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REVIEW ARTICLE

Phytochemical, Pharmacological and Toxicological Aspects of *Hibiscus sabdariffa* L.: A Review

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This article reviews the reported phytochemical, pharmacological and toxicological properties of *Hibiscus sabdariffa* L. (English: roselle, red sorrel; Arabic: karkade), the calyces of which are used in many parts of the world to make cold and hot drinks. Nutritionally, these contain ascorbic acid (vitamin C). In folk medicine, the calyx extracts are used for the treatment of several complaints, including high blood pressure, liver diseases and fever.

The pharmacological actions of the calyx extracts include strong *in vitro* and *in vivo* antioxidant activity. In rats and rabbits, the extract showed antihypercholesterolaemic, antinociceptive and antipyretic, but not antiinflammatory activities. In rat and man a strong antihypertensive action has been demonstrated. The effects of the calyx extracts on smooth muscles *in vitro* are variable, but they mostly inhibit the tone of the isolated muscles. In healthy men, consumption of *H. sabdariffa* has resulted in significant decreases in the urinary concentrations of creatinine, uric acid, citrate, tartrate, calcium, sodium, potassium and phosphate, but not oxalate. Oil extracted from the plant's seeds has been shown to have an inhibitory effect on some bacteria and fungi *in vitro*.

The plant extracts are characterized by a very low degree of toxicity. The LD_{50} of *H. sabdariffa* calyx extract in rats was found to be above 5000 mg/kg. A single report has suggested that excessive doses for relatively long periods could have a deleterious effect on the testes of rats.

In view of its reported nutritional and pharmacological properties and relative safety, *H. sabdariffa* and compounds isolated from it (for example, anthocyanins and *Hibiscus* protocatechuic acid) could be a source of therapeutically useful products.

Keywords: Hibiscus sabdariffa; antioxidant; anticancer; anthocyanins; *Hibiscus* protocatechuic acid; glycinebetaine.

INTRODUCTION

Hibiscus sabdariffa L. (family Malvaceae), commonly known in English as roselle or red sorrel and in Arabic as karkadeh, is widely grown in Central and West Africa, South East Asia, and elsewhere. The plant (family Malvaceae) is an erect annual herb, the botanical features of which have been described by Ross (2003). The thick, red and fleshy, cup-shaped calyces of the flower are consumed worldwide as a cold beverage and as a hot drink (sour tea). These extracts are also used in folk medicine against many complaints that include high blood pressure, liver diseases and fever (Dalziel, 1973; Wang *et al.*, 2000; Ross, 2003). The red anthocyanin pigments in the calyces are used as food colouring agents (Esselen and Sammy, 1975).

The purpose of the present article is to gather together the available published information on the constituents of the plant and its pharmacological and toxicological properties.

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CONSTITUENTS

There are many published reports on the constituents of different plant parts of *H. sabdariffa*, which have been summarized briefly by Ross (2003). Citric and malic acids have been reported as the major organic acids in aqueous extracts of the flowers by Buogo and Picchinenna (1937), Indovina and Capotummino (1938) and Reaubourg and Monceaux (1940), but tartaric acid was found only as a trace component by Indovina and Capotummino (1938), although it was detected, along with citric and oxalic acids, by paper chromatography in flower extracts from Taiwan (Lin, 1975). Kerharo (1971) reported high concentrations of organic acids in the calyx, with citric acid predominating, but with malic and tartaric acids also present. Khafaga and Koch (1980a) detected citric, hibiscus, malic and tartaric acids in the calyces of five strains of *H. sabdariffa* var. *sabdariffa.* In all strains, the concentration of acids increased during development of the calyces, but declined after they reached ripeness. Ascorbic acid was reported in aqueous extracts by Buogo and Picchinenna (1937) and Reaubourg and Monceaux (1940), but was not detected by Indovina and Capotummino (1938).

