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Ethnomedicine, phytochemistry, and bioactivities of *Hibiscus sabdariffa* L. (Malvaceae)

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ABSTRACT

Hibiscus sabdariffa L., belonging to the Malvaceae family, has been long used as herbal medicine, food, beverage, flavouring, and colouring agents. This study aims to review and document the evidence regarding the potential use of *H. sabdariffa* as ethnomedicine in some countries and its bioactive constituents and therapeutic properties. The electronic databases were used to search for the relevant information to the aims of this review up to March 2022. The high usage of *H. sabdariffa* as traditional medicine is due to its easy accessibility and low price. The plant is often used to treat intestinal problems, stomach disorders, and blood or liver toxicities. The plant contains phenolic compounds, including anthocyanins, flavonoids, and phenolic acids. The *in vivo, in vitro,* and clinical studies provide evidence that *H. sabdariffa* possesses therapeutic effects such as antihypertensive, antihyperlipidemic, antioxidants, antimicrobial, and antitumor activities. The studies provided scientific evidence for the statement of *H. sabdariffa* and its bioactive constituents in treating various diseases.

Implication for health policy/practice/research/medical education:
This contribution is to construct a literature review that discusses the importance of *Hibiscus sabdariffa* to various ethnicities in different countries and continents. This article also reports the latest discovery of the phytochemicals and pharmacological properties of the plant, which may be taken into consideration in future clinical studies and therapies involving this plant.
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Introduction

Plants are one of the sources containing chemical constituents providing benefits to humans in the use of food preparation or as traditional medicine. In developing countries, especially in rural areas, most people rely on consuming or using a plant as medicine. *Hibiscus sabdariffa* L. is one of the medicinal plants used in countries like India, Jordan, Egypt and some African countries as traditional medicine and also as a high nutritional food (1-3). *H. sabdariffa* contains a high amount of bioactive compounds that exert its therapeutic effects (4). The calyces are found to be rich in carbohydrates, protein, fat, and other active chemical constituents like anthocyanins and protocatechuic acid, which load nutrition to the food (5).

Hibiscus sabdariffa L. belongs to the Malvaceae family, commonly known as Rosella (Indonesia), Karkade (Egypt, Arab, and Sudan), and 'Asam paya' (Malaysia) (6). The distribution of *H. sabdariffa* is in tropical to subtropical regions like Africa, India, Jordan, and Sudan (7). *H. sabdariffa* is a plant rich in phytochemical constituents like anthocyanins, flavonoids, and phenol derivatives, with each of the class compounds exerting their specific effects to treat diseases (8). It is traditionally used to treat a wide range of diseases like hypertension, stomach disorders, blood impurity, and intestinal problems (9). The medicinal preparations and uses are different based on ethnic practices. For oral administration, the plant organs are boiled, and the decoction is made into juice. For topical applications, a paste is often made from crushed leaves. This review aims to present the ethnic usage potentials of this plant as an ethnomedicine in certain countries. To use the plant in an effective way, the study of bioactive constituents and the pharmacological activities need to be addressed to ensure the efficacy of using herbal plants. This can provide an effective strategy for the discovery of

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useful ethnomedicinal. Hence, this study tries to gather new information on traditional uses based on ethnicity, the bioactive compounds, and the pharmacological effects of *H. sabdariffa* L. Besides, this study also will be beneficial to society and to other researchers.

Methods

The search for the relevant evidence involved multiple strategies. This study reviewed the articles from electronic databases from 2004 to March 2022. The following electronic databases were used for searching the information: US National Library Medicine (PubMed), Google Scholar, and ScienceDirect. The keywords used were "*Hibiscus sabdariffa* + distribution" "*Hibiscus sabdariffa* + ethnomedicine", "*Hibiscus sabdariffa* + ethnobotany", "*Hibiscus sabdariffa* + traditional", "*Hibiscus sabdariffa* + indigenous", "*Hibiscus sabdariffa* + phytochemicals", "*Hibiscus sabdariffa* + pharmacological", "*Hibiscus sabdariffa* + bioactivity", "*Hibiscus sabdariffa* + herbal", and "*Hibiscus sabdariffa* + bioassay". The symbol (-) was put after the word "ethno" while searching to ensure not to miss any research articles containing this symbol in their title for ethnomedicine and ethnobotany. Only articles in the English language were selected. The non-primary research was excluded from this review. Findings from the reports were included in this review only if they reported the outcomes, such as pharmacological effects, bioactive compounds, and ethnomedicine. The experimental studies, *in vivo* or *in vitro* studies relevant to the aims of the present review were also included. The selection process is summarised in Figure 1.

