80 mg/100 g). The major saturated fatty acids identified in the seed oil are palmitic (20.84%) and stearic (5.88%) acids and the main unsaturated fatty acids are linoleic (39.31%) and oleic acid (32.06%) (Nzikou et al., 2011).

### 5.2. Bioactive constituents

The main constituents of *H. sabdariffa* relevant in the context of its pharmacological are organic acids, anthocyanins, polysaccharides and flavonoids (Eggensperger & Wilker, 1996; Müller & Franz, 1990).

### 5.3. Organic acids

*Hs* extracts contain a high percentage of organic acids, including citric acid, hydroxycitric acid, hibiscus acid, malic and tartaric acids as major compounds, and oxalic and ascorbic acid as minor compounds. Based on previous studies, the percentage of organic acids in "hibisci flos" varies; hibiscus acid accounts for 13–24%, citric acid 12–20%, malic acid 2–9%, tartaric acid 8% and 0.02–0.05% of ascorbic acid (vitamin C) (Eggensperger & Wilker, 1996; Schilcher, 1976).

In the late 1930s, citric and malic acids were first reported in aqueous extracts of the calyx (Buogo & Picchinenna, 1937; Indovina & Capotummino, 1938; Reaubourg & Monceaux, 1940) and also in five different strains (from Egypt, Senegal, India, Thailand and Central America) of *Hs* var. *sabdariffa* (Khafaga, Koch, El Afry, & Prinz, 1980). Ascorbic acid is also present in *cHs* but its content varies dramatically between fresh (6.7–14 mg/100 g (Ismail, Ikram, & Nazri, 2008; Morton, 1987)) and dried calyces (260–280 mg/100 g (Ismail, Ikram, & Nazri, 2008)). The amount of ascorbic acid in the latter report being much higher than the ones previously reported in the literature. The differences observed might be due to different varieties, genetics, environment, ecology and harvest conditions.

#### 5.3.1. Hydroxycitric acid

(Fig. 1) has an additional hydroxyl group at the second carbon of citric acid. This acid has four stereoisomers, (2*S*, 3*S*), (2*R*, 3*R*), (2*S*, 3*R*) and (2*R*, 3*S*), and their lactone forms. The principal organic acid found in the *cHs* is the (2**S**, 3*R*)-hydroxycitric acid (Hida, Yamada, & Yamada, 2007). It is the principal organic acid found in the calyces of Hs. It is worth noting that, (2**S**, 3*R*)-hydroxycitric acid from Hibiscus is different from the more commonly known (2**S**,3**S**)-hydroxycitric acid (HCA) extracted from, e.g., *Garcinia* sp., thus raising the question as to whether both diastereomers have identical or partially different pharmacological profiles.

#### 5.3.2. Hibiscus acid

(Fig. 1) is the lactone form of (+)-*allo*-hydroxycitric acid. It compromises a citric acid moiety with an additional hydroxyl group at the second carbon and has two diastereomers due to the existence of two chiral centers in the molecule (Boll, Sørensen, & Balieu, 1969; Eggensperger & Wilker, 1996; Griebel & Lebensm, 1939, 1942).

Hydroxycitric acid, hibiscus acid and it derivatives as the major organic acids in the leaves and calyces extracts of *Hs* (Beltran-Debon et al., 2010; Herranz-Lopez et al., 2012; Peng et al., 2011; Ramirez-Rodrigues, Balaban, Marshall, & Rouseff, 2011a,b; Rodriguez-Medina et al., 2009).

#### 5.3.3. Anthocyanins

The anthocyanins are a group of flavonoid derivatives and natural pigments present in the dried flowers of *Hs* and their colour varies with pH.

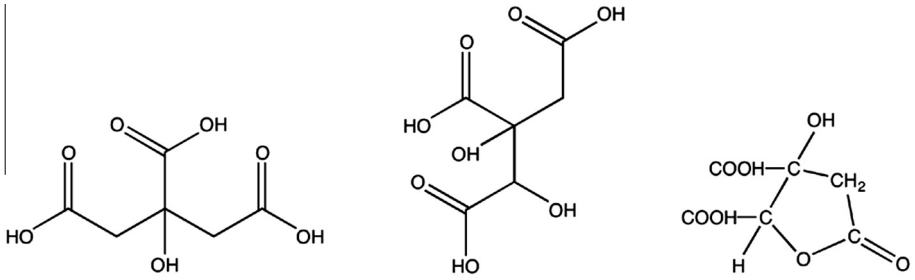


**Fig. 1.** Citric acid and its derivatives.

Delphinidin and cyanidin-based anthocynins, include delphinidin-3-sambubioside (hibiscin), cyanidin-3-sambubioside (gossypicyanin), cyanidin-3,5-diglucoside, delphinidin (anthocyanidin) and others (Williamson, Driver, & Baxter, 2009).

The first anthocyanin from the calyx of Hs to be isolated was "hiviscin", also known as "hibiscin", later named delphinidin-3-sambubioside and assigned the structure of cyanidin-3-glucoside (Yamamoto & Osima, 1932), which was later renamed as delphinidin-pentoside-glucoside (Yamamoto & Osima, 1936). From the pigments of *cHs*, three different anthocyanins were isolated: delphinidin-3-sambubioside (hibiscin), delphinidin-3-glucoside and cyanidin-3-glucoside (chrysanthenin) (Fig. 2) using material from Taiwan and Trinidad (Du & Francis, 1973; Shibata & Furukawa, 1969). The last study also identified cyanidin-3-sambubioside (gossypicyanin) (Fig. 2). Later, the presence of cyanidin-3,5-diglucoside and cyanidin-3-(2G-glucosylrutinoside) in the flower pigments of *Hs* var. *altissima* (Subramanian & Nair, 1972) was reported. A study conducted with 5 different strains of *Hs* var. *sabdariffa* reported cyanidin-3-sambubioside and cyanidin-3-glucoside as the major compounds present in this plant (Khafaga, Koch, El Afry, & Prinz, 1980). In one of the strains (Senegalese strain), delphinidin glycosides were absent. In this study, the anthocyanin content reached 1.7% to 2.5% of the dry weight in all strains. A similar anthocyanin content was observed in another study where their amount was about 1.5 g per 100 g of dry weight of *cHs*, in terms of delphinidin-3-sambubioside (Du & Francis, 1973).

Several studies have identified delphinidin-3-sambubioside (delphinidin-3-*O*-(2-*O*-β-D-xylopyranosyl)-β-D-glucopyranoise) and cyanidin-3-sambubioside (cyanidin-3-*O*-(2-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside) as the major anthocyanins present in extracts

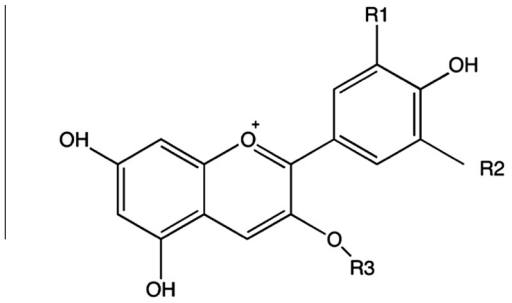


**Fig. 2.** Chemical structures of main anthocyanins.

from *cHs* (Alarcon-Aguilar et al., 2007; Alarcon-Alonso et al., 2012; Beltran-Debon et al., 2010; Degenhardt, Knapp, & Winterhalter, 2000; Herranz-Lopez et al., 2012; Peng et al., 2011) and leaves (Rodriguez-Medina et al., 2009).

*5.3.4. Flavonoids*

*Hs* contain polyphenols of the flavonol and flavanol type in simple or polymerised form. The following flavonoids have been described in *Hs* extracts: hibiscitrin (hibiscetin-3-glucoside), sabdaritrin, gossypitrin, gossytrin and other gossypetin glucosides, quercetin and luteolin (McKay, 2009; Williamson et al., 2009); as well as chlorogenic acid, protocatechuic acid, pelargonidic acid, eugenol, quercetin, luteolin and the sterols β-sitosterol and ergosterol (McKay, 2009; Williamson et al., 2009).

Earlier the flowers of *Hs* were recorded to contain 3-monoglucoside of hibiscetin (hibiscitrin) (Rao & Seshadri, 1942a,b, 1948), 7-glucoside of gossypetin (gossypitrin) and sabdaritrin, which on acid hydrolysis yielded an hydroxyflavone named sabdaretin (Rao & Seshadri, 1942a,b). The presence of these flavonol glycosides was low, with hibiscitrin being the major compound followed by gossypitrin and sabdaritrin (Rao & Seshadri, 1942a,b). In 1961, gossypetin-3-glucoside (gossytrin) was isolated (Seshadri & Thakur, 1961). The petals of *Hs* var. altissima also contain gossypetin-8-glucoside (0.4%) and gossypetin-7-glucoside (Subramanian & Nair, 1972).

From the leaves of *Hs*, β-sitosteryl-β-D-galactoside (Osman, El-Garby-Younes, & Mokhtar, 1975) and from the seeds ergosterol (Salama & Ibrahim, 1979) were reported. β-sitosterol and ergosterol were also reported in *Hs* extracts (McKay, 2009; Williamson et al., 2009).

The methanolic extract of the flowers also contains quercetin, luteolin and its glycoside (Salah, Gathumbi, & Vierling, 2002). Quercetin had already been identified in *Hs* (Takeda & Yasui, 1985). One study reported that the amount of quercetin present in *cHs* WE was 3.2 mg/g while rutin was 2.1 mg/g (Alarcon-Alonso et al., 2012). Quercetin and its conjugated glycosides (quercetin-3-glucoside), as well as, rutin (quercetin-3-rutinoside; Fig. 3) were frequently identified in *cHs* WE, alongside with kaempferol (Beltran-Debon et al., 2010; Herranz-Lopez et al., 2012; Peng et al., 2011; Ramirez-Rodrigues, Balaban, Marshall, & Rouseff, 2011a,b).

The water extract of the dried leaves showed the presence of catechin (4.25%) and ellagic acid (28.20%) (Lin et al., 2012), while *cHs* WE showed the presence of protocatechuic acid (24.24%), catechin (2.67%), gallocatechin (2.44%), caffeic acid (19.85%), gallocatechin gallate (27.98%) (Yang et al., 2010). Similar results were reported by Huang and co-workers (Huang et al., 2009).

