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A review on phytochemistry and therapeutic uses of *Hibiscus sabdariffa* L.

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ABSTRACT

Hibiscus sabdariffa L. (roselle) belonging to the Malvaceae family is widely grown in many countries. This plant is often used in the traditional medicine being rich in phytochemicals like polyphenols especially anthocyanins, polysaccharides and organic acids thus having enormous prospective in modern therapeutic uses.

The study aimed to review and document all the available evidence and information about the calyces of *Hibiscus sabdariffa* (roselle) with the special focus on their nutritional composition, bioactive constituents and therapeutic uses. The electronic database was searched up to 2017, using keywords *Hibiscus sabdariffa*, chemical constituents of roselle, therapeutic uses of roselle. Journals, books and conference proceedings were also searched.

The review provides valuable information about the nutraceutical component of *Hibiscus sabdariffa* L. and their utilization for curing various degenerative diseases like hypertension, hyperlipidemia, cancer and other inflammatory diseases of liver and kidney. Their toxicological effects have also been discussed from a safety point of view.

Most studies supported and provided the scientific basis for the statement that *Hibiscus sabdariffa* and their active constituents play an important role in the prevention of chronic and degenerative diseases that are associated with oxidative stress. Our study suggests, that good research is needed, to establish a potential strategy that can balance the pharmacological and toxic effects of roselle and standardized fingerprint of *Hibiscus sabdariffa* is required internationally for quality control.

1. Introduction

Plants have played an important role in the life of human being as they provide the basic need of mankind that is food, clothing, shelter, and medicines. They have formed the basis of traditional medicine system among which are Ayurvedic, Unani etc. that have been in existence for many decades and continue to provide mankind with new remedies. In developing countries, a large section of the population relies on medicinal plants for primary health care requirements. The traditional medicines are becoming popular among most of the world population mainly because they are cheap, abundant with less adverse effect on health. In recent years, focus on plant research has increased globally to find out the immense potentials of medicinal plants used in various traditional systems. Various medicinal plants have been studied which could be used as potent phytochemical agents in the therapeutic treatment of various diseases; one among them is *Hibiscus sabdariffa*-known for its delicacy and medicinal properties which has several health benefits [1].

There are only a few reviews available on *Hibiscus sabdariffa.* Only two previous detailed reviews are available one focusing on phytochemical, pharmacological and toxicological properties [1] and another review, on the phytochemistry, pharmacological properties and economic-botanical aspects of roselle [2]. Other systematic reviews have investigated the effect of *Hibiscus sabdariffa* in the treatment of hypertension [3], hyperlipidemia, hypertension and apoptosis [4] and hyperlipidemia and hypertension [5]. In another systematic review of human clinical trial by Walton et al. [6] assessed the effectiveness of roselle in the treatment of hypertension. A recent review by Singh et al. [7] focused on the nutritional and health benefits of *Hibiscus sabdariffa.* In a another recent review, Herranz-López et al. [8] have focused on the multi-targeted molecular effect of roselle polyphenols on obesity management. In their review they mentioned that there is a need to understand the molecular mechanism of roselle polyphenols and metabolites involved, through virtual screening and epigenetic analysis.

The present review aims to document the detail information on the calyces of the flower of roselle (*Hibiscus sabdariffa* L.). It will also focus on the traditional uses, nutritional composition, bioactive constituents and the therapeutic uses of this plant. Most of the pharmacological

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investigations on the roselle plant has been summarised that provides a scientific basis for its use as functional food and to facilitate further investigations on the therapeutic uses of this plant.

2. Origin, distribution and morphology

Hibiscus sabdariffa, with an attractive flower, is widely grown in many developing countries. More than 300 species are distributed in tropical and subtropical regions around the world [9]. They are originally native from India, to Malaysia [10] where it is commonly cultivated and was carried at an early date to Africa. It is also cultivated in Sudan, Egypt, Nigeria, Mexico, Saudi Arabia, Taiwan, West Indies and Central America [11,12]. In India it is widely grown by the tribal in the villages of Madhya Pradesh, Maharashtra, Orissa, West Bengal, Assam, Meghalaya and Andhra Pradesh [13]. It is commonly known as roselle in English speaking regions, besides it is also known as Bissap in Senegal, Jamaica in Mexico and Spain, Congo in France, Wonjo in the Gambia, Zobo in Nigeria, Karkade in Egypt, Saudi Arabia and Sudan [14–17]. In the Indian subcontinent, it is known as Indian sorrel, mesta, lal ambari, patwa, amta and amti [18,13].

Hibiscus sabdariffa belongs to the family of Malvaceae. It is an annual or perennial herb or woody-based sub-shrub, growing to 2–2.5 m tall. The leaves are deeply 3-5 palmately lobed and 8–15 cm long, arranged alternately on the smooth, cylindrical red stems [19]. The flowers are auxiliary or terminal and 8–10 cm in diameter, white to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base, 1–2 cm wide, enlarging to 3–3.5 cm, fleshy and bright red as the fruit matures (Figs. 1 and 2,). It takes about six months to mature. Roselle is cultivated at the beginning of the rainy season during mid-April and harvested for the calyces of fruits, about 3 weeks till the onset of flowering [13,18,20].

3. Uses of *Hibiscus sabdariffa*

Traditionally roselle is cultivated for its stem, leaves, calyces and seeds as all parts have industrial, medicinal and other applications [21].

3.1. Traditional culinary usage

The young leaves and tender stems of roselle are eaten raw in a salad or cooked alone or with meat or fish, while Sudanese cook it with onion or groundnut, eat it as green or dry it [11]. In India, the women folk use the tender leaves and stem for making chutney during rainy season, eaten as salad, used as vegetable and women folk dry it for use during off-season [13]. Seeds are rich in protein and after oil extraction, they are boiled and eaten in soups and are also used as a substitute for coffee in Africa [9]. The seeds are fermented to produce a meat substitute condiment [22]. The Chinese use the seeds for oil extraction [23].

Fig. 1. Roselle plant at flowering stage.

Fig. 2. Fresh roselle calyces.

The calyces are either frozen or dried in sun /artificially for out of season supply, [17].The dried calyces of roselle are utilized worldwide in the production of drinks (herbal/ice tea), jams, jellies, sauces, chutneys, wines, preserves, and a source of natural food colourant due to the presence of anthocyanins [11,24–26]. They are also used in fruit salads and in the preparation of syrup. These sauces or syrups are added to the puddings, cake frosting and in ice-cream. In Africa, they are frequently cooked as a side-dish eaten with pulverized peanuts while in Pakistan, their calyces have been recommended as a source of pectin for fruit-preserving industry [9]. In West Indies and tropical America, roselle is primarily used for cooling lemonade like beverages made from the calyces and is an important drink during Christmas [27]. Lo Shen is the processed drink, used by the Chinese [28]. In the Indian state of Madhya Pradesh, roselle calyces are sold in the local market as indigenous vegetables and the majority of females prepare beverages from the flowers of *Hibiscus sabdariffa* [13].