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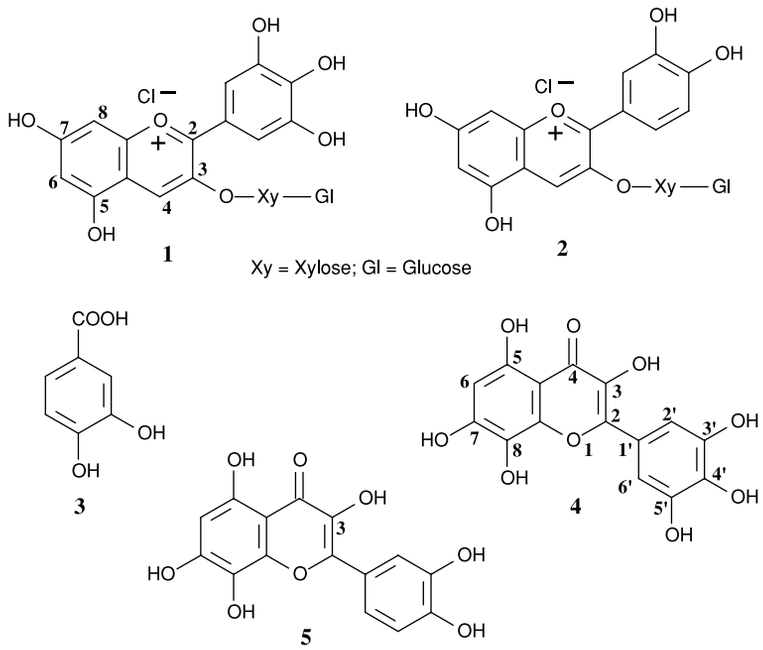

Figure 1. Chemical structures of some major constituents of *Hibiscus sabdariffa* flowers.

Most of the chemical investigations of the flower constituents have been directed towards characterization of their pigments. Yamamoto and Oshima (1932) isolated an anthocyanin, to which they assigned the structure, cyanidin-3-glucoside. Later, they changed this to delphinidin-pentoside-glucoside (Yamamoto and Oshima, 1936). Delphinidin and cyanidin were reported as constituents of plants grown in Trinidad (Forsyth and Simmonds, 1954) and Seshadri and Thakur (1961) isolated delphinidin-3-glucoxyloside (delphinidin-3-sambubioside: Figure 1, **1**), also known as hibiscin. Shibata and Furukawa (1969) studied the pigments of Taiwanese roselle and also reported the presence of delphinidin-3-sambubioside, along with small amounts of delphinidin-3-monoglucoside, cyanidin-3-monoglucoside and delphinidin. The pigments of the calyces of plants grown in Trinidad were examined by Du and Francis (1973), who also isolated delphinidin-3-sambubioside (major component), delphinidin-3-monoglucoside and cyanidin-3-monoglucoside, but, in addition, characterized cyanidin-3-sambubioside (Figure 1, **2**) as the second most abundant anthocyanin in the extract. Subramanian and Nair (1972) studied the pigments present in *H. sabdariffa* var. *altissima* and reported the presence of cyanidin-3, 5-diglucoside and cyanidin-3-(2^G-glucosylrutinoside). Khafaga and Koch (1980b) reported the anthocyanins found in the calyces of five strains of *H. sabdariffa* var. *sabdariffa.* Cyanidin-3-sambubioside was the major pigment, followed by cyanidin-3-glucoside. Delphinidin glycosides were lacking in the Senegalese strain, but were present in the other four strains. During calyx growth, the anthocyanin content reached 1.7% to 2.5% of the dry weight in all strains.

From the flowers of *H. sabdariffa*, Rao and Seshadri (1942a) isolated the flavonol glycoside, hibiscitrin, which on hydrolysis yielded the aglycone, hibiscetin (Figure 1, **4**). In a further publication, Rao and Seshadri (1942b) reported the isolation, from the flower petals of the same species, of hibiscitrin, gossypitrin and sabdaritrin. The last of these, on acid hydrolysis, yielded an hydroxyflavone to which the name sabdaretin was given. Gossypitrin had been shown earlier to be the 7-glucoside of gossypetin (Figure 1, **5**) (Rao and Seshadri, 1939). Hibiscitrin was proved later to be the 3-monoglucoside of hibiscetin (Rao and Seshadri, 1948a, b). In 1961, Seshadri and Thakur isolated gossytrin from the flower petals of *H. sabdariffa*, which they showed to be gossypetin-3-glucoside. Subramanian and Nair (1972) reported the isolation of gossypetin-8-glucoside, gossypetin-7-glucoside and gossypetin-3-glucoside from *H. sabdariffa* var. *altissima.* The 8-glucoside, gossypin, had been first isolated from *Gossypium indicum* by Neelakantam and Seshadri (1936).