Phenolic acid: Protocatechuic acid (PCA) is an important phenolic acid present in *Hs* extract (Fig. 4) (Lee et al., 2002; 2003; McKay,

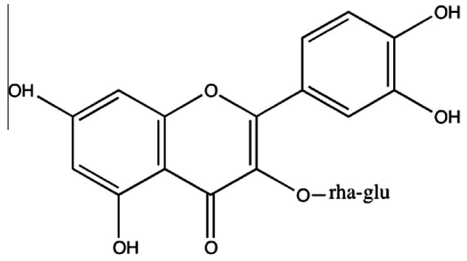


**Fig. 3.** Quercetin-3-rutinoside.

2009; Williamson et al., 2009). It was isolated from the dried flowers of *Hs* and assigned the structure of 3,4-dihydrobenzoic acid) (Osman, El-Garby-Younes, & Mokhtar, 1975; Tseng, Wang, Kao, & Chu, 1996).

Chlorogenic acid is another phenolic acid present in both leaf and *cHs* extracts and belongs to a family of esters formed between certain *trans*-cinnamic acids (caffeic acid, ferulic acid and *p*-coumaric acid) and quinic acid (Clifford, Johnston, Knight, & Kuhnert, 2003). Several studies reported the presence of this acid and its derivatives in extracts of *cHs* (Beltran-Debon et al., 2010; Herranz-Lopez et al., 2012; Peng et al., 2011; Ramirez-Rodrigues, Balaban, Marshall, & Rouseff, 2011a,b; Salah et al., 2002) and leaves (Rodriguez-Medina et al., 2009). In one study, the amount of chlorogenic acid in the extract was reported to be 2.7 mg/g (Alarcon-Alonso et al., 2012).

### 5.3.5. Mucilage, pectin and carbohydrates (polysaccharides)

Polysaccharides are another key group of compounds present in large quantities in the *cHs* WE. In one study, the ethanol-precipitated water extract yielded 10% of reddish polysaccharides. The following compounds were identified in two different fractions, arabinose, galactose, glucose, rhamnose and smaller amounts of galacturonic acid, glucuronic acid, manose and xylose (Müller, Kraus, & Franz, 1989). Similar results were obtained in two other studies (Brunold et al., 2004; Müller & Franz, 1992).

The mucilage content was determined in the calyces of five strains of *Hs* var. *sabdariffa*, reaching 24–28% in strains from Central America and Egypt but only 15% in an Indian strain. This amount was only reached at a later stage of development in the strains from Senegal and Thailand. The pectin content only accounted for 2–4% while the sugars reached a maximum of 3–5% in these five strains. Mucilage and pectin consisted of 60–80% anhydrouronic acid (Afry, Khafaga, Koch, & Prinz, 1980).

The petals of *Hs* yielded 65% of dry weight of mucilage, which on hydrolysis produced galactose, galacturonic acid and rhamnose, while the leaves only yield 10% (El-Hamidi, Saleh, & Ahmed, 1967; Sengupta & Banik, 2011).

### 5.3.6. Volatile compounds

Volatile compounds are responsible for the aroma of *Hs*. In a study conducted in 1992, more then twenty-five volatile compounds (accounting for less than 8% of total *Hs* seeds composition) were reported in seed oil of *Hs*. They were mainly unsaturated hydrocarbons, alcohols and aldehydes from $C_8$ to $C_{13}$. (Jirovetz et al., 1992) Subsequently, thirty-seven volatile compounds from five different groups from the *cHs* WE were characterised. These compounds included fatty acid derivatives (such as 2-ethylfuran and hexanal), sugar derivatives (furfural and 5-methyl-2-furaldehyde), phenolic derivatives (eugenol), terpenes (such as 1,4-cineole, limonene) and miscellaneous compounds (e.g. acetic acid) (Chen, Huang, Ho, & Tsai, 1998). In another study, the volatile profile was examined in four aqueous extracts from fresh and dried calyx using two different, time–temperature extraction conditions by GC–MS. A total of thirty-two compounds were identified and could be divided into five chemical groups: aldehydes (fourteen compounds), alcohols (ten compounds), ketones (five compounds), terpenes (two compounds) and acids (one compound) (Ramirez-Rodrigues, Balaban, Marshall, & Rouseff, 2011a,b). A total of seven aromatic volatiles were common to all four samples tested (hexanal, 3-octanone, octanal, 1-octen-3-one, nonanal, 2,4-nonadienal (*E*,*E*), and geranylacetone).

The following table (Table 1) shows an overview on the constituents present in *H. sabdariffa* water extract (*cHs* WE), which are relevant for use in herbal teas, detected on RP HPLC coupled with the photodiode array detection (DAD) and ESI-TOF-MS in positive and negative mode.

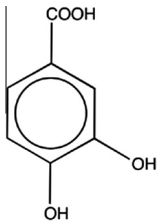


**Fig. 4.** Protocatechuic acid.

**Table 1**
Overview on constituents in *H. sabadriffa* calyces water extract.

| Class | Compound | References |
|---|---|---|
| *Organic acid* | | |
| | Hydroxycitric acid | 1, 4 |
| | Hibiscus acid | 1, 2, 3, 4 |
| | Hibiscus acid glucoside | 3 |
| | Hibiscus acid 6-methyl ester | 2, 3, 4 |
| *Anthocyanins* | | |
| | Delphinidin-3-sambubioside | 1, 2, 4 |
| | Cyanidin-3-sambubioside | 1, 2, 4 |
| *Flavonoids and phenolic acid* | | |
| | Gallic acid | 2, 3 |
| | Chlorogenic acid isomer I | 1 |
| | Chlorogenic acid | 1, 2, 4 |
| | Chlorogenic acid isomer II | 1 |
| | 5-Hydroxymethylfurfural | 2 |
| | Methyl gallate | 4 |
| | 2-*O*-*trans*-Caffeoyl-hydroxicitric acid | 4 |
| | 5-Caffeoylquinic acid | 2, 3 |
| | Myricetin-3-arabinogalactoside | 1, 4 |
| | 3-Caffeoylquinic acid | 3 |
| | Protocatechuic acid | 2 |
| | Protocatechuic acid glucoside | 3 |
| | Coumaroylquinic acid | 4 |
| | Quercetin-3-sambioside | 1, 4 |
| | Quercetin-3-rutinoside | 1, 3, 4 |
| | 5-*O*-Caffeoylshikimic acid | 1, 4 |
| | Leucoside(kaempferol-3-*O*-sambubioside) | 4 |
| | Quercetin-3-glucoside | 1, 4 |
| | Kaempferol-3-*O*-rutinoside | 1, 4 |
| | Feruloyl derivative | 2 |
| | Methyl(AS in Methylepigallocatechin) | 4 |
| | Myricetin | 4 |
| | *N*-Feruloyltyramide | 1, 4 |
| | 4-Caffeoylquinic acid | 2, 3 |
| | Caffeoylquinic acid isomer | 3 |
| | Kaempferol-3-*p*-coumarylglucoside | 1 |
| | Quercetin | 1, 4 |
| | Caffeic acid | 2 |
| | Galloyl ester | 2 |
| | Feruloyl quinic acid derivative | |
| | Kaempferol-3-glucoside | 2 |
| | Quercetin derivative | 2 |
| | Tiliroside | 2 |

Legend: 1 – Beltran-Debon et al. (2010); 2 – Peng et al. (2011); 3 – Ramirez-Rodrigues, Balaban, Marshall, & Rouseff, (2011a,b); 4 – Herranz-Lopez et al. (2012).

It is important to keep in mind that while researching the pharmacological actions of Hs extract (next section), the polyphenol content of the extract was, for some activities, reported as the one responsible for the effect. Nevertheless, the polyphenol content is a very general and often poorly defined term, as it includes a complex mixture of anthocyanins, organic acids, phenolic acids and flavonoid compounds.

## 6. Biological and pharmacological activities

A detailed review of the pharmacological effects of *Hs* extracts is presented in this section with additional information on the studies reviewed in the supplementary data (Supplementary Tables 1–15) section.

### 6.1. Effects on smooth muscles

Early studies showed that the alcoholic extract of *Hs* flowers had an antispasmodic effect by relaxing the uterus and intestine stips *in vitro* (Sharaf, 1962). This was also observed in rabbit aortic smooth muscle (Obiefuna, Owolabi, Adegunloye, Obiefuna, & Sofola, 1994). Interestingly, from various isolated muscle preparations, the extract of *Hs* inhibited the tone of rabbit aortic strip, rhythmically contracting rat uterus, guinea-pig tracheal chain and rat diaphragms, but it stimulated the tone of solated quiescent rat uterus and frog rectus abdominis (Ali, Salih, Mohamed, & Homeida, 1991).

More recently, the *Hs* WE (1–100 mg/kg) was found to inhibit rat bladder and uterine contractibility in a dose dependent manner, but via a mechanism unrelated to local or remote autonomic receptors or calcium channels (Fouda, Daba, & Dahab, 2007) as previously suggested by Salah (Salah et al., 2002).

Later, it was shown that *Hs* crude extracts mainly induced the endothelium-dependent relaxant effect in the isolated thoracic aorta of rats, via stimulation of NOS enzyme by the Pi3-K/Akt pathway. It was suggested that this was due to polyphenols. The non-endothelium dependent relaxation is a direct smooth muscle activation and results in the activation of smooth muscle potassium channels (Sarr et al., 2009).

### 6.2. Antibacterial, antifungal and antiparasitic activity

The *cHs* WE and protocatechuic acid (5 mg/ml) inhibited the growth of methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Liu, Tsao, & Yin, 2005). Moreover, protocatechuic acid (in a dose dependent manner) showed greater antimicrobial activity against these pathogens in broth than in human plasma. The study also revealed that the antibacterial effect was independent from temperature, as shown by a heat treatment. Hibiscus extract also demonstrated antibacterial effect against *Streptococcus mutans*, cariogenic bacteria from the oral cavity, with a minimum inhibitory concentration of 2.5 mg/ml (Afolabi, Ogunsola, & Coker, 2008) and *Campylobacter* species (*Campylobacter jejuni*, *Campylobacter coli* and *Campylobacter fetus*) that contaminates meat like poultry, beef and pork at a concentration range of 96–152 μg/ml (Yin & Chao, 2008). This time, the aqueous-methanol extract of dried *cHs* also showed an *in vitro* inhibitory effect against several bacterial strains, such as *S. aureus*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *Serratia mascences*, *Clostridium sporogenes*, *Escherichia coli*, *K. pneumonia*, *Bacillus cereus* and *Pseudomonas fluorescence*, but did not effect the growth of fungus *Candida albicans* (Olaleye, 2007). The fresh *cHs* WE, ethanol extract and protocatechuic acid (20 mg/ml) was effective in inhibiting the growth of food spoilage bacteria such as *Salmonella typhimurium* DT104, *E. coli* O157:H7, *Listeria monocytogenes*, *S. aureus* and *B. cereus*. Again the antibacterial effect was not affected by heat treatment and the ethanolic extract showed greater antimicrobial effect than the aqueous extract. The study further suggests that both, ethanolic extract and protocatechuic acid, might be potent agents for use as food additives to prevent contamination from these bacteria (Chao & Yin, 2009; Yin & Chao, 2008).