3.2. Traditional therapeutic usage

Various parts of the roselle plant have been used in traditional medicine to treat colds, toothaches, urinary tract infections and hangovers. It is claimed to be a Thai traditional medicine for kidney and urinary bladder stones [29]. However in India, traditionally the tribal utilizes roselle for curing diseases and use as ethnic food. They are used to relieve pain in urination and indigestion and the Mexicans, traditionally use the infusions of the calyces and leaves for curing hypertension and various other diseases [2]. The powder of dried calyx or fresh flowers is used for curing flatulence in cow, goat, and sheep. The extract of the calyces added with common salt is beneficial to cure diarrhoea and dysentery of animals and human. It is also used to cure waist pain and other gynaecological disorders in post-delivery cases [13]. The Calyx infusion (Sudan Tea) is taken to relieve coughs and remedy for biliousness [14,15,30] and are also used to lower the body temperature [31]. The drinks are also used to treat liver disease, fever, hypercholesterolemia, hypertension, antispasmodic and antimicrobial agent [32–34].

4. Nutritional composition

The calyces of roselle are rich in carbohydrate, dietary fibre,

protein, vitamins, minerals and bioactive compounds [35]. A study on the nutritional analysis of roselle plant by Luvonga et al. [36], highlighted that carbohydrate content (68.7%) was the highest followed by the crude fibre (14.6%), and ash content (12.2%). In another study, Abou-Arab et al. [37] have reported the proximate composition of calyces of roselle on dry weight basis, containing protein (7.51%), fat (0.46%) carbohydrate (69.62%), fiber (11.17%) and ash (11.24%). An earlier study by Adenipeku [38] reported similar results. However, the typical literature value of carbohydrate, protein and fat as reported by them are (68.75%), (6.71%) and (1.01%) respectively. [39] attributed these differences, to the source of calyces. More recently, Jabeur et al. [40] in their study, for the first time reported the presence of Glucose (6.5 g/100 g), fructose and fatty acids in *Hibiscus sabdariffa*. They also stated carbohydrate (87 ± 1 g/100 g dw), to be the most abundant macronutrient followed by protein (5.5 g/100 g) and fat (0.47 g/100 g). However, Sayago-Ayardi et al. [41] in their study reported that roselle flower contained dietary fiber as the largest component (33.9%) which is rich in insoluble compounds (85.6%) and soluble dietary fiber (SDF) was 14.4% of total dietary fiber (DF) content. This SDF is associated with polyphenols which present antioxidant activity thus producing a healthy effect in the colon. Nutritionists have found roselle calyces to be high in vitamins like niacin, riboflavin [9] and ascorbic acid. Niacin and pyridoxine are present in an appreciable amount in the calyces of *Hibiscus sabdariffa* [36]. α- and β-Tocopherols are also present in roselle, with α-isoform (39.19 mg/100 g) being most abundant [40]. The high amount of minerals especially, calcium, iron, potassium and magnesium are also found. Various workers reported different values of ash and mineral content [42–44]. Carvajal-Zarrabal et al. [4] suggested these differences in the value were due to the type of soil influencing the ash and mineral content within the same species. The calyces of roselle are rich in polyphenols especially in anthocyanins like delphinidin-3-sambubioside and cyanidin-3-sambubioside which are strongly hydrophilic antioxidants [45].

The roselle calyces are also rich in organic acids viz., citric acid, malic acid, tartaric acid and polyphenolic acids (hibiscus acid and protocatechuic acid) [46]. Thus roselle calyces being rich in the nutritional composition can be used as nutritional supplements and also as a functional food or functional food ingredient.

The roselle leaves are a good source of various nutrients like protein, fat, carbohydrates phosphorus, iron, β-carotene, riboflavin and ascorbic acid [47]. They contain high levels of polyphenolic compounds mainly chlorogenic acid and its isomers quercetin and kaempferol glycosides that contribute to the antioxidant capacity and anti-inflammatory activity [48].

The roselle seeds are a good source of 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 followed by sodium, calcium, phosphorus, and magnesium. 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 oleic acid (32.06%) and linoleic (39.31%) [49].

5. Bioactive components

The main constituent of roselle in context of therapeutic importance are a polysaccharide, organic acid and flavonoids mainly anthocyanins [50–53]. The extracts of dried calyces have been known to contain chemical constituents such as organic acids (citric acid, ascorbic acid, maleic acid, hibsicic acid, oxalic acid, tartaric acid) besides, phytosterols, polyphenols, anthocyanins and other water-soluble antioxidants [10,28]. The organic acids together with bioactive components have free radical scavenging activity [36]. The beneficial health effect is mainly attributed to these bioactive molecules. Table 1 shows the polyphenolic fraction (bioactive compounds) present in the extracts of *Hibiscus sabdariffa* as reported by different research groups.

5.1. Organic acid

Roselle extracts contain a high percentage of organic acids including malic acid and citric acid (13% on the dry weight basis) in the calyces as reported by Salma and Ibrahim [56]. Besides, Abou-Arab et al. [37] reported that the calyces also contain ascorbic acid (140.13 mg/100 g). Earlier data also show that the calyces are rich in ascorbic acid [47,56,57]. Some authors have reported high concentration of organic acids such as oxalic acid, succinic acid, tartaric acid; malic acid, citric acid with the last of these predominating [1]. Recently Jabeur et al. [40] have reported oxalic acid, shikimic and fumaric acids as the main organic acid with malic acid (9.10 g/100 g) to be the most abundant acid in the calyces of roselle.

5.2. Polyphenols and flavonoids

The origin of many therapeutic substances is due to the secondary metabolisms in the plant. Roselle calyces are an interesting source of potential bioactive molecules with antioxidant, hypocholesterolemic antihypertensive, antimicrobial, anti-inflammatory, antidiabetic and anticarcinogenic activities.

Many scientific investigations have revealed that the calyces of roselle are rich in polyphenols and flavonoids that enhanced the nutritive value of roselle as these compounds are correlated with their antioxidant property. The phenolic content in the plant consists mainly of anthocyanins like delphinidin-3-glucoside, sambubioside, and cyanidine-3- sambubioside [40,54] and other flavonoids like gossypetine, hibiscetin and their respective glycosides; protocatechuic acid, eugenol, and sterol like β-sitoesterol and ergoesterol [1,10,58].

The colourful anthocyanins are the members of the flavonoids group of phytochemicals. The anthocyanins are flavylium or 2-phenylbenzopyrylium cation with hydroxyl and methoxyl group present at position R_1 and R_2 of the basic structure shown in Fig. 3 [59]. Anthocyanins molecules are susceptible to degradation. Their stability depends on pH, temperature, the presence of enzyme, light, and structure, the presence of other flavonoids, phenolic acids and metals [60].

