

## The nutritional composition and biological activities of *Hibiscus sabdariffa* calyces

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### ABSTRACT

The extract of *Hibiscus sabdariffa* calyces is used as a cold or hot drink in various parts of the world. While some individuals drink it for pleasure, many others drink the extract to treat certain ailments. The calyx contains various potential bioactive compounds which include anthocyanins (especially delphinidin- and cyanidin-3-sambubiosides), flavonoids, saponins and vitamin C. In this review, literature related to the potential benefits of *Hibiscus sabdariffa* as hypotensive, hypolipidaemic, anti-inflammatory, antidiabetic, hepatoprotective and antioxidant is presented. Significant benefits of the consumption of *Hibiscus sabdariffa* calyces have been reported, although the majority of studies used cell or animal models. Potential mechanisms of action and bioactive components have also been examined and identified where possible. The LD50 of the extract of *Hibiscus sabdariffa* calyces was reported to be above 5000 mg/kg. Daily intake of 150-180 mg/kg was considered safe for humans. This review suggests that *Hibiscus sabdariffa* calyces have the potential for use as a nutraceutical. However, bioavailability

and robust human intervention studies are needed to confirm the antihypertensive, antidiabetic and hypolipidaemic benefits of the consumption of the extract of *Hibiscus sabdariffa* calyces.

**KEYWORDS:** *Hibiscus sabdariffa* calyces, nutrient composition, hypertension, dyslipidaemia, diabetes, cancer, oxidative stress, mode of action

### Scope of the review

This review focuses on the composition of the calyces of *Hibiscus sabdariffa* and its role in health and disease conditions. Web of Knowledge/ Science and Google Scholar were searched in October 2015 using the terms *Hibiscus*, *Hibiscus sabdariffa* calyces and Roselle calyces, and over 160,000 articles were identified. The search was narrowed to 135 articles that investigated *Hibiscus sabdariffa* calyces (not any other parts of the plant) either directly or indirectly. Article publication date was not a criteria for selection. Some articles are presented in more than one of the following sections:

1. Introduction
2. The composition of *Hibiscus sabdariffa* calyces
3. Hypotensive activity of *Hibiscus sabdariffa* calyces
4. Hypolipidaemic activity of *Hibiscus sabdariffa* calyces
5. Antidiabetic effect of *Hibiscus sabdariffa* calyces
6. *Hibiscus sabdariffa* calyces in apoptosis and cancer

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7. Hepatoprotective effect of *Hibiscus sabdariffa* calyces
8. Antioxidant activity of *Hibiscus sabdariffa* calyces
9. Other activities of *Hibiscus sabdariffa* calyces
10. Toxicity of *Hibiscus sabdariffa* calyces
11. Possible modes of action of bioactive compounds present in the extract of *Hibiscus sabdariffa* calyces

## Introduction

*Hibiscus* is a member of the Malvaceae family with over 300 species, among which *Hibiscus sabdariffa* is the most commonly known. Its common names include Roselle/Red sorrel (English), I'Oiselle (French), Jamaica (Spanish), Karkade (Arabic), Bissap (Wolof), Yakuwa/Zobo (Hausa), Ishapa (Yoruba), Okworo-ozo (Igbo), Sour Tea (Farsi) [1], Gongora in Hindi [2] and Krachiap daeng in Thai [3]. *Hibiscus sabdariffa* is a herb that is cultivated for leaf, fleshy calyx or seed [4]. It is a crop widely cultivated in Sub Saharan Africa and other tropical and subtropical parts of the world. African farmers cultivate *Hibiscus sabdariffa* mainly for income generation [5].

Most hibiscus species are used as ornamental plants, but many are believed to have certain nutritional and medicinal properties; among them is *Hibiscus sabdariffa* [4, 6]. Infusions of the thick red and fleshy cup-shaped calyces of the flower are consumed worldwide as a cold beverage and/or hot drink (sour tea) [7]. In Nigeria, *Hibiscus sabdariffa* is mainly cultivated in the northern part of the country and the production was estimated to be 138, 000 metric tonnes per annum [8]. The extract of *Hibiscus sabdariffa* calyces is a common local drink popularly known as Zobo in Nigeria and the average consumption of these beverages is 150-180 mg/kg per day [1]. Zobo drink is usually made by boiling and filtering the calyces of *Hibiscus sabdariffa* flower. The drink is gaining wide acceptance, being consumed by several millions of people from different socio-economic classes and background in the West Africa. Zobo drink is considered as an alternative source of cheap and relaxing non-alcoholic drink in social gatherings [9]. In Africa, the leaves and calyces of *Hibiscus sabdariffa* are frequently cooked as a side dish eaten with pulverized peanuts [7]. Villanueva-Carvajal *et al.* [10] suggested the consumption of the whole

calyces of *Hibiscus sabdariffa* instead of infusion for maximum benefit. *Hibiscus sabdariffa* is considered as an ideal yet under-exploited candidate for nutraceutical applications [11] but more well-designed controlled clinical trials are needed to confirm the health benefits of its consumption [12]. A recent review proposed that in the near future the Hibiscus calyces industry will be one of the most important in India and that the calyces could be an alternative or additional beverage to be considered by the beverages industries [13]. The published findings on the nutritional and health benefits of consumption of the extract of *Hibiscus sabdariffa* calyces have attracted the attention of scientists, farmers and food industries worldwide.