A methanol-water extract of *Hs* was effective against *E. coli* O157:H7 isolates from food, veterinary and clinical samples (Fullerton, Khatiwada, Johnson, Davis, & Williams, 2011), with the highest concentration (10%) being the most effective.

The crude extracts of *Hs* seeds (200 mg/l) also showed antimicrobial effect against three types of Gram-negative bacteria. The extract exhibited higher activity against *Salmonella* followed by *Shigella* and *Enterobacter* (Nwaiwu, Mshelia, & Raufu, 2012).

### 6.3. Antipyretic, antinociceptic and anti-inflammatory activities

Despite the claims that *Hs* is effective in the relief of pyrexia in popular medicines, limited studies are available. The antipyretic and anti-inflammatory potential of the *cHs* extract were studied *in vivo*. The ethanol (more potent) and aqueous extracts showed antipyretic effects by significantly reversing yeast-induced fever in rats. The mechanism is different from the one of aspirin, a prostaglandin inhibitor. Nevertheless, fever entails enhanced formation of cytokines such as interleukins (IL), interferons and tumour necrosis factor-α (TNF-α). The *cHs* extract may be involved in the inhibition of some of these substances, resulting also in an anti-inflammatory effect (Reanmongkol & Itharat, 2007). Similar results were obtained by Dafallah (Dafallah & al-Mustafa, 1996), suggesting that the flavonoids, polysaccharides and organic acids might be the compounds responsible for the pharmacological activity. In a more recent study the ethanolic extract from the calyces also showed antinociceptive effect in a rat model (Ali, Ashraf, Biswas, Karmakar, & Afroz, 2011).

Another *in vivo* study showed that the two fractions of the crude aqueous-ethanolic extract of the dried *cHs* exhibited impressive immunostimulatory activity by increasing the production of IL-10 and decreasing the production of TNF-α (Fakeye, 2008). Another mechanism in which the polyphenol extract exhibit its anti-inflammatory activity is by impairing cyclooxygenase-2 induction by down-regulating JNK and p38 MAPK (Kao et al., 2009).

### 6.4. Clinical studies

A study conducted with 10 healthy volunteers also supports the claim for its anti-inflammatory activity. The ingestion of *cHs* WE (dried) decreased plasma monocyte chemoattractant protein 1 (MCP-1) concentration, a biomarker in the evaluation of inflammatory diseases (Beltran-Debon et al., 2010).

### 6.5. Antioxidant activity

Several studies both *in vitro* (Duh & Yen, 1997; Farombi & Fakoya, 2005; Hirunpanich et al., 2005; Mohd-Esa, Hern, Ismail, & Yee, 2010; Sayago-Ayerdi, Arranz, Serrano, & Goni, 2007; Steenkamp, Fernandes, & van Rensburg, 2004; Tseng et al., 1997) and *in vivo* (Farombi & Fakoya, 2005; Mossalam, Aty, Morgan, Youssaf, & Mackawy, 2011; Olalye & Rocha, 2007; Usoh, Akpan, Etim, & Farombi, 2005) have shown that extracts of *Hs* have a potent antioxidant effect.

The antioxidant activity of the extract is due to its strong scavenging effect on reactive oxygen and free radicals (Farombi & Fakoya, 2005; Mohd-Esa, Hern, Ismail, & Yee, 2010; Olalye & Rocha, 2007; Sayago-Ayerdi, Arranz, Serrano, & Goni, 2007; Tseng et al., 1997; Usoh, Akpan, Etim, & Farombi, 2005), inhibition of

xanthine oxidase activity, protective action against *tert*-butyl hydroperoxide (*t*-BHP)-induced oxidative damage (Tseng et al., 1997), protection of cell from damage by lipid peroxidation (Duh & Yen, 1997; Farombi & Fakoya, 2005; Olalye & Rocha, 2007), inhibition in $Cu^{2+}$-mediated oxidation of LDL and the formation of thiobarbituric acid reactive substances (TBARs) (Hirunpanich et al., 2005; Ochani & D'Mello, 2009; Olalye & Rocha, 2007), inhibition of the formation of malondialdehyde content (100–300 mg/kg) (Farombi & Fakoya, 2005; Usoh, Akpan, Etim, & Farombi, 2005), reduction of glutathione depletion, increase of the liver and decrease blood activity of superoxide dismutase and catalase (Usoh, Akpan, Etim, & Farombi, 2005) while in the liver it increased superoxide dismutase, catalase and glutathione and decreased malondialdehyde (Mossalam, Aty, Morgan, Youssaf, & Mackawy, 2011). The effects were observed for both water and ethanolic extracts from flowers of *Hs,* as well as from the seeds or leaves (Mohd-Esa, Hern, Ismail, & Yee, 2010).

### 6.6. Clinical studies

One single randomised, open-label, two-way cross-over study was conducted with 8 healthy volunteers. One single dose (0.05 g/ml) of a *Hs* WE significantly increased the systemic antioxidant potential in plasma and urine, increasing the hippuric acid excretion with decreased malondialdehyde concentration in urine (biomarker for oxidative stress) (Frank et al., 2012).

Polyphenols in particular the anthocyanins (eg. delphinidin-3-glucoside) and protocatechuic acid (Degenhardt et al., 2000; Mohd-Esa, Hern, Ismail, & Yee, 2010; Sayago-Ayerdi, Arranz, Serrano, & Goni, 2007; Tseng et al., 1997) are key classes of compounds linked to the antioxidant activity. This activity is also the basis of many other activities including hepatoprotective and nephroprotective activities from the extract.

### 6.7. Hepatoprotective activity

*cHs* WE (100–800 mg/kg) showed hepatoprotective effects in a range of models based on toxin-induced hepatitis including, *tert*-butylhydroperoxide, lipopolysaccharides, azathioprine, carbon tetrachloride, cadmium, ammonium chloride, acetaminophen and irradiation *in vivo* (Adaramoye, Ogungbenro, Anyaegbu, & Fafunso, 2008; Ajiboye et al., 2011; Ali, Mousa, & El-Mougy, 2003; Amin & Hamza, 2005; Asagba, Adaikpoh, Kadiri, & Obi, 2007; Essa et al., 2006; Lin et al., 2003; Liu, Wang, Chu, Cheng, & Tseng, 2002, 2010; Liu et al., 2006; Olaleye & Rocha, 2008; Tseng, Wang, Kao, & Chu, 1996; Wang et al., 2000) and *in vitro* (Ajiboye et al., 2011; Lee et al., 2012; Yin, Cao, Xu, Jeney, & Nakao, 2011).

This effect is due to a strong antioxidant activity, which reduces cellular damage by reducing oxidative stress and by attenuating mitochondrial dysfunction through decreasing Bax and tBid expression in the liver (Lee et al., 2012). The extract also increased the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and *d*-aminolevulinate dehydratase (*d*-ALA-D) enzymes while decreasing lipid peroxidation in induced models of liver damage (Adaramoye, Ogungbenro, Anyaegbu, & Fafunso, 2008; Ajiboye et al., 2011; Olaleye & Rocha, 2008), and decreased liver marker enzymes such as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in experimental hyperammonemia (Essa et al., 2006). An *Hibiscus* anthocyanin extract also induced phase II drug-detoxifying enzymes, such as glutathione S-transferase, NAD(H):quinone oxidoreductase, and uridyl diphosphoglucuronosyl transferase in an induced liver damage model ($CCl_4$-mediated toxicity model) (Ajiboye et al., 2011).

The anthocyanins present in the extract seem to be the ones responsible for this effect (Ajiboye et al., 2011; Ali et al., 2003; Wang et al., 2000). Another compound that has been identified to have this effect was protocatechuic acid, a phenolic compound present in the *Hs* extract (Lin et al., 2003; Liu, Wang, Chu, Cheng, & Tseng, 2002; Tseng, Wang, Kao, & Chu, 1996).

### 6.8. Nephroprotective activity

Two studies were reported on the nephroprotective activity of *Hs* extracts on diabetic nephropathy in streptozotocin-induced type 1 diabetic rats (Lee et al., 2009; Wang et al., 2011). Nephropathy may progress to end-stage renal disease. A study was conducted to investigate the effect of the polyphenol extract of *Hs* (100 and 200 mg/kg/day) in streptozotocin-induced diabetic nephropathy in rats. The extract revealed beneficial effects as the kidney mass was reduced and the hydropic change of renal proximal convoluted tubules was improved, it reduced serum triglyceride, total cholesterol and LDL as well as increased the activity of catalase and glutathione and reduced lipid peroxidation in the kidney (Lee et al., 2009). It was found that the extracts reduced kidney mass and improved hydropic change of renal proximal convoluted tubules in this rat model. The positive effect shown by the extracts might be via improving oxidative status and regulating Akt/Bad/14-3-3$\gamma$ signalling (anti-apoptotic mechanisms). Another *in vivo* study also revealed that its nephroprotective effect is a result of the protection of the kidney from the oxidative stressed (Mossalam, Aty, Morgan, Youssaf, & Mackawy, 2011).

### 6.9. Renal effects/diuretic effect (incl. clinical studies)

The renal effect of *Hs* has been characterised pharmacologically both in clinical trials (Herrera-Arellano, Flores-Romero, Chávez-Soto, & Tortoriello, 2004; Kirdpon, Nakorn, & Kirdpon, 1994; Prasongwatana, Woottisin, Sriboonlue, & Kukongviriyapan, 2008) and in pre-clinical experiments in rats (Aguwa, Ndu, Nwanma, Udeogaranya, & Akwara, 2004; Laikangbam & Damayanti Devi, 2012).