Rao and Seshadri (1942a) showed that the content of flavonol glycosides in the calyces was very low and that these compounds were to be found primarily in the flower petals.

Milletti *et al.* (1959) examined extracts of *H. sabdariffa* by paper chromatography and reported the detection of hibiscin, gossypetin, gossypetrin, quercetin, probably myricetin, hibiscetin, hibiscetrin, sabdaritrin, possibly sabdaretin and an unidentified compound. Salah *et al.* (2002), using TLC and HPLC fingerprint analysis of an extract of *H. sabdariffa* flowers, showed the presence of quercetin, luteolin, a luteolin glycoside and chlorogenic acid, in addition to other compounds previously recorded, such as the anthocyanins delphinidin-3-sambubioside and cyanidin-3-sambubioside (Du and Francis, 1973), the flavonoids gossypetin, hibiscetin and their respective glycosides (Subramanian and Nair, 1972), protocatechuic acid (Tseng *et al.*, 1996), eugenol (Chen *et al.*, 1998) and the sterols β-sitosterol and ergosterol (Salama and Ibrahim, 1979). Quercetin had also been recorded by Takeda and Yasui (1985) in roselle colour.

Hibiscus protocatechuic acid (3, 4-dihydroxybenzoic acid; Figure 1, **3**) was isolated from the dried flowers of *H. sabdariffa* and its structure elucidated by Tseng *et al.* (1996).

The seed oil of *H. sabdariffa* was analysed by Jirovetz *et al.* (1992). More than 25 volatile compounds were detected, mainly unsaturated hydrocarbons, alcohols

and aldehydes, predominantly from C_8 to C_{13}. Chen *et al.* (1998) studied the composition of the volatile constituents of roselle tea. More than 37 compounds were characterized, which were classified into four groups: fatty acid derivatives, sugar derivatives, phenolic derivatives and terpenoids. Four differently treated samples were analysed, untreated, frozen, hot air-dried at 50 °C and hot air-dried at 75 °C. The volatile composition of each sample varied considerably, for example significant amounts of furfural and 5-methyl-2-furfural were found in the oven-dried samples, whereas only minimal amounts were detected in the fresh and frozen samples. The major components of the fresh sample were (*Z*)-3-hexenol, 2-hexenol and 1-hexenol, whereas these were present either in small amounts or were absent from the other samples. α-Terpineol was a major constituent of the fresh and frozen calyces, whereas the yields from the oven-dried samples were substantially lower. Eugenol was found in all four samples in about similar amounts.

The petals of *Hibiscus sabdariffa* yielded 65% (dry weight) of mucilage, which on hydrolysis yielded galactose, galacturonic acid and rhamnose (El-Hamidi *et al.*, 1966).

Three water-soluble polysaccharides have been extracted from the flower buds of *H. sabdariffa.* The neutral compounds are composed of arabinans and arabinogalactans of low relative molecular mass. The major fraction was shown to be a pectin-like molecule ($M_r = 10^5$ Da). The main chain is composed of α-1, 4-linked galacturonic acid (24% methyl esterified) and α-1, 2-linked rhamnose. Side chains are built of galactose and arabinose and are connected to the main chain via C-4 of every third rhamnose (Muller and Franz, 1992).

The sterols of the seed oil of *H. sabdariffa* were studied by Salama and Ibrahim (1979), who reported the presence of cholesterol, campasterol, stigmasterol, β-sitosterol, α-spinasterol and ergosterol.

As glycinebetaine and trigonelline have been found as constituents of the flowers of *Hibiscus rosa-sinensis* L. (Blunden *et al.*, 2001), their possible presence in the calyces of *H. sabdariffa* was investigated. Two commercial samples of roselle (Elnasr Hibiscus, Khartoum, Sudan) were extracted and processed using the same procedures as those described by Blunden *et al.* (2001). This entailed purification of each extract by passage through a column of cation exchange resin, examination of the semi-purified extracts by two-way thin layer chromatography (TLC), isolation of the Dragendorff-positive compounds by preparative TLC, and identification of them by ^{1}H-NMR spectroscopy (D_2O; 400 MHz) and fast atom bombardment mass spectrometry. The content of each betaine present in the original extracts was determined using a ^{1}H-NMR spectroscopic assay (Blunden *et al.*, 1986). Both glycinebetaine and trigonelline were detected with yields (dry weight) of 0.7% and 1.1% and 0.007% and 0.02%, respectively.