Morphology, origin, and distribution

Hibiscus sabdariffa L. is an annual or perennial herb, bushy and mostly branched (Figure 2). Its stem is typically reddish in colour and can grow up to 3 to 5 meters high. The leaves range from dark green to red in colour, sometimes with red veins and palmately divided into 3 to 7 lobes. The flowers are white or yellow and the calyces are red or pale yellow (10,11). The fleshy calyces are red in colour, and the most utilized part often used in food or drink preparations. For instance, in India the fleshy calyces are used to produce sauces, jellies, and beverages (12). *H. sabdariffa* takes about 3 to 4 months before it reaches the maturity stage and is harvested for its calyces,10 days after the flower blooms (13).

Hibiscus sabdariffa is usually grown in a warm and humid tropical climate. It can tolerate floods and heavy winds but is most susceptible to fog and frost conditions. The plant grows best in permeable soil, a friable sandy loam with humus, and requires rainfall averaging about 10 inches (25 cm) each month throughout the growing season (15). Also, it requires direct sunlight for 13 hours during the first month of growth to avoid premature flowering. *H. sabdariffa* is probably native to West and East Africa, South-East Asia, and Northeast India. It is cultivated in other countries, such as Sudan, Egypt, Malaysia, Thailand, and Saudi Arabia (16).

The most usage of this plant is in India, followed by Egypt, Sudan, Thailand, and Malaysia. The plant is used as traditional medicine or in food preparations. It is often used to treat hypertension, stomach disorder, and for blood or liver purification (17). In food preparation, the calyces are used as flavouring and colouring agents, besides being a good carbohydrate and fibre source (18).

Ethnomedicine of *Hibiscus sabdariffa* L.

Ethnomedicinal practices are well known in rural areas in some ethnic groups due to the easy accessibility of the plants, difficulty reaching the urban area, and high cost of modern treatment (19-21). The influence is also affected by the direct experience of the individuals, family members, neighborhood, and social networks (22). The traditional use of *H. sabdariffa* has been widely distributed,

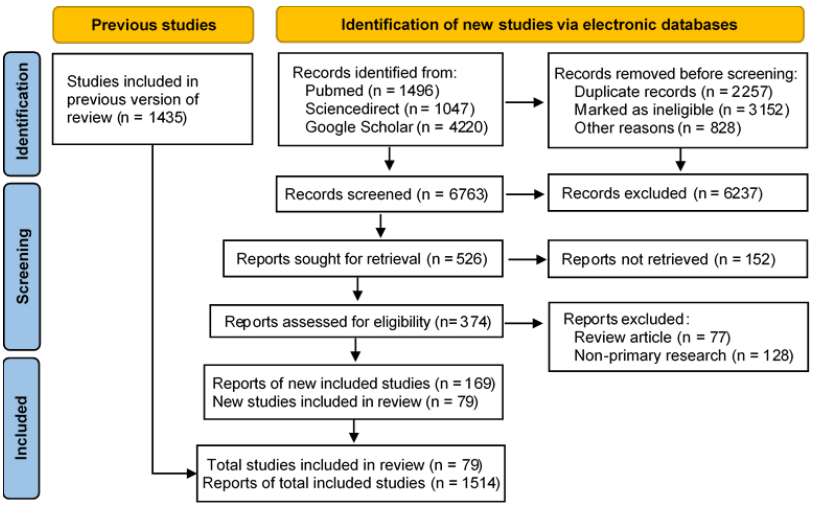

Figure 1. PRISMA diagram showing the library collection method.

Figure 2. A voucher specimen of *H. sabdariffa* L. (14).

ranging from India, Jordan, Trinidad, and Tobago, but it is predominantly used in India. In Andaman and Nicobar island of India, the rural people and tribal communities use *H. sabdariffa* to treat pile and dyspepsia (Table 1) (23). In Northeast India, Zeliang tribe of Nagas ethnic people use *H. sabdariffa* to treat stomach disorders and blood purification. They boil leaves and fruits of *H. sabdariffa* with potatoes to treat stomach disorders. Boiled fruits and fresh calyces are taken on a weekly basis to maintain blood purity (24). The Meiteis ethnic people in Northeast India use *H. sabdariffa* to treat urinary tract disease by extracting the plant pulp calyces and leaves in a cup of hot water (25). The people in Kabui Naga tribe of Manipur, India, boil one glass of leaves or fruits to treat intestinal problems (26). They also boil the leaves with crab to treat the formation of calculi in the kidney (27). The Bhoxa communities in North India use the powdered seeds of *H. sabdariffa* to treat intestinal problems (28). Karbis ethnicities in Singhason hills, India, crush the plant leaves to treat bite and bee sting poisoning. They believe that the leaves are the antidote for the poison (29).