A two-phase study in Thailand with thirty-six healthy men was conducted to evaluate the changes in urine after consumption of *Hs* juice (16 g/day and 24 g/day) to determine its effect on the treatment and prevention of renal stones. Despite the fact that the consumption of *Hs* caused a decrease in creatinine, uric acid, citrate, tartrate, calcium, sodium, potassium and phosphate it did not affect the concentration of oxalate in urinary excretion. The authors suggested that there was no beneficial effect in preventing renal stone formation and that long term and higher doses should be investigated (Kirdpon et al., 1994). Another intervention study carried out in Thailand with eighteen subjects with or without history of renal stones revealed that *Hs* tea drinking, at a dose of 3 g/day for 15 days, did not show evidence for antilithiatic or diuretic effects. No significant difference in serum sodium and urinary volume were observed during this study. However, *Hs* tea consumption produced a uricosuric effect (Prasongwatana, Woottisin, Sriboonlue, & Kukongviriyapan, 2008). Similar results were observed in albino rats when given a decoction of dried calyces at an oral dose of 1 g/kg (Caceres, Giron, & Martinez, 1987).

However, *in vivo* an antilithiatic effect was observed. In Wistar rats, which were given extract of *Hs* orally at a dose of 3.5 mg daily, the oxalate retention in the kidney decreased with increased excretion into urine and decreased calcium crystal deposition in the kidneys (Woottisin et al., 2011). The *cHs* WE (250, 500 and 750 mg/kg body weight) also effectively prevented the development of urolithiasis (stone-disorder) in male albino rats (Laikangbam & Damayanti Devi, 2012). A decrease in renal $Ca^{2+}$ and $Mg^{2+}$-ATPase activity and unaltered renal calcium handling in rats after administration of *cHs* WE at 25 and 50 mg/kg was shown. Renal function was also enhanced by reduction of serum

urea and creatinine concentrations (Olatunji, Usman, Adebayo, & Olatunji, 2012). In another pre-clinical study in rats, *cHs* WE produced diuretic and natriuretic effects at the dose range of 500 to 2500 mg/kg b.w. with a potassium-sparing effect (Alarcon-Alonso et al., 2012). This diuretic effect is in accordance with previous studies in experimental animals (Aguwa, Ndu, Nwanma, Udeogaranya, & Akwara, 2004; Caceres et al., 1987; Onyenekwe, Ajani, Ameh, & Gamaniel, 1999; Ribeiro Rde et al., 1988) and one clinical trial (Herrera-Arellano, Flores-Romero, Chávez-Soto, & Tortoriello, 2004). In this single clinical trial, assessing a chemically characterised extract of *Hs* (9.6 mg of total anthocyanins) in patients with mild to moderate hypertension, the treatment demonstrated a natriuretic effect with no effects on chloride, potassium and pH (Herrera-Arellano, Flores-Romero, Chávez-Soto, & Tortoriello, 2004).

### 6.10. Cancer-preventive activity

*Hs* is rich in phenolic compounds, such as protocatechuic acid. This compound demonstrated *in vitro* protective effects against cytotoxicity and genotoxicity of hepatocytes induced by *tert*-butylhydroperoxide (*t*-BHP), through inhibiting action on DNA repair synthesis caused by *t*-BHP and by showing radical quenching effect (Tseng, Wang, Kao, & Chu, 1996). It also inhibited 12-*O*-tetradecanolyphorbol-13-acetate (TPA)-induced skin tumour formation in CD1-mice (Tseng et al., 1998) and inhibited the survival of human promyelocytic leukaemia HL-60 cells (Tseng et al., 2000). The mechanism by which it exerted anticancer properties might be through antitumour promotion by reducing reactive oxygen species (ROS), DNA fragmentation, $G_1$ arrest and apoptosis. The apoptosis-inducing activity was associated with the phosphorylation and degradation of RB and the suppression of *Bcl*-2 protein. Similar effects were observed in human gastric carcinoma (AGS) cells in which the apoptotic effect may be mediated via *p*53 signaling and *p*38 MAPK/*Fas*L cascade pathway (Lin, Huang, Huang, Chen, & Wang, 2005). Another group of compounds present in *cHs* extracts are anthocyanins such as delphinidin-3-sambubioside. They induced apoptosis against human leukaemia cells (Chang, Huang, Hsu, Yang, & Wang, 2005; Hou, Tong, Terahara, Luo, & Fujii, 2005) via the *p*38-*Fas*L and *Bid* pathway and ROS-mediated mitochondrial dysfunction pathway and against smooth muscle cells (SMC) via *p*38 and *p*53 pathway (Lo, Huang, Lin, Chien, & Wang, 2007).

Recently, the anti-cancer activity of *Hs* leaf extracts were assessed against human prostate cancer cells *in vitro* and *in vivo* (Lin et al., 2012). The study showed the anti-apoptotic effect to be mediated via both intrinsic (*Bax*/cytochrome *c*-mediated caspase 9) and extrinsic (*Fas*-mediated caspase 8/*t-Bid*) pathways, as well as by inhibiting the growth of prostate tumour xenograft in athymic nude mice. The extract from leaves instead of calyces represented a possible source of greater polyphenolic compounds.

### 6.11. Lipid metabolism – anticholesterol effects/effects on lipid metabolism

Several studies have showed that extracts of *Hs* have a lipid lowering activity, which could prevent diseases like hyperlipidemia and cardiovascular diseases (atherosclerosis and coronary heart disease) (Carvajal-Zarrabal et al., 2005; Chang, Huang, Huang, Ho, & Wang, 2006; Chen et al., 2003, 2004; el-Saadany et al., 1991; Gosain et al., 2010; Hirunpanich et al., 2006; Ochani & D'Mello, 2009; Yang et al., 2010).

The extracts (water and ethanolic extracts of dried calyces or leaves) were able to decrease low-density lipoprotein cholesterol (LDL-c), triglycerides (TAG), total cholesterol (TC) and lipid peroxidaxion *in vivo*. A few of them even reported that the extract was also able to reduce very-low density lipoprotein cholesterol (VLDL-c) (Farombi & Ige, 2007; Ochani & D'Mello, 2009) along with an increase in serum level of high density lipoprotein cholesterol (HDL-c) levels (Ochani & D'Mello, 2009; Yang et al., 2010). Additionally, it also reduced foam cell formation and inhibited smooth muscle cell migration and calcification in blood vessels of treated rabbits. A possible explanation for the decrease in LDL-c could be related to the inhibition of the triacylglycerol synthesis or other hypolipidemic effects, through the antioxidant activity against LDL-c oxidation and hepatic liver clearance. Several groups of compounds in the extract, such as anthocyanins and protocatechuic acid, have been implicated as responsible for these effects (Chang, Huang, Huang, Ho, & Wang, 2006; Lee et al., 2012; Tseng et al., 1997).

### 6.12. Clinical studies

In one clinical trial using an oral preparation of *Hs* flower extract capsules (with a defined composition) serum cholesterol level was reduced after 4 weeks. The best results were obtained with the dosage of two capsules per meal (Lin et al., 2007). Along with this study, another randomised clinical trial with 53 patients with type II diabetes also showed that a tea of *Hs* had a significant effect on the blood lipid profile. Patients were given *Hs* (one tea sachet (2 g) in water) twice a day for a month (Mozaffari-Khosravi, Jalali-Khanabadi, Afkhami-Ardekani, & Fatehi, 2009a). Despite this, in one randomised clinical trial, ninety hypertensive patients were given *Hs* tea or black tea for 15 days, with the results showing no hyperlipidemic effect as previously reported *in vivo*. In addition, no differences in creatinine level, Na and K electrolytes were observed after administration of the tea. However, the short-term administration did not show any harmful effect (Mohagheghi, Maghsoud, Khashayar, & Ghazi-Khansari, 2011). Similar results were found in a double-blind placebo controlled randomised trial with a dose of 1 g/day for 90 days, in which the leaf ethanol-water extract of *Hs* did not appear to have a blood lipid lowering effect over and above the effect of standard dietary and lifestyle advice (Kuriyan, Kumar, Rajedran, & Kurpad, 2010). The difference between this clinical trial and the one conducted in 2007 is that in the second, the flower water extract administered at a dose of two capsules (1 g) three times a day (total of 3 g/day) for 30 days significantly lowered serum cholesterol levels. Differences might be due to different dosage being administered, the size of the test group and duration of the study.

The use of the *Hs* extract was also assessed in a rat model (Oppliger et al., 2012) and in patients (Gurrola-Diaz et al., 2010) with metabolic syndrome. Metabolic syndrome has been characterised as a combination of several metabolic risk factors such as hypertension, insulin resistance, dyslipidemia, excess adipose tissue and cardiovascular disease. Both studies showed a beneficial effect of standardised *cHs* extract on the lipid profile. Consumption of 100 mg/kg for 6 weeks was able to decrease the total cholesterol and triglycerides level in rats in a high fat diet, while similar results were observed in humans taking 100 mg of an extract (1.4 mg/kg) orally for 30 days. Additionally, the basal HDL-c levels after treatment also increased in human subjects. This increase in HDL-c levels after treatment was also observed in a clinical trial with type II diabetic patients (Mozaffari-Khosravi, Jalali-Khanabadi, Afkhami-Ardekani, & Fatehi, 2009a). The anthocyanin content of the extract has been implicated as responsible for its effects, but an extended chemical profile characterisation is needed.

### 6.13. Anti-obesity activity

Pre-clinical data from Brazil indicates a potential role in the control of certain conditions associated with obesity, such as hyperlipidemia. However, further studies were suggested (Dickel, Rates, & Ritter, 2007).

A report showed that a standardised (33.64 mg of total anthocyanins per each 120 mg) water extract of *cHs* was able to reduce weight gain in obese mice while at the same time it increase the liquid intake in healthy and obese mice (Alarcon-Aguilar et al., 2007). This effect is probably achieved through the modulation of PI3-K/Akt and ERK pathway, which play pivotal roles during adipogenesis (Kim et al., 2007).