PHARMACOLOGICAL PROPERTIES

Effect on smooth muscle

The aqueous extract of *H. sabdariffa* calyces inhibited the tone of various isolated muscle preparations that included rabbit aortic strip (Obiefuna *et al.*, 1994) and rat ileal strip (Salah *et al.*, 2002). The extract also rhythmically contracted rat uterus, guinea-pig tracheal chain and rat diaphragm. The same extract stimulated quiescent rat uterus and frog *rectus abdominus* muscle (Sharaf, 1962; Ali *et al.*, 1991). The tonic effects on rat uterus were partially reduced by hydrocortisone and indomethacin. The mechanism of action of *H. sabdariffa* aqueous extract on smooth muscles is not certain. However, as the extracts contain organic acids and minerals (for example, Reaubourg and Monceaux, 1940), the effect of the extract on different smooth muscle preparations would be expected to be variable. The overall effect is a direct relaxation of the smooth muscles, which may explain, at least partially, the hypotensive action of the extract (see below). It was suggested that the relaxant response was related to endothelium-dependent and endothelium-independent mechanisms (Obiefuna *et al.*, 1994), or mediated through calcium channels, possibly generated by constituents such as quercetin and eugenol (Salah *et al.*, 2002). However, the presence of stimulatory substance(s) in the extract has also been demonstrated using the frog *rectus abdominus* preparation (Ali *et al.*, 1991). Further studies of the mechanistic aspects of the extract on smooth muscles are warranted.

Effect on blood pressure

Intravenous injection of aqueous extracts of *H. sabdariffa* calyx to anaesthetized cats (Ali *et al.*, 1991) and anaesthetized rats (Adegunloye *et al.*, 1996) lowered blood pressure in a dose-dependent manner. This effect was resistant to a number of standard receptor blocking agents, but the hypotensive effect was partially blocked by atropine (Ali *et al.*, 1991), and atropine and antihistamine (H_1 blockers) (Adegunloye *et al.*, 1996). Therefore, the hypotensive action may be mediated, at least partially, by a cholinergic and/or histaminergic mechanism. Sectioning of the left and right vagi nerves did not have a significant effect on the fall in mean arterial blood pressure (Adegunloye *et al.*, 1996). It was also postulated that the hypotensive action of *H. sabdariffa* could be ascribed to a direct vaso-relaxant effect (Adegunloye *et al.*, 1996). Another possible mechanism for the hypotensive activity may be inhibition of angiotensin I converting enzyme (ACE). The latter action has been demonstrated *in vitro* with a crude hydroethanol extract of *H. sabdariffa* calyces, and was ascribed to flavones present in the extract. In addition, a beneficial cardioprotective effect of this extract was shown *in vivo*, and was attributed to flavonoids and anthocyanins (Jonadet *et al.*, 1990). More recently, the antihypertensive action of *H. sabdariffa* has been confirmed in rats with experimental hypertension (Odigie *et al.*, 2003) and in spontaneously hypertensive rats (Onyenekwe *et al.*, 1999) given the aqueous extracts at doses of 250–1000 mg/kg for up to 14 weeks.

In a single clinical trial involving 54 patients with moderate essential hypertension, Haji-Faraji and Haji-Tarkhani (1999) have reported that daily consumption of an aqueous *H. sabdariffa* extract (two spoonfuls of blended 'sour tea' boiled in one glass of water for 20–30 min) resulted in about an 11% decrease in

systolic and diastolic blood pressure 12 days after beginning the treatment. Three days after cessation of the treatment, the blood pressure rose again by about 6–8%. The authors did not investigate the possible mechanism(s) of action of the plant extract, but a diuretic, vasodilator and/or an inhibitory effect on ACE was postulated.

The effectiveness of an aqueous extract of *H. sabdariffa* on mild to moderate hypertension was recently confirmed in a clinical trial involving 39 Mexican patients (Herrera-Arellano *et al*., 2004). The extract was made from 10 g dried calyx in 0.5 L water (9.6 mg anthocyanin content) and was given daily for 4 weeks before breakfast. For comparison, 36 hypertensive patients were given the ACE inhibitor, captopril (25 mg twice daily for 4 weeks). The extract treatment reduced the systolic blood pressure from 139 to 124 mm mercury, and the diastolic from 91 to 80 mm mercury. These results were not significantly different from those obtained by captopril treatment. No adverse effects were found with either treatment, confirming the effectiveness and safety of the extract.