## The composition of *Hibiscus sabdariffa* calyces

Many studies have determined the nutrient and bioactive composition of the calyces of *Hibiscus sabdariffa*. These include phytochemicals, volatiles, macro and micronutrients. There were marked inconsistencies in the reported concentrations of carbohydrate, protein and fat content of the calyces of *Hibiscus sabdariffa* which make comparison difficult. These variations may be attributed to differences in the samples, extraction and analytical methods.

Carbohydrate contents of the calyces of *Hibiscus sabdariffa* differ markedly among research findings (0.5 to 68.7%) by approximately 150-fold [14-16]. This variation may be attributed to the differences in the type and source of the calyces of *Hibiscus sabdariffa* used. However, the whole calyces of *Hibiscus sabdariffa* have higher total carbohydrate than the aqueous extract. This is due to the fact that the extract may contain only soluble fibres while the whole calyx contains both soluble and insoluble fibres. However it seems very unlikely (as indicated in one of the findings) that the calyces of *Hibiscus sabdariffa* have comparable or even higher than the reported carbohydrate content of some cereals like brown wheat flour, oatmeal or white bread [17]. Protein contents of the calyces were found to range from as low as 0.000004% to about 10% [14-16, 18-20]. This variation is unexpected as most researchers used a similar method of analysis. Another study found 3026.11 mg/100 g as the total amount of 16 amino acids detected in the extract of *Hibiscus sabdariffa* calyces with aspartic acid predominating [21]. It is worthy of note that

most of the reported carbohydrate and protein contents were found by a single determination method which would not account for experimental error. Fat content of the extract of *Hibiscus sabdariffa* calyces showed about 10-fold difference between the lower and upper reported levels which were 0.59 and 9.26%, respectively [15, 19, 20]. The high fibre and ash contents of the calyces [15, 16, 18-20, 22, 23] are indicative of their potential as a source of both soluble and insoluble fibres. The large differences in the reported macronutrient content of the extract of *Hibiscus sabdariffa* calyces reported in the literature are probably due to the considerable variability in the preparation methods, source of the calyces and analytical techniques employed. This makes it difficult to estimate their composition with any degree of confidence. The macronutrient content of the extract of *Hibiscus sabdariffa* calyces seem to be higher than that of the black tea, which contain only traces of the macronutrients [17].

Approximately 30 phytochemicals were reported to be present in *Hibiscus sabdariffa* calyces (Table 1) and the acceptability of hibiscus drink was found to correlate positively with its anthocyanin content [24]. Most of the biological activities of the calyces are related to one or more of these compounds. Notable among them are the anthocyanins delphinidin- and cyanidin-3-sambubiosides. Extraction of hibiscus calyces with water at 100 °C was recommended to get optimal extraction of the anthocyanins in the calyces [25]. Similar to the macronutrient content, variation in the phytochemical concentrations, for example in total phenolic contents, was remarkable and attributable to differences in the sample sources, preparation and methods of analysis. For example, pH differential method was employed by some researchers to determine the anthocyanin content [26, 27] while others used high performance liquid chromatography (HPLC) in the analysis of anthocyanin and other phenolics in the calyces [27-30]. It is worthy of note that the natural pH of the extract of *Hibiscus sabdariffa* calyces may temper with the accuracy of results from the pH differential method. It has also been shown that a natural non-caloric sweetener stevia can increase the stability of hibiscus phenolics [31]. Furthermore, Folin-Ciocalteu method was used by some researchers to determine the total phenolics in the extract of the calyces [19, 32, 33]. However,

vitamin C in the calyces may interfere with the results obtained through this method and may lead to falsely elevated values. Over fifty aroma/volatile compounds which include aldehydes, ketones, alcohols and mineral acids were reported to be present in the calyces [34-36]. This could explain the sensory attributes of the calyces.

The vitamin C content of *Hibiscus sabdariffa* calyces has been reported by many groups (Table 2). This may be due to consideration of its role as an antioxidant in relation to some biological activities of the calyces. Table 2 shows variations in vitamin C content which can be attributed to the differences in sample types, handling, processing and analytical methods. Other vitamins are either absent or not widely studied. Irrespective of the variation in minerals content (Table 2), the calyces could be said to be a source of important minerals like iron and calcium. However, the bioavailability of these minerals has not been established and the contribution to daily intake could be small.