*In vitro* and *in vivo* studies showed that *Hibiscus* extract (or tea) inhibited the activity of α-amylase, blocking sugars and starch absorption, which may assist in weight loss (Hansawasdi, Kawabata, & Kasai, 2000, 2001; Preuss, Echard, Bagchi, & Stohs, 2007). A study conducted in Mexico using an ethanol extract of *Hs* concluded the extract could be considered as a possible anti-obesity agent due to its effects on fat absorption-excretion and body weight of rats (Carvajal-Zarrabal et al., 2009).

The therapeutic use of the extract, possibly due to polyphenols, was also evaluated in patients with metabolic syndrome, an obesity-associated collection of disorders (Perez-Torres, Ruiz-Ramirez, Banos, & El-Hafidi, 2012). Meanwhile a study showed that the aqueous extract was more efficient in inhibiting triglyceride accumulation when devoid of fibre and polysaccharides, but when polyphenols were fractionated and isolated, the benefits of the whole extract was greater than the sum of its parts (Herranz-Lopez et al., 2012).

### 6.14. Lactating activity

The ethanolic seed extract of *Hs* (200–1600 mg/kg) increased the serum prolactin level ($p < 0.01$) when compared to the control in a dose-dependent manner in lactating Albino Wistar rats (Gaya, Mohammad, Suleiman, Maje, & Adekunle, 2009).

### 6.15. Anti-diabetic activity

Diabetes mellitus can be defined as an endocrine and metabolic disorder characterised by chronic hyperglycaemia, dyslipidemia, and protein metabolism that results from defects in both regulations of insulin secretion and/or insulin action.

The protective effect of a polyphenol extract of *Hs* was studied in a type II diabetic rat model (high fat diet model). At a dose of 200 mg/kg, the extract demonstrated anti-insulin resistance properties as it reduced hyperglycaemia and hyperinsulinemia. It decreased serum triacylglycerol, cholesterol and the ratio of low-density lipoprotein/high-density protein (LDL/HDL), as well as reduced the plasma advanced glycation end products (AGE) formation and lipid peroxidation (Peng et al., 2011).

The currently accepted therapeutic strategy for the control of postprandial hyperglycaemia is based on the inhibition of α-glucosidase and α-amylase. This results in an aggressive delay of carbohydrate digestion to absorbable monosaccharide. With this in mind, a study was conducted to determine the effect of *Hs* extract on intestinal α-glucosidase and pancreatic α-amylase activity *in vitro*. As a result, *Hs* extract was shown to be a potent pancreatic α-amylase inhibitor (Adisakwattana, Ruengsamran, Kampa, & Sompong, 2012). Similar results were found for hibiscus acid (*hibiscus*-type (2*S*,3*R*)-hydroxycitric acid lactone) (Yamada, Hida, & Yamada, 2007), which inhibited pancreatic α-amylase and intestinal α-glucosidase enzyme (Hansawasdi et al., 2000, 2001).

Diabetes mellitus is a risk factor for coronary heart diseases as well as atherosclerosis. An ethnobotanical study conducted in the Caribbean for urinary problems and diabetes mellitus revealed that *Hs* is traditionally used to 'clean' the liver and blood within a group of plants used for "cooling", high cholesterol and urinary problems. When the respondents were asked which medicinal plants were used for high blood pressure, diabetes and jaundice, *Hs* was referred to hypertension (Lans, 2006). A study in alloxan-induced diabetic rats showed that an ethanolic extract of *Hs* flowers (200 mg/kg) had a strong hypolipidemic as well as antioxidant effect. Thus, *Hs* extract showed therapeutic promise in decreasing and preventing the development of atherosclerosis and possible related cardiovascular pathologies linked with diabetes. The authors suggest that this activity might be linked to polyphenolic compounds and dihydrobenzoic acids, like protocatechuic acids, but further identification of the active compounds is warranted (Farombi & Ige, 2007). A similar effect was reported (Huang et al., 2009) with the extract suppressing the high-glucose-induced migration in a vascular smooth muscle cell model.

### 6.16. Clinical studies

Recently a double-blind, randomised, controlled trial was carried out to compare the anti-hypertensive efficacy of *Hs* (tea) in diabetic patients. The results demonstrated that the consumption of the sour tea had positive effects on blood pressure in type II diabetic patients with mild hypertension (Mozaffari-Khosravi, Jalali-Khanabadi, Afkhami-Ardekani, Fatehi, & Noori-Shadkam, 2009b). Following this study another randomised clinical trial (no control group or double-blinding) in identical patients (100 individuals) showed that consuming three glasses of green or hibiscus (sour) tea daily for a period of 4 weeks significantly decreased systolic and diastolic blood pressures in those patients (Mozaffari-Khosravi, Ahadi, & Barzegar, 2013). However, sodium, potassium or calcium concentrations were neither qualified nor specific amounts of the other active constituents of tea, such as caffeine, were taken into consideration. Other clinical trial was conducted to investigate the hypolipidemic effects of sour tea in patients with diabetes. Again, the beneficial effect of the sour tea in diabetic patients was found. The sour tea was able to significantly effect the blood lipid profile by increasing high-density lipoprotein-cholesterol, decreasing total cholesterol, low density lipoprotein-cholesterol, triglycerides and Apo-B100, with no effect on apolipoprotein-A1 and lipoprotein a (Mozaffari-Khosravi, Jalali-Khanabadi, Afkhami-Ardekani, & Fatehi, 2009a).

### 6.17. Delayed puberty activity

A few studies with rats have shown that consumption of *Hs* WE during pregnancy and lactation resulted in increased postnatal weight gain, delayed onset of puberty and elevated body mass index at onset of puberty in the female offsprings (Iyare & Adegoke, 2008a,b,c; Iyare & Nwagha, 2009). The consumption of the extract during pregnancy and lactation caused decrease of maternal fluid and food intake with increased plasma $Na^+$ and corticosterone concentration, while the accelerated growth and delayed puberty observed in the offspring could be due to increased corticosterone and decreased leptin delivery through breast milk (Iyare & Adegoke, 2008b; Iyare, Adegoke, & Nwagha, 2010). These studies however require confirmation as no observations have been reported in the literature up to-date pointing to the presence of respective effects in humans.

### 6.18. Anti-hypertensive activity

Decoctions of *Hs* have been used traditionally in West Africa and Mexico as an anti-hypertensive remedy. Several *in vitro* (Jonadet et al., 1990; Obiefuna, Owolabi, Adegunloye, Obiefuna, & Sofola, 1994) and *in vivo* studies have shown that the extract of the calyces (ranging from 125 to 500 mg/kg) indeed reduce both the systolic and diastolic pressures, lowering heart rate and working as a vasodilator (Adegunloye et al., 1996; Ajay, Chai, Mustafa, Gilani, & Mustafa, 2007; Inuwa et al., 2012; Mojiminiyi et al., 2007; Onyenekwe, Ajani, Ameh, & Gamaniel, 1999; Shehata & El

Menoufy, 2008). The anti-hypertensive activity might be through: inhibition of angiotensin-converting enzymes (ACE) (Jonadet et al., 1990; Ojeda et al., 2010), acetylcholine-like and histamine-like mechanisms (Adegunloye et al., 1996), diuretic effect (Mojiminiyi, Adegunloye, Egbeniyi, & Okolo, 2000), reduction in the diffusion distance between capillaries and myocytes, as well as new vessel formation (Inuwa et al., 2012) and direct vaso-relaxant effects (Adegunloye et al., 1996; Ajay, Chai, Mustafa, Gilani, & Mustafa, 2007; Obiefuna, Owolabi, Adegunloye, Obiefuna, & Sofola, 1994; Adegunloye et al., 1994). The relaxant effect might be partially endothelium independent and possibly mediated by endothelium-derived nitric oxide (EDNO)-dependent action. Endothelium-dependent vasodilator component results through activation of the endothelium-derived nitric oxide/cGMP-relaxant pathway, whereas the endothelium-independent component could be due to inhibition of $Ca^{2+}$ influx (Ajay, Chai, Mustafa, Gilani, & Mustafa, 2007).

Additionally, *Hs* showed antiplatelet but no thrombolytic activity *in vitro* (Yamamoto, Yamada, Naemura, Yamashita, & Arai, 2005). One *in vivo* study reported that despite the beneficial effect as an anti-hypertensive, *Hs* might produce undesirable effects on gonadal activity (Shehata & El Menoufy, 2008).

### 6.19. Clinical studies

Several clinical trials were carried out to determine the anti-hypertensive effect of *Hs* (Haji Faraji & Haji Tarkhani, 1999; Herrera-Arellano, Flores-Romero, Chávez-Soto, & Tortoriello, 2004; Herrera-Arellano et al., 2007; Mozaffari-Khosravi, Jalali-Khanabadi, Afkhami-Ardekani, Fatehi, & Noori-Shadkam, 2009b). Both a Cochrane review and a systematic review carried out in 2010 concluded that the studies did not provided reliable evidence to support recommendation of this plant to control or lower blood pressure in hypertensive patients, when compared to placebo or no treatment (Ngamjarus, Pattanittum, & Somboonporn, 2010; Wahabi, Alansary, Al-Sabban, & Glasziuo, 2010). However, a recent randomised, double-blind, placebo-controlled clinical trial showed that *Hs* tea (1.25 g of *H. sabdariffa* per 240 mL boiled water; 3 servings a day for 6 weeks) effectively reduced blood pressure in pre- and mildly-hypertensive adults (McKay, Chen, Saltzman, & Blumberg, 2010). Similar effects on decreasing systolic and diastolic blood pressures were observed in mildly hypertensive type II diabetic individuals when taking green or hibiscus (sour) tea for 4 weeks (three times a day, 2 h after each meal) (Mozaffari-Khosravi et al., 2013). The authors also concluded that this might be useful in preventing the progression to moderate or more severe hypertension, potentially decreasing cases of cardiovascular disease. Furthermore, a recent comprehensive review on animal and human studies on the effect of *Hs* in the treatment of hypertension and hyperlipidemia concluded that *Hs* has great potential to reduce risk factors associated with cardiovascular diseases and warrants further studies (Hopkins, Lamm, Funk, & Ritenbaugh, 2013).

Anthocyanins, including delphinidin-3-*O*-sambubioside (hibiscin) and cyanidin-3-*O*-sambubioside (gossypicyanin), have been identified as being responsible for ACE inhibition (Herrera-Arellano et al., 2007; Ojeda et al., 2010).