5.2.1. Anthocyanins of Hibiscus sabdariffa L

Ali et al. [1] and Gradinaru et al. [61] have reported two major anthocyanins compounds, (delphinidine-3-sambubioside and cyanidine-3-sambubioside) and two minor compounds (delphinidine-3-glucoside and cyanidine-3-glucoside) present in the calyces of roselle. Earlier Pouget et al. [62] and Bridle and Timberlake [63], have reported the similar findings. According to a study conducted by Tsai et al. [64], have reported that 85% of anthocyanins is delphinidine-3-sambubioside and is the major source of antioxidant capacity of roselle extract. In another study by Aurelio et al. [35] reported that the extracts of roselle calyces are rich in anthocyanins like delphinidine-3-glucoside, sambubioside and cyanidin-3-sambubioside contributing to their antioxidant properties.

The researchers have mainly used an aqueous or organic solvent to extract the polyphenols and anthocyanins from the roselle calyces. The different extraction techniques and different varieties of *Hibiscus sabdariffa* used in various studies make it difficult to compare. Luvonga et al. [36] reported total phenolic content to be 6.06 mg/g in roselle extract. Dried roselle contained total anthocyanins as cyanidine 3-glucoside 622.91 mg/100 g and 37.42 mg/g total phenolic content in dry weight sample [37]. Recently Jabeur et al. [40] in their study identified delphinedine-3-o-sambubioside, delphinidine-3-o glucoside and cyanidine-3-o sambubioside in the concentration of (7.03 mg/g), (1.54 mg/g) and (4.40 mg/g) respectively.

6. Therapeutic benefits of *Hibiscus sabdariffa*

Available evidence suggest that the polyphenols and anthocyanins present in the calyces of roselle exhibit multiple biological effects. Many

Table 1
Concentration of bioactive compounds in *Hibiscus sabdariffa* calyces.

Calyces Extract	Class	Bioactive compound	Concentration	Reference
Aqueous	Anthocyanins	Delphinidin-3-sambubioside	2701.21 ± 165.55 ppm	[45]
Aqueous			0.78 mg/m	Sinela et al. [54]
Hydroethanolic			7.03 ± 0.04 mg/g	Jabeur et al. [40]
Infusion			7.0 ± 0.2 mg/g	–
Aqueous		Cyanidine-3-sambubioside	1939.15 ± 39.27 ppm	[45]
Aqueous			0.46 mg/ml	Sinela et al. [54]
Hydroethanolic			4.40 ± 0.02 mg/ml	Jabeur et al. [40]
Infusion			4.08 ± 0.07 mg/g	–
Aqueous	Flavonoids & Phenolic acid	Chlorogenic acid isomer I	2755.15 ± 62.42 ppm	[45]
		Chlorogenic acid	1923.72 ± 38.69 ppm	–
		Chlorogenic acid isomer II	1041.19 ± 16.96-ppm	–
		5-O-Caffeoyl-shikimic acid	171.47 ± 6.92 ppm	–
Aqueous		3-Caffeoylquinic acid	0.36 mg/ml	Sinela et al. [54]
Hydroethanolic			2.6 mg/g	Jabeur et al. [40]
Infusion			2.88 ± 0.03 mg/g	–
Aqueous		5-Caffeoylquinic acid	0.30 mg/ml	Sinela et al. [54]
Hydroethanolic			1.53 ± 0.06 mg/g	Jabeur et al. [40]
Infusion			1.14 ± 0.01 mg/g	–
Aqueous		4-Caffeoylquinic acid	0.10 mg/l	Sinela et al. [54]
			1.44 ± 0.08 mg/g	Jabeur et al. [40]
			1.00 ± 0.02 mg/g	–
Methanolic		Caffeic acid	18.24%	Kuo et al. [55]
		Protocatechuic acid	8.62%	–
		Catechin	9.86%	–
		Epigallocatechin gallate	20.34%	–
Aqueous		Quercetin	121.24 ± 2.01 ppm	[45]
		Quercetin-3-sambubioside	304.02 ± 5.90 ppm	–
Aqueous		Quercetin-3-rutinoside	495.76 ± 4.34 ppm	–
Hydroethanolic			1.07 ± 0.1 mg/g	Jabeur et al. [40]
Infusion			1.00 ± 0.01 mg/g	–
Aqueous		Quercitin-3-glucoside	143.74 ± 2.16 ppm	[45]
		Kaempferol-3-O- rutinoside	91.86 ± 2.28 ppm	–
		Kaempferol-3-p-coumarylglucoside	28.37 ± 0.48 ppm	–
Hydroethanolic		Myricetin-pentosylhexoside	0.961 ± 0.001 mg/g	Jabeur et al. [40]
Infusion			0.951 ± 0.003 mg/g	–
Hydroethanolic		Quercetin-pentosylhexoside	1.031 ± 0.002 mg/g	Jabeur et al. [40]
Infusion			0.987 ± 0.001 mg/g	–
Aqueous		Hydroxycitric acid	8288.03 ± 397.63 ppm	[45]
		Hibiscus acid	31122.02 ± 1128.39 ppm	–

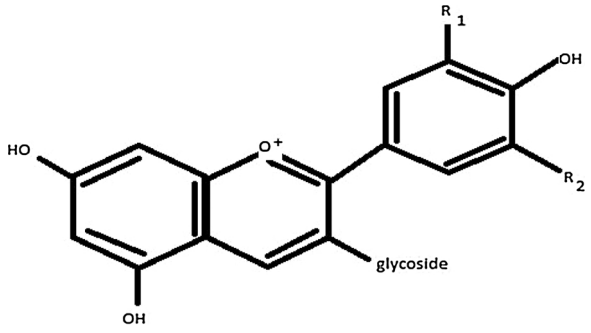

Fig. 3. Structure of flavylium cation (aglycones)

Anthocyanins	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Peonidin	OCH_3	H
Delphinidin	OH	OH
Petunidin	OCH_3	OH
Malvinidin	OCH_3	OCH_3

research reports highlighted the dried calyces as the potential source of bioactive molecules that exert potent antioxidant-antiradical activity, anti-inflammatory action, antiobesity, antihyperlipidemic, antihypertensive, inhibition of blood platelets aggregation, diuretic, antiurolithicatic, antimicrobial, anticancer, hepatoprotective, renoprotective, antitumour, immunomodulatory properties [65–73]. The extract of roselle has been effectively used against hypertension, inflammation, liver disorders, diabetes and metabolic syndrome [74].