#### **Hypotensive activity of *H. sabdariffa* calyces**

Several human studies have reported the potential hypotensive benefits associated with the consumption of the extract of *Hibiscus sabdariffa* calyces [42-49]. Recent studies [50, 51] have shown that consumption of the extract of *Hibiscus sabdariffa* calyces at a daily dose of 150 mg/kg body weight for 4 weeks by newly diagnosed mild and moderate hypertensive Nigerians (who have not started treatment) was more effective in blood pressure reduction than 25 mg/day for 4 weeks of antihypertensive drug hydrochlorothiazide (HCTZ) and comparable to 10 mg/day for 4 weeks of lisinopril in Angiotensin Converting Enzyme (ACE) inhibition. In a randomised, controlled, double blinded human clinical trial, consumption of the extract of *Hibiscus sabdariffa* calyces (containing 250 mg anthocyanins) by 171 hypertensive men and women aged 25 to 61 years daily for four weeks resulted in an absolute decrease of 17.14 and 11.97 mmHg in systolic and diastolic blood pressures, respectively [47] and did not cause any pathological changes related to renal and hepatic functions. In a related study, a significant blood pressure reduction was reported as a result of the consumption of the extract of *Hibiscus sabdariffa* calyces (containing 9.6 mg anthocyanins) daily for four weeks by 39 hypertensive patients who were

**Table 1.** Polyphenols content of *Hibiscus sabdariffa* calyces (g/100 g).

Group	Class	Sub-class	Minimum reported	Maximum reported	Reference(s)
Flavonoids	Anthocyanins	Delphinidin-3-sambubioside	0.27	0.40	[27, 28]
		Cyanidin-3-sambubioside	0.17	0.20	[27, 28]
		Total anthocyanins	0.01	0.71	[22, 26]
	Anthocyanidins	Total anthocyanidins	0	30.80*	[19, 37]
	Flavonols	Quercetin-3-rutinocide	0.03	0.05	[27, 28]
		Quercetin-3-sambubioside	0.03	0.03	[28]
		Quercetin-3-glucoside	0.01	0.01	[28]
		Quercetin	0.01	0.01	[28]
		Kaempferol-3-O-rutinoside	0.01	0.01	[28]
		Kaempferol-3-(p-coumarylglucoside)	$0.28 \times 10^{-2}$	$0.28 \times 10^{-2}$	[28]
		Myricetin-3-arabinogalactose	0.01	0.01	[28]
	Total flavonols	5.87*	22.00*	[19]	
	Total	Total flavonoids	0.419	5.36	[33, 37]
Phenolic acids	Hydroxycinnamic acids	3-Caffeoylquinic acid	0.20	0.29	[27]
		5-Caffeoylquinic acid	0.15	0.18	[27]
		4-Caffeoylquinic acid	0.05	0.08	[27]
		5-O-Caffeoyl shikimic acid	0.02	0.02	[28]
		Caffeoylquinic acid isomer	0.02	0.05	[27]
		Chlorogenic acid	0.19	20.18	[28, 38]
		Chlorogenic acid isomer I	0.28	0.28	[28]
		Chlorogenic acid isomer II	0.10	0.10	[28]
		Hydroxycoumarin	0.18	0.18	[28]
		Total hydroxycinnamic acids	21.00*	30.60*	[19]
	Hydroxybenzoic acids	Gallic acid	$0.12 \times 10^{-2}$	$0.12 \times 10^{-2}$	[27]
		Protocatechuic acid glucoside	$0.02 \times 10^{-2}$	$0.76 \times 10^{-3}$	[27]
		Total hydroxybenzoic acids	28.00*	32.60*	[19]
Total	Total phenolics	Total phenolics	0.019	10.0	[16, 19, 26, 27, 32, 33, 37, 39]
Other organic acids and their derivatives	Other organic acids and their derivatives	Hibiscus acid	0.01	3.11	[27-29]
		Hibiscus acid glucoside	$1.84 \times 10^{-3}$	0.02	[27]
		Hibiscus acid-6-methyl ester	$0.92 \times 10^{-3}$	$4.64 \times 10^{-3}$	[27]
		Hydroxycitric acid	0.83	0.83	[28]
Phenolic amine	Phenolic amine	N-Feruloyltyramine	$0.99 \times 10^{-2}$	$0.99 \times 10^{-2}$	[28]

\*Percentage of total extractable phenolics.

**Table 2.** Vitamins and minerals in *Hibiscus sabdariffa* calyces.

Micronutrient	Name	Minimum reported	Maximum reported	Reference(s)
Vitamins (mg/100 g)	Vitamin C	$7.26 \times 10^{-5}$	$7.50 \times 10^{-3}$	[14, 16, 18, 20, 22, 26, 40, 41]
	Carotene	0.03	0.03	[20]
	Vitamin B1	0.12	0.12	[20]
	Vitamin B2	0.28	0.28	[20]
	Vitamin B3	3.77	3.77	[20]
Minerals (ppm)	Iron	0.40	$8.98 \times 10^4$	[18, 20, 22, 23, 40, 41]
	Calcium	2.00	$7.05 \times 10^4$	[18, 20, 22, 41]
	Zinc	28.00	$3.73 \times 10^4$	[22, 23, 41]
	Sodium	25.11	59.40	[18, 41]
	Potassium	219.00	235.00	[18]
	Magnesium	5.75	$2.68 \times 10^4$	[18, 22]
	Phosphorus	$2.73 \times 10^3$	$2.73 \times 10^3$	[20]

not on medication for at least one month prior to participation in the study when compared to 36 apparently healthy volunteers [43]. These data suggest that consumption of the extract of *Hibiscus sabdariffa* calyces containing as little as 9.6 mg anthocyanins can significantly lower blood pressure in humans. Consumption of the extract of *Hibiscus sabdariffa* calyces in the form of sour tea for twelve days caused a reduction in systolic and diastolic blood pressures by 11.2% and 10.8%, respectively in patients with essential hypertension. Three days after the tea withdrawal, systolic and diastolic blood pressures increased by 5.6% and 6.2%, respectively [48]. A randomised, double blinded, placebo-controlled clinical trial (conducted in 65 pre- and mildly hypertensive adults aged 30 to 70 years and not on any antihypertensive medication) showed that daily consumption of 720 mL of the extract of *Hibiscus sabdariffa* calyces for six weeks significantly lowered systolic and diastolic blood pressures by 5.5 and 4%, respectively [45]. This further strengthens the health claim for the usage of the extract of *Hibiscus sabdariffa* calyces as an antihypertensive agent.