### 6.20. Anti-anaemic activity

A preliminary study on the use of *Hs* decoctions as an alternative source of iron for the treatment of anaemia and some other mineral deficiency diseases was conducted and showed that dry fermented calyces of hibiscus exhibited a very low pH value which enhanced mineral availability. Another reason for enhancing mineral (iron, zinc, calcium and magnesium) bioavailability is the high concentration of ascorbic acid (Falade et al., 2005).

The effect of *cHs* extract (200 to 1000 mg/kg body weight) on some haematological parameters in rats was studied to determine its medicinal usefulness in the treatment of anaemia. The study suggested that at a comparatively high dose range of 200 to 400 mg/kg, the extract had a beneficial effect on the red cells, but this was not sustained at even higher doses (Adigun, Ogundipe, Anetor, & Odetunde, 2006). Another study using a rat model of infection with *Trypanosoma congolense* showed that the use of *Hs* WE (equivalent to 9.61 mg/100 g/day of ascorbic acid for 3 weeks) prevented the disease-induced anomalies with increase of serum creatinine and urea levels. It was concluded that consumption of the extract ameliorated the pathological changes in blood as well as hepatic and renal structures of *T. congolense*-infected rats. The observed effects might be due to the ascorbic acid component or other antioxidants present, which presumably kept the free radical load in infected rats low as well as preventing the disease-associated depletion in systemic antioxidants. Nevertheless, further studies are needed to determine the long-term effects and the mechanism of action before recommendations could be made (Umar et al., 2009).

### 6.21. Others

The clinical use of *Hs* was reported for conjunctivitis (Fraunfelder, 2004).

A *Hs* flower tea was shown to be a very efficient oral negative contrast agent for magnetic resonance cholangiopancreaticography (MRCP) study in reduction of high intensity fluid in the stomach and duodenum. The authors suggested that this was natural, safe, inexpensive and palatable for oral administration (Varavithya et al., 2005).

The effect of WE of dried *cHs* was also assessed on intestinal transit in experimental rats providing a potential use in the control of diarrhoea, as there was a reduction in percentage transit point indicating a reduction in intestinal motility and increased transit time. At an appropriate dose it could therefore become a constipating agent (Owulade, Eghianruwa, & Daramola, 2004).

A study reported that crude polysaccharides from *Hs* flowers had a potent stimulator effect on keratinocytes proliferation with an influence on the early differentiation behaviours of the cells. Thus, polysaccharide-containing extracts from *Hs* could be used for dermatological or cosmetic applications (Brunold et al., 2004).

Another unorthodox use proposed for *Hs* flowers is as a pH indicator for environmental protection. The flowers of this plant contain anthocyanine pigments. As these pigments change colour depending on pH, they could work as a environmental indicator replacing synthetic indicators, therefore being safer for the user's health, minimising chemical pollution and become more cost effective (Soltan & Sirry, 2002).

Genome shuffling of *Streptomyces* sp. U121 was used to achieve improvements on the production of hydroxycitric acid, an organic acid present in *Hs* (Hida et al., 2007). This technique is of great interest to the biotechnological production of this compound in the food industry as a safe food additive. As previously reported, hibiscus acid, the (+)-*allo*-hydroxycitric acid lactone, has been demonstrated to have an inhibitory effect on pancreatic α-amylase and intestinal α-glucosidase (Hansawasdi et al., 2000, 2001), resulting in reduction of carbohydrate metabolism and blood insulin levels.

## 7. Safety

*H. sabdariffa* preparations, predominantly the infusion and aqueous extracts, have a long standing traditional use both in food and in medicine, and in general are considered to be safe. The

available toxicological data, however limited, are in support of this assessment. The literature search for this review did not reveal any case reports of adverse reactions following oral consumption of *H. sabdariffa* preparations.

### 7.1. Toxicology

In a bioassay for screening plant extracts for their biological activity, the lethal dose ($LD_{50}$) of three different types of *Hs* extract was assessed in the brine shrimp toxicity assay. Aqueous *Hs* extract (i.e., infusion) produced an $LD_{50}$ of 9.59 μg/ml, while for the dichloromethane extract it was 24.51 μg/ml and 4.75 μg/ml for the ethanolic extract (Serrano, Ortega, & Villar, 1996). Given the very limited value of the brine shrimp assay for complex mixtures like plant extracts (Manilal, Sujith, Kiran, Selvin, & Shakir, 2009) and the incomplete information on the mode of preparation of the extracts, this work is mentioned here for completeness only.

The $LD_{50}$ in mice (b.w. 30 g) was reported to be about 0.4–0.6 ml on intraperitoneal administration of a 30% aqueous *Hs* decoction (20 min in distilled water) (Sharaf, 1962). The same authors observed a lowered blood pressure in dogs (b.w.: 7 kg) with no side effects after administering (i.p.) 10 ml of a 10% solution of the *Hs* decotion.

According to a study of Onyenekwe and coauthors, no deaths were observed in Albino mice after fourteen day's administration (i.p.) at doses of 1000–5000 mg/kg b.w./d., thus the calculated $LD_{50}$ of cHs aqueous extract was >5000 mg/kg b.w. The same authors assessed the effect of the extract on blood pressure in spontaneously hypertensive and normotensive Wistar-Kyoto rats. As part of this study it was observed that between the seventh and the twenty-first day after extract administration, the highest dose of 1000 mg/kg resulted in spontaneous deaths in hypertensive but not in normotensive rats. With reference to the well known increased risk of sudden cardiac death in patients receiving non-potassium sparing diuretics, the authors speculate that the death of the animals may have been due to a diuretic effect of the extract (Onyenekwe, Ajani, Ameh, & Gamaniel, 1999), however, the dose found to be active is excessively high. Although Kirdpon and co-authors report a decrease of potassium and sodium in 36 healthy young men after successive administration of 16 g/d Hibiscus "Juice" (while surprisingly no such effect was seen in the high dose group with 24 g/d), this interpretation remains questionable in view of a much larger and well-documented controlled clinical study in which Hibiscus extract showed a natriuretic effect with no effects on chloride, potassium and pH in 171 men with mild to moderate hypertension (Herrera-Arellano, Flores-Romero, Chávez-Soto, & Tortoriello, 2004). Lack of acute toxicity with calculated $LD_{50}$ values >5000 mg/kg b.w. was reported for a methanolic dried flower extract in adult albino mice on a herb-drug interaction study after 24 h administration (i.p.) (Ndu, Nworu, Ehiemere, Ndukwe, & Ochiogu, 2011) and for an ethanolic extract of *Hs* seed in albino Wistar rats while studying the effect of the extract on lactogenic activity (Gaya, Mohammad, Suleiman, Maje, & Adekunle, 2009).

Administration of the water-soluble fraction of a concentrated cHs extract (extraction solvent: MeOH 80%) given orally at up to 15 successive doses of 250 mg/kg/d to Wistar albino rats showed no pathological features in both liver and heart after 24 h (Akindahunsi & Olaleye, 2003). The authors observed a dose-related increase in the levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) when compared to the control group. However, levels of the same enzymes decreased slightly in the liver. In view of the extraordinarily high serum AST value in the control group as compared to reference values from the literature (Boehm et al., 2007), the conclusions of the authors regarding a putative cardio- or liver toxicity of the tested extract have to be read with caution.

In recent years the effect of Hibiscus extracts on reproduction and development in rats has been subject to *in vivo* studies by several authors. In female rats, addition of hibiscus extracts to drinking water resulted in a dose-dependent reduction of fluid and feed intake. The effects observed in the pups included increased weight gain and delayed onset of puberty. As the authors rightly state, these effects have likely been caused by the reduced fluid and feed intake in accordance with earlier publications. Rather than representing specific toxicologic properties of hibiscus, the decreased fluid and feed intake and subsequent adverse effects observed in this study may be the result of the animals dislike for the flavour of hibiscus (Iyare & Adegoke, 2008a,b; Iyare et al., 2010). Despite the fact that earlier studies showed the $LD_{50}$ to be above 5000 mg/kg and doses as high as 4600 mg/kg were administered to rats in drinking water for 12 weeks with no increase in mortality, the extract induced testicular toxicity (reduced sperm counts and spermatogenesis with evidence of marked degenerative histological changes) at all concentrations tested (1150–4600 mg/kg) (Orisakwe, Husaini, & Afonne, 2004). Additionally, deleterious effects on the testis and spermatozoa and an adverse influence in the male reproductive fertility of albino mice were also reported after a *cHs* WE was administered daily for 4 weeks in a dose of 200 mg/kg (Y. I. Mahmoud, 2012). In contrast to these studies, long term administration of *Hs* WE for 10 weeks and hibiscus anthocyanins (50–200 mg/kg b.w.) for 5 days did not affect the male reproductive system in rats (Ali et al., 2012).

Fakeye and co-authors assessed toxicological effects of a 90-day oral administration of aqueous, ethanol or 50% ethanol extracts of dried *cHs* at doses of 300 and 2000 mg/kg b.w., respectively, in male Charles Foster rats. Strikingly contrasting with previous studies by various other authors, they observed a strong toxicity with total mortality in all 2000 mg groups until day 28, and in the aqueous and 50% ethanol groups at 300 mg, until day 60 and 40, respectively. (Fakeye, Pal, Bawankule, Yadav, & Khanuja, 2009). At a dose of 300 mg/kg (b.w.) all extract types produced a significant increase of plasma creatinine after 30 days administration of the extracts. High creatinine blood levels may be associated with muscular dystrophy, loss of kidney function or even mortality. However, since the ethanol extract produced the strongest increase in creatinine but no mortality at the 300 mg dose level, it is unlikely that the elevated creatinine levels have been the cause of death in the other groups (Fakeye, Pal, Bawankule, Yadav, & Khanuja, 2009). Overall, the results of this study remain highly questionable. The possible reasons considered by the authors, including differences in anthocyanin content, are not convincing in view of the still moderate anthocyanin doses provided with the extracts.

A similar effect was observed on rat testis in a cisplatin (CIS)-induced rodent model of reproductive toxicity when ethanol extracts of *Hs* was administered for 26 days (1 g/kg per day) (Amin & Hamza, 2006). The extract also revealed anti-mutagenic activity *in vitro* against 1-nitropyrene, a potent mutagen (Olvera-Garcia et al., 2008) and reduction of micronuclei in polychromatic erythrocytes (PCEs) (Adetutu, Odunola, Owoade, Adeleke, & Amuda, 2004). The administration of ethanolic *Hs* extracts (200 mg/kg and 300 mg/kg, orally) in rabbits over a period of eight weeks did not show any toxic effect when dyslipidemia and oxidant stress associated with prolonged excessive intake of cholesterol was studied in these animals (Ekor et al., 2010).