6.1. Antihyperlipidemic

Various studies have shown the hypolipidemic efficacy of *Hibiscus sabdariffa* extract (HSE) suggesting the possibility of roselle as an antiobesity agent. Obesity is characterized by a gain in the body weight due to excessive accumulation of body fat. High-fat diet and energy imbalance, results in obesity which is associated with various metabolic syndromes like cardiovascular risk factors (hyperlipidemia and hypertension), fatty liver and insulin resistance [75,76]. These metabolic disorders are characterized by early cellular events and dysregulation of normal cellular homeostasis. Polyphenols derived from *Hibiscus sabdariffa* has pleiotropic character and could become auxiliary in this pathology as they have multi-targeted effects on obesity affecting human health and this multi-targeted mechanism involves regulation of energy metabolism, oxidative stress and inflammatory pathways, transcription factors, hormones and peptides, digestive enzymes, as well as epigenetic modifications [8]. There are evidence from animal studies and human clinical trials that HSE reduced hyperlipidemia. In a

study it was reported, that when hypercholesterolemic rats were fed with a dose of 5–10 % of HSE for 9 weeks lowered the different lipid fraction in plasma, heart, brain, kidney and liver [77]. Similarly, in another study, it was reported that when rabbits fed with cholesterol for 10 weeks and then treated with 0.5 or 1% HSE showed that the levels of, total cholesterol, triglyceride and low-density lipoprotein (LDL) in the serum were reduced [33]. Hirunpanich et al. [58] in their study highlighted that aqueous extracts of the dried calyx of roselle posses both antioxidant effects against LDL oxidation and hypolipidemic effects in vivo. They have reported HSE at doses of 500 and 1000 mg/kg for 6 weeks in hypercholesterolemic rats significantly decreased serum cholesterol; triglycerides and LDL level, however serum high-density lipoprotein (HDL) level was not affected. His findings were in agreement with the findings of Chen and co-workers. Similarly, later Fernandez-Arroyo et al. [45] in their study, showed that aqueous extract of *Hibiscus sabdariffa* (10 g/l) had reduced more than 50% of serum triglyceride concentration in mice under hypercaloric diet for several weeks. In a study on animal model [78] fed hypercholesterolemic male Wistar rats (200–250 mg) with cellulose, *Agave tequilana* dietary fibre (ADF) and *Agave tequilana* dietary fibre along with Jamaica calyces (*Hibiscus sabdariffa*) (ADF-JC) for 5 weeks. They observed a significant reduction in weight of hypercholesterolemic Wistar rats that consumed ADF-JC. They reported a reduced concentration of cholesterol transporters in caecum tissue with no change in the plasma lipid profile. According to Lin et al. [79] roselle has an inhibitory effect on LDL oxidation and hence can be used as potent phytochemical agents in the therapeutic treatment of atherosclerosis. Recently Moyano et al. [67] in their study on animal model highlighted, that grounded calyces of *Hibiscus sabdariffa* reduce the body weight, adiposity, plasma total cholesterol and glucose in C57BL6 mice, subjected to a high-fat diet.

Sabzghabaee et al. [80] conducted a randomized triple masked placebo controlled trial on 72 patients and gave 6 g of roselle powder per day in a divided doses for a period of 4 weeks that significantly lowered total cholesterol, LDL-C and triglyceride levels without any significant decrease in HDL and suggested the beneficial effects of HSE on lipid profile of the hyperlipidemic patients. Gurrola-Diaz et al. [81] in their study included the population with or without metabolic syndrome and compared diet and a combination of *Hibiscus sabdariffa* extract powder and diet. A total of 100 mg HSEP was orally administered in the form of capsule for one month. The metabolic syndrome patients showed a significant reduction in total cholesterol and glucose level and increased the HDL-c level. However, triglyceride lowering effect was seen in metabolic syndrome patients treated with a combination of *Hibiscus sabdariffa* extract polyphenols HSEP and diet. In one of the RCT's HSE capsules were given to hypercholesterolemic patients to assess the cholesterol-lowering effect of *Hibiscus sabdariffa* extract. Each HSE capsule (500 mg) contained anthocyanins (20.1 ± 3.0 mg), flavonoids (10.0 ± 2.5 mg) and polyphenols (14 ± 2.8 mg). They concluded with a remark that a dosage of 2 capsules of HSE (with a meal) for 1 month can significantly reduce the serum cholesterol [82].

The studies were lacking the uniformity in the extract, hence it becomes very difficult to compare the results between the studies and most of the cholesterol trials were of very short duration lasting for 4 weeks only. However, in patients treated with statin or change in diet and lifestyle, it takes up to two months for lipid profile to change [5]. Therefore studies with longer duration should be conducted. Roselle may be beneficial and the adverse effects are mild and infrequent with short-term use [71].

Some researchers have proposed the following mechanisms to explain the antihyperlipidemic effect of *Hibiscus sabdariffa*. Ochani and D'Mello [83] in their study suggested, that the increased level of high density lipoprotein (HDL-C) in the serum, transfers the excess cholesterol from the peripheral cells to the liver for its catabolism via reverse cholesterol transport pathway, thus increasing the inhibition of absorption of cholesterol, interference with lipoprotein production and increased expression of hepatic low density lipoprotein (LDL) receptors/their production, resulting in increased degradation and removal of LDL-C from the blood. All these events either individually or together decreased the LDL-C and total cholesterol level in the serum. Yang et al. [84] in their study suggested the antihyperlipidemic effect of aqueous roselle extract decreases the hepatocyte lipid content due to fatty acid synthase HMG-CoA reductase via adenosine monophosphate-protein kinase (AMPK) activation and sterol regulatory element binding protein reduction. However Sari et al. [85] suggested that *Hibiscus Sabdariffa* extract has lipase inhibitory activity that hydrolyses 50–70% of total dietary fat. Antihyperlipidemic effect has been attributed to the anthocyanins and protocatechuic acid present in the roselle extract. Also, Isomers of hydroxycitric acid (-)-HCA has an inhibitory effect on citrate lyase that inhibits acetyl-CoA generation and hence triglyceride and cholesterol biosynthesis [4]. Recently, Herranz-López et al. [8] from their computational result based on free energy, validated that polyphenols from *Hibiscus sabdariffa* acts as putative ligands for various digestive and metabolic enzymes and have the potential to inhibit these enzymes and highlighted the need for in vivo and vitro studies to substantiate this silicio approach. They further suggested that integrated approaches (transcriptomic, proteomic and targeted metabolomic) with the support of virtual screening techniques of metabolites on selected protein targets and epigenetic analysis should be used to explicate the exact mechanism of polyphenols against obesity-related disorders.

6.2. Antihypertensive

Hypertension is a chronic medical condition of the heart in which the arterial blood pressure is raised. This is a common health problem in both developed and developing countries. Traditionally *Hibiscus sabdariffa* (HS) is used as the antihypertensive agent. The ancient use of HS is related to cardiovascular diseases. Several scientific investigation reports have provided evidence that *Hibiscus sabdariffa* extract (HSE) significantly reduces blood pressure in both animal and human model. Hs aqueous extract had shown the antihypertensive and cardioprotective effects in the 2-kidney-1-clip (2k- 1c) rats, [86].