In support of these human studies, administration of the extract of *Hibiscus sabdariffa* calyces at a daily dose of 250 mg/kg for eight weeks caused an approximate 34.4 mmHg reduction in systolic

blood pressure in 2-Kidney, 1-Clip renovascular hypertensive rats [42]. In addition, intravenous injection of the aqueous extract of *Hibiscus sabdariffa* calyces at a dose of 1-25 mg/kg to rats with salt-induced and L-NAME ( $N^0$ -L-arginine methyl ester)-induced hypertension showed a fall in arterial pressure [44]. This animal data supports the potency of the calyx as an antihypertensive agent. In addition, consumption of the extract of *Hibiscus sabdariffa* calyces reduced blood pressure in spontaneously hypertensive rats [52], normal rats [53] and anaesthetised cats [54].

Even though a great deal of evidence suggests that the extract of *Hibiscus sabdariffa* calyces possesses antihypertensive properties, a systemic review by Wahabi *et al.* [55] highlighted that out of the human intervention trials that investigated the benefits of the consumption of the extract of *Hibiscus sabdariffa* calyces only one study [47] was a fully randomised, blinded clinical trial. Therefore, the need to conduct more robust high quality randomised clinical trials was recommended.

#### **Hypolipidaemic activity of *H. sabdariffa* calyces**

Dyslipidaemia has been implicated as a risk factor for metabolic syndrome (MeSy), type 2 diabetes and cardiovascular diseases (CVD). Consumption of the aqueous extract of *Hibiscus sabdariffa* for 12 weeks

at a dose of 20 g/kg diet reduced serum triacylglycerol (TAG) concentration in rats fed with a high-fructose diet. In addition, it reduced serum cholesterol in rats fed with a high-cholesterol diet. Serum low density lipoprotein cholesterol (LDL-c) concentrations and LDL-c/high density lipoprotein cholesterol (HDL-c) ratio were reduced in both high-fructose and high-cholesterol diet-fed rats [56]. This suggests that the consumption of the extract of *Hibiscus sabdariffa* calyces may be beneficial in reducing some hyperlipidaemic states in animal models. A related study in rats showed the same effect [57]. The extract of *Hibiscus sabdariffa* calyces administered at a daily dose of 1000 mg/kg for six weeks lowered serum TAG, total cholesterol (TC) and LDL-c in animal models [3, 58, 59]. Consumption of the aqueous extract of *Hibiscus sabdariffa* calyces at a dose of 10 g/L for 14 weeks reduced serum TAG concentration by 50% [28]. In another study, an aqueous extract of red or green *Hibiscus sabdariffa* calyces at a daily dose of 1 and 1.5 mg/kg for 28 days lowered TC in rats. This was strongly associated with decreased LDL-c with no significant alteration in TAG and HDL-c levels [60].

A limited number of human studies investigating the impact of the consumption of the extract of *Hibiscus sabdariffa* calyces on lipid profile have been performed, the results of which suggested benefits in individuals with MeSy [61]. A significant reduction in plasma glucose and TC concentrations, increased HDL-c levels and improved TAG/HDL-c ratio (insulin resistance marker) was reported in 51 patients with MeSy who consumed the extract of *Hibiscus sabdariffa* calyces at a dose of 100 mg/day for one month [62]. Consumption of the extract of *Hibiscus sabdariffa* calyces twice daily for one month caused 7.6% reduction in TC, 8.0% reduction in LDL-c, 14.9% reduction in TAG, 3.4% reduction in Apo-B100, 16.7% increase in HDL-c, and 4.2% increase in Apo-A1 in patients with type II diabetes mellitus [63]. Furthermore, in a randomised clinical trial, the increase in both TC and HDL-c in hypertensive subjects were significant after consumption of the extract of *Hibiscus sabdariffa* calyces (twice daily for 15 days) and its withdrawal at day 30 (i.e. 15 days after extract administration stopped) relative to their initial/baseline values [64]. Increase in TC in this study may be due to withdrawal of the extract administration, increase

in HDL-c, which is a component of TC concentration and/or lack of dietary control in the subjects studied. Although the quantity of hibiscus in a capsule is not clearly stated, research findings showed that a daily dosage of 1 or 2 capsules of *Hibiscus sabdariffa* calyces (each capsule containing 20.1, 10 and 14 mg of anthocyanins, flavonoids and polyphenols, respectively) for one month lowered serum cholesterol level by 11 to 15% in hypercholesterolaemic men and women. This suggests that the capsules of *Hibiscus sabdariffa* calyces may be effective hypolipidaemic agents [59]. However, recent reviews and meta-analysis [65, 66] suggested the need for more rigorously designed trials to confirm the benefits of the consumption of the extract of *Hibiscus sabdariffa* calyces on lipids profile.