Antioxidant and anticancer activity

An 80% ethanol extract of *H. sabdariffa* was effective in reducing about 60%–90% of the mutagenicity induced by heterocyclic amines at a concentration of 12.5 mg/plate in the salmonella mutation assay. Below this dose, neither significant antimutagenic nor antibacterial effects were observed (Chewonarin *et al*., 1999). The extract of the plant also inhibited the formation of colon cancer at the initiation stage.

Fractions of the ethanol extract of dried flowers of *H. sabdariffa* were evaluated by their capacity to quench 1, 1-diphenyl-2-picrrylhydrazyl free radical and inhibiting xanthine oxidase activity. The ethyl acetate fraction of the ethanol extract showed the greatest ability of scavenging free radical and the chloroform fraction showed the strongest inhibitory effect on xanthine oxidase activity. The antioxidant activities of the various extracts were also investigated using a model of *tert*-butyl hydroperoxide-induced oxidative damage in rat primary hepatocytes. Both fractions were shown to be active, indicating that the extract of dried *H. sabdariffa* flowers protect rat hepatocytes from *tert*-butyl hydroperoxide-induced cytotoxicity and genotoxicity by different mechanisms (Tseng *et al.*, 1997).

The calyces of *H. sabdariffa* contain anthocyanins (for example, Shibata and Furukawa, 1969; Du and Francis, 1973) and *Hibiscus* protocatechuic acid (Tseng *et al.*, 1996). It was demonstrated that *Hibiscus* protocatechuic acid has a protective effect against cytotoxicity and genotoxicity induced by *tert*-butylhydroperoxide in a primary culture of rat hepatocytes (Tseng *et al.*, 1996) and it was proposed that one of the mechanisms of this protective effect was associated with the scavenging of free radicals. *Hibiscus* protocatechuic acid also inhibits lipopolysaccharide-induced rat hepatic damage (Lin *et al.*, 2003) and *tert*-butylhydroperoxide-induced rat hepatotoxicity (Liu *et al.*, 2002). *Hibiscus* protocatechuic acid has also been shown to inhibit the carcinogenic action of various chemicals in different tissues of the rat, including diethylnitrosamine in the liver (Taneka *et al.*, 1993), 4-nitroquinoline-1-oxide in the oral cavity (Tanaka *et al.*, 1994), azoxymethane in the colon (Kawamori *et al.*, 1994), *N*-methyl-*N*-nitrosourea in glandular stomach tissue (Tanaka *et al.*, 1995) and *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in the bladder (Hirose *et al.*, 1995). Topical application of *Hibiscus* protocatechuic acid prior to treatment with 12-*O*-tetradecanoylphorbol-13-acetate to female mice, initiated with benzo[α]pyrene, inhibited the incidence of tumours (Tseng *et al.*, 1998). Tseng *et al.* (2000) also demonstrated that *Hibiscus* protocatechuic acid inhibits the survival of human promyelocytic HL-60 cells in a concentration- and time-dependent manner. The data presented by Tseng *et al.* (2000) suggest that the compound is an apoptosis inducer in human leukaemia cells and that RB phosphorylation and Bcl-2 protein may play a crucial role in the early stage.

Oxidation of low-density lipoprotein can increase the incidence of atherosclerosis. Lee *et al.* (2002) have reported that *Hibiscus* protocatechuic acid inhibits this oxidation induced by either copper or a nitric acid donor.