Haji faraji and Haji Tarkhani [87] have evaluated the effect of HS tea on hypertension. The first single trial was studied in 54 patients with moderate hypertension which revealed a reduction in (systolic by 11.2% and diastolic by 10.7%) blood pressure in 12 days after beginning of the treatment and when the treatment was stopped, after three days the systolic and diastolic blood pressure was elevated by 5.6% and 6.2% respectively. In a randomized trial of 75 patients, Herrera-Arellano et al. [88] have shown that HS aqueous extract effectively reduced the blood pressure by more than 10% and was safe when compared with 25 mg of captopril. In another study again in Herrera-Arellano et al. [89] highlighted the effect of standardized *Hibiscus sabdariffa* extract in a randomized, double-blind, lisinopril-controlled clinical trial involving 171 hypertensive patients for 4 weeks. HS extract lowered the blood pressure (BP) but the magnitude of the effect was lower when compared with the patient treated with 10 mg lisinopril concluding that Hs exerted important antihypertensive effect with a wide margin of tolerability and safety. Wahabi et al. [3] in their review have mentioned that current trials of HS effects in reducing blood pressure were not up to the standard except for one trial. In a study in McKay et al. [16] had done a first randomized, double-blind, placebo-controlled clinical trial in 65 pre and mildly hypertensive adults (age 30–70 years), not taking blood pressure lowering medication for 6 weeks and they confirmed the anti-hypertensive effect of hibiscus tea. The Blood pressure lowering effects were greater in this study as compared to the study of Haji Faraji and Herrera- Arellano. Joven et al. [90] verified the effectiveness of polyphenolic compound from *Hibiscus sabdariffa* in hypertension management, in patients with metabolic syndrome and in spontaneously hypertensive rats. The dose of 125 mg/kg/day was used for patients with metabolic syndrome for a period of 4 weeks and the dose of 125 or 60 mg/kg in a single dose or daily for 1 week. Recently Ali et al. [91],

evaluated the beneficial effect of aqueous extract of *Hibiscus sabdariffa* and anthocyanin isolated from it in an adenine-induced chronic kidney disease (CKD) in male wistar rat and they reported that a dose of 50 mg/kg and 200 mg/kg of anthocyanins abolished the systolic blood pressure (SBP) that significantly increased due to adenine treatment. Also, anthocyanins (50 mg/kg) significantly reduced the pulse rate.

Comparative analysis of above studies is not possible because they are lacking the standardization of the active chemical components in the extract. Only a few studies have reported the active component concentration in HSE.

The studies on antihypertensive effect put forward that *Hibiscus sabdariffa* is comparatively effective as other pharmaceutical antihypertensive drug and is a safe and well-accepted treatment option for mild to moderate essential hypertension. Roselle could be considered as a first line defence against rising hypertension in otherwise well individuals [6].

However, Ojeda et al. [92], demonstrated for the first time that antihypertensive effect of *Hibiscus sabdariffa* extracts in humans was because of angiotensin-converting enzyme (ACE), thus providing a scientific basis for the use of roselle extract in folk medicine for lowering the blood pressure. They have recommended the following possible mechanisms that occur in the renin-angiotensin system (RAS) involved in the regulation of the plasma sodium concentration and arterial blood pressure, probably as aldosterone antagonistic (diuretic) and angiotensin-converting enzyme (ACE) inhibitor that are complementary to each other. The anthocyanin-rich fraction (delphinidin-3-O-sambubiosides and cyanidin-3-O-sambubiosides) inhibit the enzyme activity by competing with the active site in a dose-dependent manner. The angiotensin I is converted to angiotensin II by the enzyme ACE. This angiotensin II is a potent vasoconstrictor and also stimulates the secretion of aldosterone. The other mechanism of action is attributed to a vasodilator effect via endothelium-dependent and independent pathway. Ajay et al. [93] also reported the mechanisms of blood pressure lowering effect of methanolic extract of HS in an isolated aorta from spontaneously hypertensive rats. They highlighted that the vasodilator effect of roselle extract is probably through activation of endothelium-derived nitric oxide/cyclic guanosine monophosphate (cGMP) relaxant pathway and the independent pathway that involves the inhibition of calcium ion flow into vascular smooth muscle cells. However in another study conducted by Sarr et al. [94] also reported *Hibiscus sabdariffa* extract stimulate endothelium-dependent relaxant effect by activation of lipid kinase (Pi3-Kinase/Akt pathway) that leads to phosphorylation of endothelial nitric oxide synthase (eNOS). Joven et al. [90] reported that roselle polyphenols induced favourable endothelial responses and considered this mechanism of action, to be more important along with antioxidant and anti-inflammatory activity as compared to diuresis and ACE inhibitor activity.

More double-blind randomized placebo-controlled clinical trials along with standardized active ingredient doses for a longer period is recommended to determine the effectiveness of HS extract as antihypertensive agent and their possible toxicological effects.

6.3. Anti-inflammatory

Inflammation is a physiological response of the body against damage or disturbance outside factors. Diabetes trigger inflammatory compounds (hs-CRP, IL-6, TNF-α and IL-18). In the study conducted by Mardiah et al. [65] to assess the effect of roselle extract on the streptozotocin induced diabetic rats have shown that a dose of 72 mg roselle/day/200 g body weight and 288 mg/day/200 g body weight decreased the level of tumour necrosis factor-α (TNF-α) thus implying the anti-Inflammatory activity of roselle. In another the study conducted by Yang et al. [95] on type 2 diabetic rat model have shown that a dose of 200 mg/kg of *Hibiscus sabdariffa* polyphenol extract (HPE) prevents early diabetic nephropathy, inhibiting albuminuria and the elevation of clearance of creatinine and also inhibited fat deposition and glycation end-products (AGE) in kidney. They reported that HPE reversed collagen accumulation and also ameliorated the increase of tubular connective tissue growth factors (CTGFs) and the glomerular cluster of differentiation 31 (CD31), decreased angiotensin II type 1 receptor(AT-1) elevation and improved oxidative stress. Earlier they had shown the beneficial effect of HPE reducing hyperglycaemia and hyperinsulinaemia. Dafallah and Al-Mustafa [96] studied the effect of *Hibiscus sabdariffa* on rats and highlighted the significant inhibitory effect on yeast induced pyrexia. The anti-inflammatory and anti-pyre effect of roselle drink was basically due to the inhibition of production of cytokines. Beltran-Debon et al. [97] characterized the active components in *Hibiscus sabdariffa* extract. In vitro, they have shown that HSE effectively protects peripheral blood mononuclear cells (PBMCs) from the cellular death induced by H_2O_2 via modulating the production of inflammatory cytokines. And also in clinical trial on healthy human volunteers they have shown the decreases of plasma monocyte chemoattractant protein 1 (MCP-1) concentration which is a biomarker in the evaluation of inflammatory diseases.

In a study, Chou et al. [98], for the first time, investigated the application and mechanism of roselle on urinary tract infection (UTI) by applying a clinical observation-guided transcriptomic study. They reported that roselle drink with a concentration of 4.84 μg/ml of delphinidine-3-sambubioside improved lipopolysaccharide (LPS) induced renal inflammation in mice through down-regulation of cytokine network (iNos, NO,IL-6, MCP-1 and TNF-α), pro-inflammatory product production and nuclear transcription factor ($NF\text{-}_KB$) pathways in mice, in a dose-dependent manner. They also analysed traditional therapeutic use of roselle drink in the prevention of UTI in residents with urinary catheters in long-term care facilities through survey questionnaire and reported the decreases in the incidence of UTI by 36%. In a more recent study Ali et al. [91] have shown that *Hibiscus sabdariffa* aqueous extracts (HSE) and their isolated anthocyanins significantly reduced the severity of adenine-induced chronic kidney disease (CKD) in mice to a similar extent as lisinopril (commonly used in human CKD) in a dose-dependent manner by antagonizing oxidative stress marker and inflammatory cytokines reaction associated with it. The compositions of anthocyanins used in the study were delphinidine-3-sambubioside (47.3 ± 1.0%), cyanidine-3-sambubioside (12.9 ± 9%), rutoside (3.2 ± 0.02%) and chlorogenic acid (1.3 ± 0.06%).