#### **Antidiabetic effect of *Hibiscus sabdariffa* calyces**

Even though human studies are lacking, animal studies support the potential beneficial impact of the consumption of the extract of *Hibiscus sabdariffa* calyces on diabetes pathophysiology. Administration of the extract of *Hibiscus sabdariffa* calyces at a dose of 200 mg/kg for seven weeks improved insulin sensitivity and lowered blood glucose levels in type 2 diabetic rat model [67]. The extract of *Hibiscus sabdariffa* calyces administered at a daily dose of 400 mg/kg for 8 weeks attenuated diabetic nephropathy in streptozotocin-induced type I diabetic rats through its antioxidative and anti-apoptotic activities [68, 69]. Related studies showed similar effect in type 2 diabetic rats [70, 71]. In another study, aqueous extract of *Hibiscus sabdariffa* calyces were reported to have beneficial action against damage caused by free radicals in alloxan-induced diabetic rats. This action was related to the *in vitro* and *in vivo* antioxidant activity of the extract [72]. These data suggest the potential use of the extract of *Hibiscus sabdariffa* calyces in the dietary management of diabetes, although this requires confirmation in robust controlled human dietary intervention studies.

#### ***Hibiscus sabdariffa* calyces in apoptosis and cancer**

The role of *Hibiscus sabdariffa* calyces in apoptosis has been studied predominantly in cell models. Several cell studies have demonstrated the potential of the extract of *Hibiscus sabdariffa* calyces as

anti-tumorigenic. Anthocyanins in the extract of *Hibiscus sabdariffa* calyces caused cancer cell apoptosis, especially in human leukaemia cells (HL-60). Twenty-four-hour exposure to 0.05-4 mg/ml of hibiscus anthocyanins markedly induced dose- and time-dependent apoptosis in HL-60 cells [73]. In a related study, delphinidin-3-sambubioside, an anthocyanin present in *Hibiscus sabdariffa* calyces, induced a dose- and time-dependent apoptosis in HL-60 cells probably through a reactive oxygen species-mediated mitochondrial dysfunction pathway [74]. Tsai *et al.* [75] demonstrated the antileukaemic ability of the extract of *Hibiscus sabdariffa* calyces in rats. Protocatechuic acid (3,4-dihydroxybenzoic acid), a simple phenolic found in *Hibiscus sabdariffa* calyces was found to inhibit most of the cancers especially of the digestive system at a dose of 200-2000 ppm irrespective of the stage of carcinogenesis [76-78].

The extract of *Hibiscus sabdariffa* calyces have anti-proliferative activity on oestrogen-receptor positive and oestrogen-receptor negative human breast cancer (MCF-7 and MDA-MB-231, respectively), human cervical cancer (HeLa), and human ovarian cancer (Caov-3) cell lines [79]. Based on its safety as evident in being non-toxic to experimental animals at high doses and its ability to inhibit MCF-7 cells in a selective, concentration- and time-dependent manner, the extract of *Hibiscus sabdariffa* calyces might be a good candidate for anti-breast cancer drug development [80]. At a dose of 1 g/kg body weight, *Hibiscus sabdariffa* calyces exhibit antimutagenic activity against mutagens methylazoxymethanol acetate and heterocyclic amines and decreased the number of aberrant crypt foci induced by some colon carcinogens in rats [81]. The extract of *Hibiscus sabdariffa* calyces at graded doses of 0 to 5 mg/ml exhibited a dose-dependent cell apoptosis induction activity by proliferating smooth muscle cells of rat [82, 83]. These results suggested the potential benefit of *Hibiscus sabdariffa* calyces in cancer treatment but require confirmation through human intervention trials.

### **Hepatoprotective effect of *Hibiscus sabdariffa* calyces**

Age-related changes in the structure and function of liver are related to various disease conditions [84]. The consumption of the extract of *Hibiscus sabdariffa*

calyces has demonstrated hepatoprotective effects in numerous animal studies [57, 85-87]. A daily dose of 200 mg/kg of the aqueous extract of *Hibiscus sabdariffa* calyces for four weeks restored paracetamol-induced hepatotoxicity in rats [88]. Protocatechuic acid (PCA), a polyphenolic compound present in *Hibiscus sabdariffa* calyces, protected rats against *tert*-butyl-hydroperoxide (t-BHP)-induced hepatotoxicity. This effect was related to PCA's antioxidant and anti-inflammatory properties accompanied by stress signal transduction blockage [89]. In a related research, anthocyanins present in *Hibiscus sabdariffa* calyces exhibited hepatoprotective effect against t-BHP-induced cytotoxicity in rats [86, 90]. Both PCA and anthocyanins present in *Hibiscus sabdariffa* calyces reduced the incidence of liver lesions including inflammation, leucocyte infiltration, and necrosis induced by t-BHP in rats [86].