The anthocyanins of *H. sabdariffa* were also shown to have a protective effect against *tert*-butylhydroperoxide-induced hepatic toxicity in rats (Wang *et al.*, 2000). The anthocyanins were able to quench the free radicals of 1, 1-diphenyl-2-picrylhydrazyl and this antioxidant effect was also demonstrated by the ability of the anthocyanins to reduce the cytotoxicity induced by *tert*-butylhydroperoxide in rat primary hepatocytes and to attenuate hepatotoxicity in rats (Wang *et al*., 2000). Administration of the anthocyanins isolated from the plant (100 or 200 mg/kg/day for 5 days) significantly reduced the activities of the serum enzymes indicative of liver damage, ameliorated histological lesions and reduced oxidative liver damage. Similar dosages of *H. sabdariffa* anthocyanins were effective in significantly mitigating the pathotoxicity induced by paracetamol in mice (Ali *et al*., 2003). It has also been reported that anthocyanins protect against DNA damage induced by *tert*-butylhydroperoxide in rat smooth muscle and hepatoma cells (Lazze *et al*., 2003). In view of the established strong antioxidant and antilipid peroxidation actions of *H. sabdariffa* extracts and the compounds they contain (Tseng *et al*., 1997; Wang *et al.*, 2000; Suboh *et al*., 2004), and because many diseases and conditions (for example, diabetes and aging) are thought to involve lipid peroxidation and the generation of free radicals (Poon *et al*., 2004; Vincent *et al*., 2004), the anthocyanins (from this and other plants) and *Hibiscus* protocatechuic acid may potentially be useful in ameliorating or preventing these diseases and conditions.

Glycinebetaine has also been shown to protect against hepatotoxicity induced by bacterial lipopolysaccharides (Kim and Kim, 2002), by ethanol (Kanbak *et al.*, 2001), niacin therapy (McCarty, 2000), chloroform (Kim and Kim, 1998) and methotrexate (Barak *et al.*, 1984). Glycinebetaine in extracts of *Hibiscus* calyces could, therefore, have a significant therapeutic role.

Antipyretic, antinociceptive and antiinflammatory activities

Dafallah and Al-Mustafa (1996) have reported that, in rats, an aqueous extract of *H. sabdariffa* was effective

in inhibiting yeast-induced pyrexia, and in reducing the reaction time in a hot plate, tail-flick assay, indicating that the extract has antipyretic and antinociceptive actions. The extract, however, was without significant effect in the rat paw carrageenan-induced oedema test (Dafallah and Al-Mustafa, 1996), which is one marker used for assaying antiinflammatory action. However, in one clinical trial involving 50 patients, administration of a decoction of dried fruit (3 g/person, three times every day for 7 days to 1 year) was shown to produce antiinflammatory activity (Anon, cited in Ross, 2003). More work on this aspect, using several models for the assay of antiinflammatory activity is warranted. It was suggested that the above antipyretic and antinociceptive actions could be attributed to flavonoids, polysaccharides and organic acids (Dafallah and Al-Mustafa, 1996). Further work is required to study the effects of fractions and isolated compounds in experimental antiinflammatory, antipyretic and antinociceptive tests and their possible mechanism(s) of action.

Renal effects

Workers studied, in six normal Thai subjects, the changes in urine composition that follow the consumption of *H. sabdariffa* extract at different concentrations and for various periods of time (Kirdpon *et al*., 1994). This work indicated that consumption of *H. sabdariffa* extract resulted in significant decreases in the urinary concentrations of creatinine, uric acid, citrate, tartrate, calcium, sodium, potassium and phosphate, but not oxalate. It was noted that the low dose of *H. sabdariffa* (16 g/day) caused a more significant decrease in salt output in the urine than a high dose (24 g/day). A significant uricosuric action was noted in rats given a decoction of the dried calyx at an oral dose of 1 g/kg (Caceres *et al*., 1987; Mojiminiyi *et al.*, 2000).

Anticholesterol effects

H. sabdariffa calyx (5% or 10%) was fed to rats with hypercholesterolaemia for 9 weeks (El-Saadany *et al*., 1991). The treatment progressively lowered the different lipid fractions in plasma, heart, brain, kidney and liver; and also decreased the activities of several plasma enzymes used in tests as markers of tissue function. This treatment, however, slightly raised the content of plasma phospholipids. Although the mechanism of action of *H. sabdariffa* as a cholesterol-lowering agent was not elucidated in this work, it was hypothesized, albeit with no experimental evidence, that the extract may contain some compounds that activate hormonal secretions, such as adrenocortical hormones, which stimulate the metabolic pathway of cholesterol by conversion into other compounds.

The anticholesterol action of *H. sabdariffa* (0.5% or 1%) was confirmed in rabbits fed cholesterol for 10 weeks. This treatment was effective in reducing the serum concentrations of triglycerides, total cholesterol and low-density lipoprotein cholesterol, and in mitigating atherosclerosis in the aorta. Histopathologically, it was found that feeding *H. sabdariffa* had reduced foam cell formation and inhibited smooth muscle cell migration and calcification in the blood vessel of treated rabbits (Chen *et al*., 2003).