These studies suggest the potential application of roselle to be as safe functional supplement to attenuate the chronic inflammatory diseases in human.

6.4. Antimicrobial effect

For the first time, in vitro study conducted by Alshami and Alharbi [99] highlighted the effect of methanolic extract of *Hibiscus sabdariffa* against antifungal isolates from candiduria and biofilm inhibition activity. They used 6 strains of fluconazole-resistant *C.albicans* obtained from patients with recurrent candiduria. Minimum inhibitory concentration values ranged from 0.5 to 2.0 mg/ml and reported that *Hibiscus sabdariffa* was effective at all levels in inhibiting *C. Albicans*. This study provides a scientific basis for its use in folk medicine for treating and preventing of urinary tract infections.

Abdallah [100] in his study evaluated the antibacterial activity of 80 percent (V/V) methanolic extract of *Hibiscus sabdariffa* calyces against hospital isolates of multidrug-resistant (MDR)-*Acinetobacter baumanni*, using agar disc diffusion, minimum inhibitory concentration and maximum bactericidal concentration methods. The author reported that *Hibiscus sabdariffa* was more effective than gentamicin thus exhibiting potent antibacterial activity against the MDR strains of *A.Baumannii*, (the competitor of antibiotics), thus roselle can be used as an anti-bactericidal drug. Borras-Linares et al. [101] reported the antibacterial activity of the ethanolic extracts of 25 varieties of Mexican roselle against gram-positive and gram-negative bacteria and highlighted that the extracts were more effective against gram-positive

bacteria. Jabeur et al. [40], comparatively evaluated the antimicrobial property of lyophilized hydroethanolic extract and infusion of *Hibiscus sabdariffa*. They used 10 mg/ml of the extract of hydroethanol and infusion respectively for antimicrobial assays. The antibacterial activity, was performed on both gram positive and gram negative bacteria and for antifungal activity *Trichoderma viride* and four different species of *Aspergillus* were used. They reported that hydroethanolic extract had the same bactericidal effect for all the tested bacteria as compared to infusion in which no growth inhibitory activity was observed against *Bacillus cereus, Micrococcus flavus* and *Escherichia coli* and no bactericidal effect was seen in *Enterobacter cloacae* despite the capacity to inhibit its growth. Further, the fungicidal effect was seen against all the tested strains but infusion showed no fungicidal activity in any of the strains tested despite its capacity to inhibit their growth. They justified their result explaining the difference in the concentrations of the identified phenolic compounds present in both the extracts and highlighted that both antibacterial and antifungal properties revealed a similar concentration range of inhibition.

The antimicrobial activity of roselle extract is attributed to the flavonoids, as they have the ability to form a complex with the bacterial cell walls and permeability of bacterial cell surface to the extract. The possible mechanism of action involves the inhibition of electron transport protein translocation, phosphorylation steps and other enzyme dependent reactions followed by increased plasma membrane permeability, resulting in the leakage of an ion from bacterial cells [102]. Al-Hashimi [103] in their study highlighted that roselle contains proanthocyanidins which combine or transform the structural entity of P-fimbriae of bacterial cells thus inhibiting their adhesion to the uroepithelium and formation of biofilm in vitro.

Further research is recommended to establish that in vitro results are attainable in vivo.

6.5. Diuretic, uricosuric effect and hyperuricemia

Ribeiro et al. [69] investigated the diuretic effects of *Hibiscus sabdariffa* (HS) aqueous infusion in conscious rats. After ingestion of 6 h HS extract, urine collected was increased to 103 ml/kg which was significantly higher when compared with water placebo ((46 ml/kg) and 25 mg/kg of hydrochlorothiazide (83 ml/kg). Urinary sodium, urinary potassium and uric acid also increased compared to placebo and those treated with hydrochlorothiazide.

Prasongawatana et al. [70] had suggested a dose-dependent (3 g/day), uricosuric effects of roselle tea in humans that could be used in the treatment of hyperuricemia in gout disease. At this dose it has neither antilithiatic nor diuretic effect. Their study included two groups of the human model, one with no history of renal stones and the other with renal stones. Blood and urine samples were collected and analysed at the baseline, during tea drinking and 15 days after tea drinking was stopped. All the serum parameters were within the range with no significant difference between the two groups. The urinary parameters at the baseline were same in both groups, however after tea intake, there was an increase in oxalate and citrate, uric acid excretion and fractional uric acid excretion in both the groups was decreased to the baseline during the washout period.

Kuo et al. [55] investigated the effect of *Hibiscus sabdariffa* extract (HSE) on oxonic acid (OA)-induced hyperuricemia in rats. They treated the rats with normal saline and oxonate solution for 1 week and with or without feeding allopurinol (an XO inhibitor) or HSE (1%, 2% and 5%) for 5 weeks and reported that HSE inhibited OA induced hyperuricemia, with greater uric acid lowering effect than allopurinol treatment. These studies provide a scientific basis that HSE could be effectively used in the treatment of hyperuricemia.

The diuretic effect of roselle has been controversial as some researcher found an increase in urinary volume [69], however Prasongawatana et al. [70] reported no increase in urinary volume. Alarcon-Alonso et al. [104] in their study had shown the diuretic, natriuretic and potassium-sparing effect of aqueous *Hibiscus sabdariffa* extract. They used two experimental models (rat diuresis and renal filtration rate in kidney “in situ”) and used the doses between 500 and 2500 mg/kg that resulted in a reproducible diuretic effect. They highlighted the dose-dependent (500–2500 mg/kg) behaviour of aqueous HSE and reported that diuretic effect of roselle may be mediated by endothelium-dependent nitric oxide release.

6.6. Treatment of anaemia

Hibiscus sabdariffa (Hs) was the most cited species against anaemia being rich in iron and ascorbic acid. This ascorbic acid helps in the absorption of non-heme iron which justifies their potential as anti-anaemic in ethnomedicine. Pharmacologically, *Hibiscus sabdariffa* (HS) extract was tested in both the animal and human models results, showed the elevated hematocrit and haemoglobin [66]. Adigun et al. [105] evaluated the effect aqueous extract of *Hibiscus sabdariffa* on haematological parameters like haemoglobin, haematocrit and total and differential white blood cells count recommending a dosage of 200–400 mg/kg to be beneficial. Doses of 200–1000 mg/kg body weight were given orally to rats up to 14 days. After 14 days significant elevation were observed in haematocrit and haemoglobin in the group of animals given doses of 200–400 mg per kg. However higher doses showed a significant reduction in the haematocrit level but not in the haemoglobin. A similar study was conducted by Emelike and Dapper [106] on a Wistar albino rat for a period of 28 days, suggesting that administration of aqueous extract of *Hibiscus sabdariffa* is beneficial to the haematopoietic system.