Pre-treatment with the extract of *Hibiscus sabdariffa* calyces was reported to have a protective effect against azathioprine-induced hepatotoxicity in rats. This effect was found to be through reducing malondialdehyde (MDA) levels and reversing the inhibitory effect of azathioprine on glutathione, catalase and superoxide dismutase activities [87]. Administration of the aqueous extract of *H. sabdariffa* calyces lowered the levels of alanine and aspartate aminotransferases as well as carbon tetrachloride (CCl<sub>4</sub>)-induced lipid peroxidation inhibition in rats. These data suggest that the extract may protect rat liver against CCl<sub>4</sub>-induced fibrosis. Antioxidants in *Hibiscus sabdariffa* calyces appear to be responsible for the hepatoprotective effect [85]. Furthermore, aqueous extract of *Hibiscus sabdariffa* calyces prevented lipid peroxidation against potassium bromate-induced tissue damage in Wister rats, which was found to be dose-dependent [91] and also protected mouse liver from acetaminophen-induced acute liver damage [92].

Although the potential hepatoprotective effect of *Hibiscus sabdariffa* calyces have been demonstrated in animal studies, human studies are very limited. Consumption of 75 g of the extract of *Hibiscus sabdariffa* calyces in the form of capsules for 12 weeks improved liver steatosis in both humans and mice [21, 93]. The lack of human studies and the lack of clarity on the molecular mechanisms of action justify the need to explore this aspect from whole body system to cellular level.

### **Antioxidant activity of *Hibiscus sabdariffa* calyces**

Oxidative stress is one of the major concerns in the aetiology of age-related diseases like diabetes and cardiovascular diseases. *Hibiscus sabdariffa* calyces are reported to be a good source of antioxidants such as anthocyanins [94] and vitamin C [8, 26, 40]. Although out of fashion nowadays, the antioxidant properties of *Hibiscus sabdariffa* calyces and other hibiscus species have been previously studied [95, 96]. In a study involving 8 healthy non-smoking volunteers of both sexes (aged 22 to 27 years), a single dose (quantity not specified) of the extract of *Hibiscus sabdariffa* calyces with concentration of 10 g/200 mL enhanced the systemic antioxidant potential and reduced oxidative stress [97]. However, it is worthy of note that systemic antioxidant activities of the extract of *Hibiscus sabdariffa* calyces are not widely studied in humans.

Previous data suggested that anthocyanin is the major contributor of antioxidant capacity in hibiscus extract, accounting for 51% of the antioxidant capacity of the calyces [98]. Specifically, the anthocyanins delphinidin-3-sambubioside and cyanidin-3-sambubioside isolated from the methanolic extract of hibiscus calyces appear to contribute largely to the antioxidant activity of the extract and they were found to inhibit lipid peroxidation *in vitro* with 64% and 63% inhibition of liposome peroxidation, respectively [99]. This is comparable to another finding which reported that the extract of *Hibiscus sabdariffa* calyces inhibits lipid peroxidation between 69 and 79% [100]. Protocatechuic acid isolated from *Hibiscus sabdariffa* calyces has antioxidant property. It was found that 1 mM of protocatechuic acid significantly inhibited copper or nitric oxide (NO) donor-induced human low density lipoprotein (LDL) oxidation *ex vivo* [101]. In another study, superoxide anion scavenging chemicals in hibiscus were found to include ascorbic acid (39%), delphinidin-derivatives (51%) and cyanidin-derivatives (10%) [102].

Several other studies have supported the antioxidant activity of *Hibiscus sabdariffa* calyces [3, 32, 58, 59, 74, 90, 103-108]. At a dose of 10 and 20 g/kg of a high fructose diet, *H. sabdariffa* calyces inhibited LDL oxidation and lowered serum triacylglycerol, cholesterol and LDL-cholesterol

in rats, rabbits and humans [56, 58, 59]. Aqueous extract of *Hibiscus sabdariffa* calyces showed high reducing capacity and significant capability to scavenge peroxy radicals through hydrogen atom transfer, indicating that the extract may protect cells from damage by lipid peroxidation [28, 109]. Commercialised hibiscus juice exhibit a strong antioxidant activity attributable to its flavonoids and saponins content [79]. A previous study demonstrates that the extracts of *Hibiscus sabdariffa* calyces at a dose of 200 and 300 mg/kg for 7 days have a dose-dependent protective effect against sodium arsenate-induced oxidative damage in rat model [110]. At a dose of 0.2 mg/ml the extract of *Hibiscus sabdariffa* flower scavenged 1,1-dicylhydraxyl (DPPH) radical but was unable to scavenge nitric oxide radical *in vitro* [110]. These findings are suggestive of the possible chemopreventive and antioxidative role played by dried flower extract of *Hibiscus sabdariffa* [109, 110]. However, the selective radical scavenging activity of the extract of *Hibiscus sabdariffa* flowers observed [110] seems to be unexpected, and calls for further investigation.