Based on the data presented above, dosages up to 200 mg/kg should be safe and not show signs of toxicity, but further studies, most importantly with chemically well characterised extracts, are warranted.

### 7.2. Interactions

While there is no particular ground for suspicion of a relevant interaction potential of Hibiscus, several preclinical and one clinical study have addressed this issue. The sub-acute effect of an aqueous *cHs* extract on CYP450 activity, clinical blood chemistry and haematology was investigated in male Wistar rats at 250 and 1000 mg/kg/day for 30 days. The authors found no effect on hepatic phase I enzymes (CYP 1A1, 1A2, 2B1/2, 2E1 and 3A) and the extract did not significantly affect blood chemistry and haematology. (Prommetta, Phivthong-ngam, Chaichantipyuth, Niwattisaiwong, & Lawanpraset, 2006). More recently, the interaction of a methanolic *Hs* flower extract and hydrochlorothiazide (a diuretic drug) was examined in adult albino mice, albino rats and healthy adult rabbits. Co-administration of the extract (20–40 mg/kg b.w.) with hydrochlorothiazide (10 mg/kg) caused a significant increase in the volume of urine excreted, as well as a significant decrease in the pH of urine and the concentrations of sodium, bicarbonate and chloride ions. It also increased and prolonged the plasma concentration, the mean area under the concentration–time curve and the volume of distribution of the diuretic drug over a 24 h sampling period (Ndu, Nworu, Ehiemere, Ndukwe, & Ochiogu, 2011). Regarding the type of preparation (methanol extract) and mode of administration (intraperitoneal), the relevance of these findings for the use of traditional hibiscus preparations remains highly questionable.

The pharmacokinetics of chloroquine (600 mg) and a freshly prepared *Hs* beverage, similar in taste and concentration to the one usually consumed by Sudanese people, were studied in healthy males. The study showed a statistically significant reduction in the area under the plasma concentration versus time curve and the peak plasma concentration of chloroquine (Mahmoud, Ali, Homeida, & Bennett, 1994). In view of the very small group size ($N = 6$) and the poor information on the mode of preparation and dosage of the hibiscus beverage, the results of this study need to be interpreted with caution.

The effect of so-called Zobo Drink (sweetened aqueous extract prepared from 30 g dried hibiscus calyces/l) on acetaminophen pharmacokinetics was studied in healthy young men ($N = 6$, cross-over design). No significant kinetic changes were observed when the extract was administered concomitantly with acetaminophen, except for a slight elevation of the clearance by ca. 11%. Given the poorly described study protocol and the small sample size of $N = 6$, the conclusions of the authors on a possible interaction potential of hibiscus should be read with caution (Kolawole & Maduenyi, 2004).

Taken together, the data available today from preclinical and clinical studies does not provide substantiated evidence of any therapeutically relevant interaction potential of commonplace teas or beverages containing hibiscus and its preparations. This complements the evidence based on the complete absence of drug interaction case reports involving hibiscus in the scientific literature.

## 8. Scientific applications and translational research

In order to assess the level of translational research on *Hs* products, a patent research at the Espacenet patent database of the European Patent Office (http://worldwide.espacenet.com) incorporating 90+ national patent databases was performed in March 2013 (Worldwide – collection of published applications from 90+ countries). The result of this research on intellectual property rights regarding *Hs* shows an increasing interest in novel technological applications of potential future products derived from *Hs*.

Research queries used were: hibiscus (1450 hits) AND sabdariffa (51 hits) and common names for hibiscus or hibiscus tea (305 hits), like roselle (753 hits), karkade (22 hits), which delivered a remarkable number of relevant hits, whereas results for the terms rosemallow, flor de Jamaica, Abelmoschus cruneatus, sorrel (not *Rumex acetosa*) or bissap were not found or lead to non-relevant patents.

Based on the research outcome, further queries were performed with AND (Boolean operator) search options Tea, Extract, Food, Health, Medic* (for Medical, medicine/s, medicinal, medication/s etc.). Table 2 shows a survey of the results/hits and representative examples as selected patent titles, the nations of their application (e.g. CN-China, JP-Japan, MX-Mexico etc.) and the year of their publication.

Also recent clinical research topics are already reflected in newer patents. For instance, the hypertensive effect of hibiscus preparations, like the tea or extract can be found. The term "Hibiscus Tea" yielded in 305 hits and the query "Hibiscus Tea Hypertension", as well as, "Hibiscus AND Hypertension" yielded two relevant patents. The same patents are found starting from "hibiscus extract" with 793 hits; those connected with AND hypertension showed two hits (see Table 3 for more details), whereas AND "blood pressure" gave no hits. Regarding bioactive principles, one of the main phytochemicals found in Hs calyxes, besides the more known anthocyanins, is hibiscus acid, a (2*S*,3*R*)-hibiscus type enantiomer. Patent search for "hibiscus acid" revealed 9 patents. The query "hydroxycitric acid AND hibiscus" yielded 2 results, but only five of those results are relevant (see Table 3 for more details).

### 8.1. Future research

The available information on *H. sabdariffa* shows a wide range of traditional as well as potentially new health applications and therapeutic targets associated with such uses. More robust, randomised, controlled clinical trials would be desirable with well-characterised *Hs* preparations to corroborate its beneficial effects in pre- and mildly-hypertensive patients. The same applies for its diuretic effects.

Obesity is a growing problem affecting not only adults but also children. The effectiveness of *Hs* extract for metabolic disorders like type II diabetes should be investigated further, as previous clinical studies have shown promising effects on hyperlipidemia and hypertension, conditions strongly correlated with type II diabetes or metabolic syndrome (Hernandez-Perez & Herrera-Arellano, 2011; Hopkins, Lamm, Funk, & Ritenbaugh, 2013). Given the longstanding safe use of hibiscus preparations as food, it could be of great help in the supportive treatment of these pathologies.

Other therapeutic uses could also be pursued. In a study with *Hibiscus asper* Hook.f., a closely related species, the methanolic extract of leaves showed neuroprotective activity against 6-OHDA-induced toxicity, through its antioxidant and anti-apoptotic activities in a Parkinson's disease model. Further studies will be needed to determine if an extract of *Hs* would have similar effects. Parkinson's disease is a progressive neurodegenerative disorder with limited therapeutic options available (Hritcu et al., 2011).

*Hibiscus rosa sinensis* L. roots have been used in fertility regulation, showing strong anti-implantation and uterotropic activity (Kholkute & Udupa, 1976; Vasudeva & Sharma, 2008). This plant forms an important constituent of several Ayurvedic contraceptive preparations (Kamboj & Dhawan, 1982). Further research with *Hibiscus* species including *Hs* may be warranting with regard to gynaecologic applications.

Limited data is available that correlates the therapeutic uses of *Hs* and the chemical profile of the extracts being used. Most of the studies reported in this review only identify the type of extract and part of plant used, but do not quantify the compounds present in the extract.

**Table 2**
Results/hits and representative examples as selected patent titles of *Hibiscus sabdariffa* L.

| Hits | *Hibiscus sabdariffa* (total of 51 hits (Hibiscus 1459 hits)) | Roselle (746 hits) | Karkade (22 hits) |
|---|---|---|---|
| AND tea | 3 hits: relevant only JPS6212767 (B) of Mutsuo (see Table 3) | 46 hits: 39xCN,3xJP,2xRU,2xTW; published 2000–2012. Selected patent titles: Flower/fruit/natural tea drink and preparation method, blood nourishing and facial beautifying composition, refreshing composition with low glycaemic index, weight reducing tea, health protection herbal tea, radiation-resistant functional tea beverage, liver-nourishing tea, natural colourful total nutrient, tea drink capable of removing toxin and beautifying, wisdom brain enlightening drink, tea for benefiting wisdom and awakening brain, natural tea for nourishing entrails and reducing blood fat, blood sugar and blood pressure, tea drink for promoting mentality and building body, health-care tea and its making method, quick dissolving roselle tea bag, Roselle tea and method for producing the same, etc | 19 hits: 14 RU patents all of inventors: Butina, et al. published 2010–2012. Functional food product with antitoxic radioprotective, immunomodulating, hypoglycaemic, ergogenic, hepato-protective, hypocholesteremic, interseptum protective, hypolipidemic, antioxidant, herophophylactic, hypotensive properties, anti-inflammatory properties against digestive tract diseases, others: sweet liqueur or alcoholic beverage 2xRU//2xJP Dough improving agent,//1xEP drink//1986–2011 RU, JP, GE |
| AND extract | 31 hits: EU, US, mainly Asian: CN, KR, TW; published 1981–2013. Selected patent titles: Patents on extract use in for hepatic, inflammatory, metabolic disorders, related to lipase activity inhibitor, fat metabolism improving composition, anti-allergic effect, anti-aging, cosmetic use as activators of aconitase and their use in anti-ageing skin care, skin protecting agent, hair care, active oxygen scavenger cosmetic, as meat quality preservative due to its antioxidant effect | 19 hits: 10xCN, 4xJP, 2xMX, 1xTW, 1xRU, 1xUS, published 1987–2012. Method for extracting roselle calyx red pigment, refreshing composition with low glycaemic index, nutritional healthy beverage, fabric refreshing cabinet device, the improvement of anthocyanin content from extraction of the roselle calyx infusion, preparation capable of defaecating, adjusting qi and blood, improving internal secretion and preventing senescence, method for producting roselle chewing gum and the resultant product, production of concentrated drink from Hibiscus and Roselle, etc | 15 hits: 14 RU patents of Inventor: Butina E.A. et al.; published 2010-2012. On functional food product, 1xJP external use |
| AND food | 8 hits: JP, RU, TW, MX; published 2003–2012. Composition of *Hibiscus sabdariffa* L. calyces for the manufacture of drugs, food supplements, functional food and sweeteners for beverage, cosmetic use, and in atherosclerosis prevention | 53 hits: 25xCN, 5US, 2xMX etc.; published 1997–2012. Selected patent titles: Nutrient health care product for reducing trioxypurine, rose oral liquid, rose jam, composition and food for improving metabolic syndrome, health protection herbal tea, method of preparing healthy food, roselle red pigment, baked food, multicolored hypoglycaemic tea drink, roselle fast food, drink and food obtained by using leaf of roselle, food biologically active addition, microorganism reduction methods and compositions for food cleaning, cleaning/sanitising methods, compositions, and/or articles for fabric, articles, methods for cleaning produce and edible and edible animal protein, etc | 15 hits: 14 RU patents of Inventor: Butina E.A. et al.; published 2010–2012. On functional food product. 1xJP dough improving agent. |
| AND health | 2 hits: (1) JP2005333942; published 2005. Fermented herb vinegar and method for producing the same. (2) CN1404740, published 2003. Eight-ingredients slim tea and its making method | 51 hits: 47xCN, 2xTW, 2xJP; published 1995-2012. Selected patent titles: many of the roselle tea patents; health protection herbal tea, method of preparing healthy food, natural tea drink capable of clearing away heat, roselle mooncake, roselle eight-flavour rice dumpling, method for cultivating roselle, application of roselle for preparing health product for promoting lead-eliminating effect, process of making preserved vanilla-roselle calyx, roselle notoginseng beverage, roselle calyx jam, roselle series product, etc | 0 hit |
| AND medic* | 13 hits: mainly of Asian origin; published 2003 and thereafter. Use of the *Hibiscus sabdariffa* extract in the preparation of a medicament; mainly in atherosclerosis prevention or inhibition; mainly within mixtures of 1–8 herbals | 24 hits: 15xCN, 3xRU etc.; published 1998–2012; Roselle solid beverage, species ‘‘Dirosavit’’ eliciting general tonic effect, roselle calyx beverage, elixir for gastroenteric tract disease treatment normalizing metabolism and eliciting sedative effect, arrangement for delivering liquid to medical treatment point, nitric oxide donor compounds and pharmaceutical compositions for pulmonary hypertension and other indications, etc | 8 hits: 8 RU patents of Inventor: Butina, et al., published 2010–2012. On functional food product |