Anaemia is an iron deficiency disease having low red blood cells (RBC) in the blood. The RBC carries and delivers the oxygen to the body cells. The anti-anaemic activity of roselle extract might be because of its very low pH and high concentration of ascorbic acid that has increased the mineral bioavailability [2].

Contrary to the above two studies, a clinical trial for a period of 30 days on mild to moderate anaemic human subjects showed that ingestion of standardized aqueous extract of *Hibiscus sabdariffa* (1000 ml,1500 ml and 2000 ml) did not improve the iron status of anaemic adults in malaria-endemic region. However, no adverse effect was reported with 30 days of oral administration, suggesting roselle to be safe for human consumption [107]. They reported that there was a significant increase in serum ferritin (efficient indicator in measuring response to iron intervention) in control group but the increase in the test group was insignificant. Haemoglobin (Hb) and CRP did not change significantly either in test or control group. The incidence of malaria was found in all groups (highest in control group followed by group fed with 2000 ml of roselle extract). They justified their result mentioning that iron supplementation increases the risk of infection that lowers the Hb and increases ferritin. They finally concluded that they did not control daily diet and other vitamin deficiency in participants which could be the reason for non-responsive to iron intervention and lack of dose-dependent response in the groups.

Research on anti-anaemic activity of roselle extract is limited and better quality research is required, with bigger sample size and good study design, to investigate the possible potential of roselle in the treatment of anaemia and their mechanism of action which is an emerging area.

6.7. Anti-carcinogenic

In a study reported by Chewonarin et al. [12] investigated the chemopreventive effect of methanolic extract of roselle in F344 rats in which aberrant crypt foci (ACF) formation was induced by azoxymethane (AOM) and 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP). Colon carcinogens such as AOM, PhIP and IQ induce aberrant crypt foci (ACF), a biomarker in colon carcinogenesis. The AOM and PhIP induced ACF was significantly reduced by 17–25% and

22% respectively thus showing the antimutagenic activity against methylazoxymethanol (MAM) acetate and PhIP induced ACF in the initiation stage, however, the number of ACF was increased in the post–initiation stage. Therefore, further study on mechanisms of modulative effect of roselle is suggested.

In another study, the influence of *Hibiscus sabdariffa* anthocyanins on human tumour cells was investigated and it was found that anthocyanins cause apoptosis in HL-60 cancer cells via activating p38 MAP Kinase that phosphorylates target protein C-Jun which further activate apoptotic protein cascades that contain Fas-mediated signalling, resulting in the release of cytochrome C from mitochondria leading to the cleavage of caspase-3 [51]. Delphinidin 3-sambubioside from *Hibiscus sabdariffa* induces a dose-dependent apoptosis in human promyelocytic leukaemia cells (HL-60) through reactive oxygen species (ROS-mediated) mitochondrial dysfunction pathway [108]. The above two findings were in agreement with each other.

Roselle juice has the anti-proliferative effect on the breast (MCF-7and MAD-MB-231), ovarian (Caov-3) and cervical (HeLa) cancer cells [109]. Cytotoxic effects of *Hibiscus sabdariffa* aqueous extract on human breast adenocarcinoma cell line (MCF-7) and fetal foreskin fibroblast (HFFF) was investigated by Khaghani et al. [110]. They found that at the concentration 0.5 mg/ml of the extract induces apoptosis in MCF-7 cells which were significantly reduced to less than 50% but the extract was not cytotoxic against normal HFFF cells. Formagio et al. [73] reported that the methanolic extract of the *Hibiscus sabdariffa* calyces had cytotoxic effects on leukaemia cells (K-562) with concentration-dependent, cytotoxic and cytocidal effects.

The protective effect of roselle anthocyanins on N-nitrosomethylurea (NMU) induced leukaemia in male Sprague-Dawley rats had shown that administration of (0.2%) of the extract has inhibited the progression of leukemia by 33.3%. Moreover, the Hibiscus anthocyanins improved the impairment of morphology, haematology and histopathology. However, aspartate aminotransferase (AST), alanine transaminase (ALT), uric acid and myeloperoxidase (MPO) level was reduced [72]. This is the first report that demonstrated the antileukemic activity in vivo. Wu et al. [111] have reported for the first time that *Hibiscus sabdariffa* anthocyanins modified mitochondrial function and stimulated cell death by autophagy and necrosis in MCF-7 cells instead of programmed cell death. The above studies provide evidence that anthocyanins from *Hibiscus sabdariffa* have an anticarcinogenic effect and more in vivo studies are needed in this area to further support the anticarcinogenic effect of anthocyanin extract of roselle.

6.8. Used against cadmium poisoning

In a study conducted by Omonkhua et al. [112] on male Wistar rat, reported that the pre-treatment of rats with *Hibiscus sabdariffa* extract (0.2 g/kg) significantly reduced the hepatotoxicity of cadmium. It also protected rats against liver and testis lipoperoxidation. Thus the antioxidant ability of roselle can be used to treat cadmium poisoning. Recently, a new pharmacodynamic activity of this plant has been reported as an immunoprotective effect of anthocyanins-rich extract. The extract has increased the viability of the cells that was suppressed by cadmium and reduced the cadmium-mediated production of the markers of macrophage-activation when compared to quercetin dihydrate in a dose-dependent manner [113].

6.9. Reproductive ability

In the study conducted by Amin and Hamza [114] on male albino rats had shown that *Hibiscus sabdariffa* extract (1 g/kg/day) reduced the extent of cisplatin-induced sperm abnormality and enhanced the sperm motility via antioxidant capacity, increasing the activities of testicular antioxidant enzymes, showing their possibility to be used in fertility disorder. More preclinical and clinical trials are required to confirm the ability of roselle extract in improving the fertility disorder.

7. Toxicology of *Hibiscus sabdariffa*

Apart from the promising outcome of roselle to modulate the risk factors concerned with metabolic syndrome, its safety and toxicological aspects should be taken into account. A large number of products with *Hibiscus sabdariffa* in their composition are available in the market, sometimes accounting to 50% of the total ingredient and is frequently used as a basic constituent of herbal tea [115]. Roselle tea is widely consumed dietary item in some parts of the world assuming it to be safe and natural with some potential health benefits. However, the natural product might not be safe for medicated individuals or vulnerable groups (children and pregnant women) due to the possibility of the herbs interacting with the synthetic drugs [115]. Herb-drug interactions occur when the pharmacokinetics profile of either product is altered significantly as a result of their co-administration that leads to increased side effects, toxicity or therapeutic failure [116]. Aqueous extract of *Hibiscus sabdariffa* caused the reduction in the elimination of acetaminophen, diclofenac and hydrochlorothiazide [5,117,118]. Conversely, Johnson et al. [116] in their in vitro studies on the inhibitory effect of roselle extract on cytochrome P450 (CYP) reported that this may not cause significant herb-drug interaction. Further, more clinical studies are required in this area for the safe usage of aqueous extract of *Hibiscus sabdariffa* with concomitant use of other drugs. The labelling of the herbal product should be done according to the European Union (EU) food legislation that will help the consumer to understand the product-specific benefits. As per EC No. 1924/2006 health claims must be warranted by appropriate human intervention studies, reasonable in terms of the relationship between intervention studies and results (dose, placebo, frequency, effects) and meet the methodological scientific standards [115].