Antibacterial, antifungal and antiparasitic actions

Oil extracted from seeds of *H. sabdariffa* has been shown to have an *in vitro* inhibitory effect on *Bacillus anthracis* and *Staphylococcus albus*, but not *Proteus vulgaris* and *Pseudomonas aeruginosa* (Gangrade *et al*., 1979). An ethanol extract of the dried leaves of the plant has been shown to reduce aflatoxin formation (El-Shayeb and Mabrook, 1984), and to have an *in vitro* inhibitory effect against some fungi that include *Aspergillus fumigatus, Rhizopus nigricans* and *Trichophyton mentagrophytes* (Guerin and Reveillere, 1984). An ethanol extract of the dried leaves was found to be ineffective against *Lumbricus terrestris* (Boum *et al*., 1985).

An aqueous extract of dried sepals of *H. sabdariffa* (100 ppm) was active against *Schistosoma mansoni.* However, an aqueous extract of dried seeds (10 000 ppm) was inactive against this trematode (Elsheikh *et al*., 1990).

Interaction with drugs

The interaction of three Sudanese beverages, including *H. sabdariffa*, with the kinetics of chloroquine was studied in human volunteers (Mahmoud *et al*., 1994). *H. sabdariffa* was found not to have a significant effect on any pharmacokinetic parameter, indicating its safety when taken with drugs that may have the metabolic pathways of chloroquine. More recently, Kolawole and Maduenyi (2004) studied the effect of *H. sabdariffa* water extract on paracetamol (acetaminophen) kinetics in healthy men. On the whole, the administration of the extract induced no significant changes in the major kinetic parameters of paracetamol, although very minor and probably biologically insignificant alterations in some were observed. In view of the fact that *H. sabdariffa* drinks may be ingested with medicines, more studies to ascertain the presence or absence of interactions with drugs of different metabolic profiles are warranted.

Toxicological properties

The LD_{50} of *H. sabdariffa* calyx extract in rats was found to be above 5000 mg/kg (Onyenekwe *et al*., 1999), suggesting that the extract is virtually non-toxic. In spontaneously hypertensive rats, treatment with the extract at doses of 500–1000 mg/kg decreased blood pressure, and also significantly decreased serum creatinine, cholesterol and glucose levels, but significantly increased the serum content of uric acid. The treatment caused no significant effect on either water intake or urine output.

Workers from Nigeria have recently studied the effect of sub-chronic administration of aqueous extracts of *H. sabdariffa* calyx on the testes (Orisakwe *et al*., 2004), as the plant is often claimed in West African folk medicine to be an aphrodisiac (Dalziel, 1973; Orisakwe *et al*., 2004). Rats were given 1.15, 2.3 and

4.6 g/kg/day of an aqueous extract of *H. sabdariffa* calyx in the drinking water for up to 12 weeks. At the end of the treatment period there was a steady decrease in body weight, but no changes in the relative or absolute weights of the testes. However, the higher two doses of the extract caused a significant decrease in the epididymal sperm counts, histological distortion of tubules, disruption of normal testicular epithelial organization and disintegration of sperm cells. The authors postulated that these effects were related to interference by the extract with spermatogenesis that may have been caused by an oestrogenic action of the extract. Indeed, Ali *et al*. (1989) have previously alluded to this possibility. It is, however, difficult to ascribe the above testicular effects to an oestrogenic action in the absence of any significant change in testicular weight, as oestrogens are known to reduce the weights of the male reproductive organs. The relevance of the testicular toxicity of *H. sabdariffa* in humans is not certain when the relatively high amount of extract given in the drinking water for 12 weeks is taken into consideration.

Akindahunsi and Olaleye (2003) suggested that, in rats, the average consumption of 150–180 mg/kg/day of an aqueous-ethanol extract of *H. sabdariffa* calyces appeared to be safe, although higher doses might elevate the activity of some plasma enzymes indicative of tissue function (such as alanine aminotransferase and aspartate aminotransferase). However, the activity of some related plasma enzymes (alkaline phosphatase and lactate dehydrogenase) was not significantly affected, nor was there any evidence of histological damage to the heart and liver of the treated rats.

Mutagenicity of roselle colour was reported by Takeda and Yasui (1985) and the compound responsible for this activity was suggested to be quercetin.

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