Studies on the *ex vivo* inhibitory activity on lipid peroxidation and *in vivo* effects on cholesterol-induced hyperlipidaemia in rats suggested that the antioxidant and hypocholesterolaemic activities of ethanolic extracts of calyces and leaves of *Hibiscus sabdariffa* can be linked to polyphenol and flavanol content of the extract [57]. An aqueous extract of *Hibiscus sabdariffa* calyces exhibited a strong antioxidant activity in *ex vivo* copper-mediated oxidation of rat LDL. LDL oxidation inhibitory effect was found to be dose-dependent and 5 mg/ml of the extract had greater antioxidant potency than 100  $\mu$ M of vitamin E [108]. Red hibiscus was found to have greater potential health benefit than the white hibiscus. This is based on the greater polyphenol content in the methanolic extract of the calyces of red *Hibiscus sabdariffa* as compared to the white [99, 100]. According to a research finding *H. sabdariffa*-based drinks can be considered as protective beverages and regular consumption might protect against free radical damage [111]. There seems to be some evidence to suggest the role of the extract of hibiscus calyces in managing oxidative stress and age-related complications when consumed, but more human intervention clinical trials are required to ascertain the level and mechanisms of



action of hibiscus bioactive compounds at the molecular level.

#### **Other activities of *H. sabdariffa* calyces**

A number of other potential activities of *Hibiscus sabdariffa* calyces have been reported. They include antimicrobial [4, 112-115], antinociceptive, antidiarrheal, anti-inflammatory [39, 116, 117] and anti-obesity [118] effects in various animal models. *Hibiscus sabdariffa* was also reported to inhibit the contractility of rat bladder and uterus [119]. Polysaccharides from *Hibiscus sabdariffa* calyces were shown to stimulate the proliferation and differentiation of human keratinocytes [120]. Several other potential benefits of the consumption of the extract of the calyces of *Hibiscus sabdariffa* have been identified. Notable among them are the ease of bowel evacuation, stimulation of digestion in the stomach and mucous membrane protection [20, 121, 122]. A research finding concluded that the extract of *Hibiscus sabdariffa* calyces can cause significant reduction in monocyte chemoattractant protein-1 (MCP-1) plasma concentration which implies that the extract may have potential in the treatment of chronic inflammatory diseases [46].

The aqueous extract of *Hibiscus sabdariffa* calyces showed a mild cathartic (cleansing) activity in a previous study [123]. This is related to the saponin (natural detergents with emollient properties) content of *Hibiscus sabdariffa* calyces. The extract possibly acts through modifying the permeability of electrolytes and water across the intestinal membrane [123]. Consumption of aqueous extract of *Hibiscus sabdariffa* calyces caused a loss of weight due to decreased food consumption in pregnant rats compared to control [124]. The decreased food consumption observed could be due to the unpalatability of the unsweetened extract [124]. The methanol extract of *Hibiscus sabdariffa* calyces inhibit the intestinal motility in rats [125]. Research finding concluded that aqueous extract of *Hibiscus sabdariffa* calyces might prove to be a useful memory restorative agent in the treatment of dementia seen in the elderly. This activity can be attributed to the anti-acetyl cholinesterase property of the extract [126].

#### **Toxicity of *Hibiscus sabdariffa* calyces**

Consumption of the extract of *Hibiscus sabdariffa* calyces is generally considered to be safe [21, 64].

Average consumption of 150-180 mg/kg/day by human appeared to be safe but a dose of 250 mg/kg/day for 15 days showed significant increase in serum albumin level in rat [1] suggesting possible impairment in liver function. A daily dose of 2000 mg/kg caused the death of all (ten in number, 5 each for water and ethanol calyx extracts) albino rats in eight days. The death of the animals was preceded by a severe weight loss and diarrhoea [127]. In a related study, aqueous-methanolic extract of *Hibiscus sabdariffa* calyces administered at 250 mg/kg for up to 10 days only showed a mild effect on the liver of rats. However, prolonging the days of extract administration to 15 has been associated with liver injury in rats. Histopathological studies showed no pathological effects on both liver and heart of rats after administration of the extract for up to 15 days [128]. Continuous consumption of the extract of *Hibiscus sabdariffa* calyces at a dose of 1000 mg/kg was reported to cause sudden death of spontaneously hypertensive rats, but was not harmful to normal rats at even higher doses. Another study concluded that consumption of 100 mg/kg body weight for 2 weeks maintained the liver and heart muscles in rabbits exposed to crude oil [129]. The LD<sub>50</sub> of the extract was found to be above 5000 mg/kg which indicates its apparent safety for consumption [52].

According to the 53<sup>rd</sup> meeting of WHO/FAO expert committee on food additives, the permissible limits of cadmium, lead, arsenic and mercury are 0.1, 0.2, 0.1 and 0.03 µg/g, respectively. The amounts of toxic and essential metal ions in *Hibiscus sabdariffa* calyces were found to be below the World Health Organisation permissible limits and may not constitute health hazard to consumers [23, 130]. There is no data on the toxicity of *Hibiscus sabdariffa* calyces in humans.