Some pre-clinical studies reported the low degree of acute toxicity of *Hibiscus sabdariffa*. Akindahunsi and Olaleye [119] has recommended a safe dosage of 150–180 mg/kg /day and had shown the prolong usage of higher dose causes liver injury while the effect is mild at lower doses. Their experimental results show that 15 doses of 250 mg/kg *Hibiscus sabdariffa* extract in the Wistar albino rats significantly increased the level of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and albumin serum however of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were not significantly affected. No histopathological effect was seen in the liver and heart.

Fakeye et al. [117] in their study with albino rats used high doses (300–2000 mg/kg) of *Hibiscus sabdariffa* extract for 3 months and reported that aqueous extract increased the serum creatinine level while 50% alcohol had more damaging effects on liver function enzyme along with the increase in plasma creatinine levels.

The doses of 250–1000 mg/kg/day did not show any harmful effects of several important organs system like liver, kidney, blood system, electrolytes, lipid and carbohydrate metabolism [120]. They found that the dose of 250 and 1000 mg/kg/day did not modulate the activities of phase 1 hepatic CYPs involved in drug metabolisms and carcinogenic/mutagenic bioactivation in an ex vivo study in male Wistar rats. However, in a similar study, Nwachukwu et al. [121] have shown a dose of 2000 mg/kg and above increases the liver enzyme (AST, ALT, and ALP) while urea, creatinine and uric acid significantly increased at 1000 mg/kg and above. On the basis of their result they had recommended a dose below 1000 mg/kg to be safe that will not affect liver and kidney function. The above studies suggest that the extract of roselle should be administered with care as higher doses may have a toxic effect. More research is required in this area to find the appropriate dose that balances the pharmacological and toxicological effects of roselle.

8. The dosage of the extract and its bioactivity

It is difficult to elucidate dosing recommendation because of the

Table 2
Doses of the extract of *Hibiscus sabdariffa* with biological activity.

Bioactivity	Extract	Doses	Reference
Antihyperlipidemic		5–10%	El-Saadany et al. [77]
		0.5–1%	Chen et al. [33]
	Aqueous	500–1000 mg/kg body weight	Hirunpanich et al. [58]
	Aqueous	10 g/lit/day	Fernandez-Arroyo et al. [45]
	Powder	6 g powder/day	Sabzghabaee et al. [80]
	Aqueous	2 capsule of HSE (500 mg each)	Lin et al., [82]
	Aqueous ethanolic	1 capsule of HSEP (100 mg)	[81]
Antihypertensive	Aqueous	250 mg/kg/day	Odigi et al. [86]
	Infusion	2 spoonfuls	Haji faraji and Haji Tarkhani [87]
	Aqueous	10 g dry calyx standardized to 9.6 mg anthocyanin content	Herrera-Arellano et al. [88]
	Aqueous	250 mg of standardized anthocyanin	Herrera-Arellano et al. [89]
	Infusion	1.25 g/240 ml water	Mckay et al. [16]
	Freeze dried	125 mg/kg/day twice	Joven et al. [90]
	Ethanolic (anthocyanins)	50 mg/kg and 200 mg/kg	Ali et al.[91]
Anti-inflammatory	Aqueous	72 mg/day/200 g body weight 288 mg/day/200 g body weight	Mardiah et al. [65]
	–	200 mg/kg of HPE	Yang et al. [95]
	Aqueous	100 µg/ml roselle drink	Chou et al. [98]
	Aqueous	50 mg/kg & 200 mg/kg	Ali et al. [91]
Antimicrobial	80% methanol	0.5–2.0 mg/ml	Alshami & Alharbi [99]
	80% methanol	50–100 mg/ml	Abdallah [100]
	70% ethanol	20 µL	Borras-Linares et al. [101]
	Hydroethanolic	10 mg/ml	Jabeur et al. [40]
Diuretic, Uricosuric and Hyperuricemia	Aqueous infusion	40 mg/kg	Ribeiro et al. [69]
	Aqueous infusion	3 g/day	Prasongawatana et al. [70]
	Aqueous	1%, 2% and 5% of HSE	Kuo et al. [55]
	Aqueous	500-2500 mg/kg	Alarcon-Alonso et al. [104]
Anti-Anaemia	Aqueous	200–400 mg/kg	Adigun et al. [105]
	Decoction	0.6–1.8 g/100 ml	Emelike & Dapper [106]
Anticarcinogenic	Ethanolic	1.0 g/kg	Chewonarin et al. [12]
	Methanolic	3 mg/ml	Chang et al. [51]
	Alcoholic D-3-s	25–125 µM	Hou et al. [108]
	Aqueous	0.5 mg/ml	Khaghani et al. [110]
	Methanolic	0.25–250 µg/ml	Formagio et al. [73]
	Methanolic	0.2%	Tsai et al. [72]
	Methanolic	3 mg/ml	Wu et al. [111]
Hepatoprotective	Aqueous	0.25 µg/ml–10 µg/ml	Omonkhua et al. [112]
	Aqueous	0.2 g/kg	Okoko & Ere [113]
Reproductive ability	Ethanol	1 g/kg/day	Amin & Hamza [114]

heterogeneity in the studies related to the bioactivity of the polyphenolic contents of *Hibiscus sabdariffa.* Different methods of extraction and products are used in different studies. The doses that are used in each of the positive studies are given in Table 2.

9. Conclusions

Comprehensive detailed evidence has been provided about the multiple beneficial effects of *Hibiscus sabdariffa* and its presumed mechanism of action. In this paper, most of the previous work on phytochemical composition and therapeutic uses of *Hibiscus sabdariffa* has been reviewed to find out its present status. Phytochemical and pharmacological studies have validated many traditional usages of this plant. Reports on phytochemical composition have demonstrated that roselle possesses bioactive compounds that are effective in ameliorating various degenerative diseases. Studies confirmed that roselle consumption is safe at low doses without any adverse effect on liver or kidney. Thus roselle can be used as functional food or as an active ingredient in functional food that could be used in curing various degenerative diseases. Further research is suggested to establish a potential strategy that can balance the pharmacological and toxic effects of roselle. The bioavailability and dosage of the extract of roselle is yet another concern that has to be focused on. Hence there is a need for the standardized fingerprint of *Hibiscus sabdariffa* internationally for quality control.

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