Abbreviations: CN-China, JP-Japan, RU-Russia, TW-Taiwan, MX-Mexico, KR-Korea, GE-Georgia, EP-Europe, WO-World.

Preliminary studies also demonstrated very interesting pharmacologic effects for *Hs* in obesity and diabetic conditions. In relation to type II diabetes, the combined presence of nephroprotective and α-amylase inhibiting properties deserves further attention.

Overall, *Hs* preparations, particularly *Hs* tea and aqueous extracts can be considered to be safe.

An increasing body of pharmacologic and clinical studies is providing promising perspectives for an interesting range of therapeutic applications as well as potential health claims. However, this review has also identified a wide range of problems associated with the quality of many aspects of these studies, but most importantly the pharmacological or clinical approaches (or models) used

**Table 3**
Patents list that include *Hibiscus sabdariffa* L., Hibiscus acid and Hs Tea and hypertention

| Patent or application number | Issue date | Title | Summary | Inventor(s) | Applicant/assignee |
|---|---|---|---|---|---|
| US 6127553 (A) | Oct 3, 2000 | Convenient method for large-scale isolation of hibiscus acid | The invention relates to a process for the isolation of Hibiscus acid or (+)hydroxycitric acid lactone (2*S*,3*R*-dihydroxy-1,2,3-propanetricarboxylic acid lactone) from the leaves of *Hibiscus furcatus*, *Hibiscus sabdariffa* and *Hibiscus cannabinus*. Garcinia acid, one of the optical isomers of hydroxycitric acid is a potentially interesting molecule and found extensive application in the pharmacological as well as synthetic fronts. | Ibrahim Ibnusaud, Rani Rajasekharan, Teena Philip, Salini Thomas | Department of Science and Technology, Government of India |
| US 6849278 (B) | Feb 1, 2005 | Method to counter oxidation of LDL, decrease triglyceride or cholesterol and inhibit atherosclerosis using *Hibiscus sabdariffa* extract | A method for countering oxidization of low density lipoproteins, reducing cholesterol or triglyceride in plasma or inhibiting atherosclerosis comprising administering an effective amount of a *Hibiscus sabdariffa* extract | Chau-Jong Wang | Universal Biotech Co., Ltd. |
| US 2008/ 0095867 A1 | April 24, 2008 | Hydroxycitric acid compositions from garcinia cambogia and hibiscus sp., methods of making, and therapeutic uses of same | Herbal compositions that comprise one or more of extracts of hydroxycitric acid, (−) HCA and (+) HCA, from *Garcinia cambogia* and *Hibiscus sabdariffa*, and anthocyanins from *Hibiscus sabdariffa*; all constituent extracts at independently controlled concentrations which optionally include second therapeutic agents and pharmaceutically acceptable additives. Processes for the preparation of these herbal compositions, such processes including steps of extracting hydroxycitric acids and anthocyanins from *Garcinia* and *Hibiscus* species, reacting the hydroxycitric acid with IA and IIA group metals of periodic table, isolating the metal salt of hydroxycitric acid and drying and optionally mixing anthocyanins. Uses of the compositions that comprise extracts containing hydroxycitric acids (positive and negative isomers) and anthocyanins from Garcinia and Hibiscus species optionally along with secondary agents and pharmaceutically acceptable additives to manufacture a medicament for multiple therapeutic uses, as well as beverages and powdered beverage premixes, all of which provide healthful benefits, are also provided | Scott Alexander Moffett | Renaissance Herbs, Inc. |
| *Hs Tea* | | | | | |
| JPS6212767 (B) | March 20, 1987 | Tea bag of *Hibiscus sabdariffa* L. and its preparation | Purpose: To obtain the titled tea bag useful for a fancy drink, having effect of herb medicine on diabetes, hypertension, malum cordis, etc., by adhering uniformly and firmly a specific amount of stevia extract powder as a sweetener to dried ground pieces of *Hibiscus sabdariffa* L., followed by packing. Constitution: Dried ground pieces of *Hibiscus sabdariffa* L. is sprayed with 1–3 wt% aqueous solution or alcoholic solution of stevia extract powder so that the powder is adhered to *Hibiscus sabdariffa* L. uniformly and firmly. Water or alcohol is evaporated by a dryer, etc. to make water content of the prepared product 10 wt% and it is packed in tea bags.; *Hibiscus sabdariffa* L. contains no caffeine, has effect of herb medicine on diabets, hypertension, malum cordis, renopathy, respiratory diseases, diuresis, reduction of cholesterol in the blood, etc. and exhibits bright ruby and proper acidity. While stevia extract powder has sweetness about 100 times as much as that of sugar. An amount of the latter per bag is preferably 0.02–0.04 g. EFFECT: Even excess drinking is harmless | Satou Mutsuo | Hounan Shiyokuhin Kogyo KK |
| *Hibiscus acid* | | | | | |
| US6703515 (B2) | March 09, 2004 | Novel chiral derivatives of hibiscus acid bearing lactone ring moiety, process for preparing the same and a convenient method for the large-scale isolation of hibiscus acid | (see title) | Saud Ibrahim Ibnu [IN] Gopinath Chitra [IN] | Dept Science and Technology, Technology Bhavan |
| US6489493 (B2) | December 03, 2002 | Novel acyclic chiral derivatives of hibiscus acid and the process of preparing the same | (see title) | Saud Ibrahim Ibnu [IN], Nair Rani Rajasekharan | Saud Ibrahim Ibnu, Nair Rani Rajasekharan |
| US6127553 (A) | October 03, 2000 | Convenient method for large-scale isolation of hibiscus acid | (see above) | Ibnusaud Ibrahim [IN], Rajasekharan Rani [IN] | Dept Science & Tech [IN] |
| JP2000239164 (A) | September 05, 2000 | Glycosidase Inhibitor | – | Kasai Takanori Kawabata Jun | Kikkoman Corp |
| US 2008/ 0095867 A1 | April 24, 2008 | Hydroxycitric acid compositions from garcinia cambogia and *Hibiscus* sp., methods of making, and therapeutic uses of same | Herbal compositions that comprise one or more of extracts of hydroxycitric acid, (−) HCA and (+) HCA, from Garcinia cambogia and *Hibiscus sabdariffa*, and anthocyanins from *Hibiscus sabdariffa*; | Scott Alexander Moffett | Renaissance Herbs, Inc. |

| | | | | | |
|---|---|---|---|---|---|
| *Hibiscus for hypertension* WO2005102371 (A3) | January 26, 2006 | Use of the pregnane glycosides in treatment/management of obesity, obesity-related and other disorders | Doses containing pregnane glycosides alone (preferred glycoside is a caralluma plant extract containing a synergical mixture of the pregnane glycosides, caratubersides and boucerosides containing 90–95% of the former), and in combination with several other compounds for treatment of disorders/conditions such as obesity, low BMR, hyperglycaemia, hypertension, hypercholesterolemia, Type (2) diabetes, migraine, osteo-arthritis and joint degradation/inflammation, clinical depression, menopausal syndrome, ageing syndrome, circulation syndrome, capillary degeneration, reduced cognitive and memory function, hearing loss, sexual dysfunction and others are disclosed. Doses are also provided for the regulation/improvement of various physiological parameters/conditions/functions associated with said disorders and others such as skin condition, joint mobility, mood, memory function and recall, lean body mass, stamina, libido and others.; Combinations thereof with the extracts of *Hibiscus sabdariffa*, and 20 other extracts or supplements | Rajendran Ramaswamy [IN] Rajendran Kamala [IN] | Rajendran Ramaswamy [IN] Rajendran Kamala [IN] |
| JPS6212767 (B) | August 20, 1987 | Tea bag of *Hibiscus sabdariffa* L. and its preparation | Matter of the patent: combination of *Hibiscus sabdariffa* due to effect in diabetes, hypertension, malum cordis, renopathy, respiratory diseases, diuresis, reduction of cholesterol in the blood, etc. and Stevia as sweetner | Satou Mutsuo | Hounan Shiyokuhin Kogyo KK |

and a lack of linking these to a careful profiling of the extracts. Therefore, a better chemical profiling of the extracts, as well as its standardisation and the correlation between the chemical components and the pharmacologic/therapeutic action will be essential in future studies with this very promising traditional plant.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.05.002.

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