#### **Possible modes of action of bioactive compounds present in the extract of *Hibiscus sabdariffa* calyces**

It has been postulated that the hypotensive effect of the extract of *Hibiscus sabdariffa* could be associated with a number of potential mechanisms, including direct vasorelaxant effects. At a dose of 1.7 mg/ml, a maximum relaxation of pre-contracted endothelium-intact and endothelium-denuded aortic rings was observed *in vitro* [53, 131]. However, the extract relaxant effect was partly dependant on

the presence of a functional endothelium. The relaxant effect was significantly lower in endothelium-denuded aortic rings [131]. The hypotensive effect of the extract of *Hibiscus sabdariffa* calyces was proposed to be related to its ability to induce endothelium-dependant effects related to the nitric oxide synthase (NOS) activation by components within the extract. In addition the effect may be independent of the endothelium and could act through activation of potassium channels in the underlying smooth muscle cells [38]. However there is no evidence to suggest that the hypotensive effects associated with the extract of *Hibiscus sabdariffa* calyces is mediated via inhibition of sympathetic nervous system but may be mediated via nitric oxide (NO) or partly via inhibition of  $\text{Ca}^{2+}$  influx through receptor-gated channels [53, 132]. Acetylcholine-like and histamine-like mechanisms (neuronal mechanisms that are thought to be involved in blood pressure regulation) could be implicated in the hypotensive mechanism of action of *Hibiscus sabdariffa* calyces [53]. Hypotensive effect of the extract could also be through activation of endothelial NO-cGMP-relaxant pathway in which acetylcholine release lead to activation of eNOS [131]. Quercetin present in *Hibiscus sabdariffa* calyces has been related to NO release. Quercetin increases renal vasorelaxation via increasing kidney filtration rate [133].

In addition, the extract of *Hibiscus sabdariffa* calyces act as an inhibitor of angiotensin converting enzyme (ACE) *in vitro* [134, 135]. The anthocyanins (delphinidine-3-O-sambubioside and cyanidine-3-O-sambubioside) present in *Hibiscus sabdariffa* calyces competitively inhibit ACE from rabbit lung [135]. This mechanism of action is supported in human studies which related hypotensive activity of *Hibiscus sabdariffa* calyces to inhibition of ACE and decrease in serum sodium [47]. Polyphenols present in the extract of *Hibiscus sabdariffa* calyces were thought to act through multi-faceted metabolic regulation [136].

The possibility of the extract of *Hibiscus sabdariffa* calyces to serve as anti atherogenic through modulation of lipoprotein metabolism was supported by cell studies in which treatment with 0.5 and 1.0 mg/mL *Hibiscus sabdariffa* polyphenols for 18 hours enhanced the expression of LDL receptor and LDL binding of human hepatocyte cell line HepG2. This implies that *Hibiscus sabdariffa*

polyphenols may exert their hypolipidaemic effect through enhancing hepatic LDL clearance [137].

The extract of *Hibiscus sabdariffa* calyces in the form of tea has high inhibitory activity against porcine pancreatic  $\alpha$ -amylase. This activity may result in decreased glucose absorption with consequent reduction in postprandial glucose level which can aid in diabetes management [138, 139].

Induction of cell apoptosis by the extract of *Hibiscus sabdariffa* calyces appears to be possibly due to the potential of the extract to activate protein kinase pathway [82, 83]. Antihepatotoxic effect of the anthocyanins present in the extract of *Hibiscus sabdariffa* calyces is probably through their ability in quenching free radicals and decreasing malondialdehyde (MDA) formation [86, 90].

### Future prospects

The present review presents data which demonstrates the potentials of *Hibiscus sabdariffa* calyces as hypotensive, hypolipidaemic, anti-inflammatory, antidiabetic, hepatoprotective and antioxidant, yet the majority of studies have used experimental animals or cell culture studies. Extrapolation of results from these studies to humans is often questionable and hence there is a need to perform more controlled human intervention studies. There is the need to acutely assess the bioavailability of the active compounds present in *Hibiscus sabdariffa* calyces. The impacts of hibiscus drink consumption on vascular function and nitric oxide need to be investigated. Potential mechanisms of action also require elucidation. The advent of -omics techniques could aid in the determination of molecular effects of the extract of *Hibiscus sabdariffa* calyces and the possible contribution to the observed, often large variations of the effects. Valid determination of the nutrient and phytochemical content of *Hibiscus sabdariffa* calyces also needs to be addressed. Furthermore, the fact that gastrointestinal tract plays a very significant role in the metabolism and conjugation of polyphenols [140] makes the need to investigate the biotransformation of polyphenols present in the extract of *Hibiscus sabdariffa* calyces in the gastrointestinal tract to identify the active metabolites responsible for its various actions. Since flavonoid-rich foods have been reported to play positive roles in age-related decline in cognitive function [141], there is a need

to investigate the effect of the consumption of the extract of *Hibiscus sabdariffa* calyces and their polyphenols on memory and cognition.

### CONCLUSION

This review has identified potential beneficial properties of *H. sabdariffa* calyces which could be classed as a nutraceutical. The reported nutrient and phytochemical composition of the calyces supports its usage both as a source of some nutrients (such as vitamin C and iron) and as a potential medicine. However well-controlled, randomised human intervention studies are required to determine the extent to which the consumption of the extract of *Hibiscus sabdariffa* calyces is associated with the significant health benefits and chronic disease risk reduction.

### ACKNOWLEDGEMENTS

Thanks to the Tertiary Education Trust Fund (TETFund) and Bayero University Kano, Nigeria, for sponsoring S. M. Abubakar.

### CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

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