Hibiscus sabdariffa is also used traditionally in African countries. In Sudan, *H. sabdariffa* calyces are used to treat hypertension, cold, and flu (30). In Zimbabwe, the people use calyces, the edible part of the plant, to treat cancer. A survey on ethnomedicinal plants used by traditional medicine practitioners in Zimbabwe reported that *H. sabdariffa* is used in treating all types of cancer (31). Apart from treating cancers, they also use the plant to treat infections and malnutrition. In Mauritius of East Africa, due to the high prevalence rate of diabetes, people use *H. sabdariffa* to treat diabetes mellitus type 2. They use the fruits of *H. sabdariffa* in juices preparation. To prepare the juice, the seeds need to be removed from the fruit and boiled in 1 litre of water for 10 minutes. Then, the result should be filtered and drank 1 cup twice daily (32). In Mkuranga, the district of Tanzania, the leaves or calyces of *H. sabdariffa* are used to treat anaemia (33). People in Mpigi District of Uganda use the leaf of *H. sabdariffa* to treat people with communicable diseases like HIV and AIDS (34). Apart from treating HIV, the plant also used to treat illnesses related to AIDS, such as anaemia, paralysis, abdominal pain, dizziness, and urinary tract infections (UTIs). The herbalists boil the leaves and the infused water is given to the patients (35). The people of Lango tribe of Northern Uganda stew the leaves and took twice daily as soup to treat low appetite. The stew is also taken to increase milk production during lactation (36).

In Jordan, the decoction of *H. sabdariffa* calyces is used to treat hypertension (37). In Northern Egypt, due to the high prevalence rate of hypertension, the people of Beni Sueif use the flower decoction of *H. sabdariffa* to treat hypertension. They also prepare the plant with decoction or infusion of flowers for patients having microbial infections (38). The uses, however, focus on treating hypertension as compared to microbial infection. In Iran, the calyces are boiled and taken as juice to treat obesity (39).

In the Caribbean states, *H. sabdariffa* is used for blood and liver purification and hypertension in Trinidad and Tobago (40). In Brazil and Mexico, the dried calyces are macerated, and the infused water is taken orally to treat obesity (41,42). The Greeks used the flower to maintain body weight (43).

Besides traditional medicine, *H. sabdariffa* is also used in culinary preparation. In Tamil Nādu of India, the fleshy calyces are used to produce sauces and jellies, and their infusions are used as refreshments in beverage preparation (12). The tribe people of Nagaland are eaten fruit and leaves as a vegetable (23). The seeds are used as dietary fibre in the preparation of cookies (44). The leaves are used as a seasoning in curries (11), making them spicier. The dried calyces are used in preparing herbal teas (45). These calyces give a natural reddish colour in herbal teas due to the presence of anthocyanin compounds (46).

Bioactive compounds of *Hibiscus sabdariffa* L.

Anthocyanins

The extraction of *H. sabdariffa* is rich in anthocyanin compounds that provide antioxidant activity. Two major anthocyanin compounds found in the dried calyx of *H. sabdariffa*, delphinidin-3-sambubioside **1** and cyanidin-3-sambubioside **2** (44). The presence of cyanidin-3-sambubioside in the extraction of *H. sabdariffa* calyx has also been reported (47). Another anthocyanin compound found in the extraction of *H. sabdariffa* is prodelphinidin B3 **3** (48). A study by Tsai et al showed that the distribution of delphinidin-3-sambubioside was higher than cyanidin-3-sambubioside, with the total of anthocyanin being 49% and 9%, respectively (49). Hydroethanolic extract of *H. sabdariffa* showed the presence of delphinidin-3-sambubioside, cyanidin-3-sambubioside, and cyanidin-3-glucoside **4,** with concentrations of 18.1, 6.0, and 2.56 mg/g, respectively (50). Thus, delphinidin-3-sambubioside is the most abundant anthocyanin compound with its highest concentration obtained. Besides, there are two minor anthocyanin compounds in dried extracts of *H. sabdariffa,*

Table 1. Ethnomedicinal uses of *Hibiscus sabdariffa*

Region	Disease	Plant part	Preparation/administration	References
India	Pile		-	(34)
	Dyspepsia		-	(34)
	Stomach disorders	Leaves, fruits	Boil with potato and take with food	(23)
	Blood purification	Fruits, calyces	Boil and take once a week	(24)
	Urinary tract disease	Calyces pulp, leaves	Extract the plant pulp calyces and leaves in a cup of hot water	(25)
	Intestinal problems	Leaves, fruits, seeds	Boil one glass of leaves or fruits Seed are dried and ground into powder	(26) (28)
	Kidney disease	Leaves	Boil the leaves with crab to treat the formation of calculi in the kidney	(27)
	Ant bites and bee sting	Leaves	Crush the plant leaves and apply topically	(29)
Sudan	Hypertension, cold and flu	Calyces	-	(30)
Zimbabwe	Cancer,	Calyces	-	(31)
	Infections,	-	-	(31)
	Malnutrition,	-	-	(31)
Mauritius	Diabetes mellitus	Fruits	The seeds are removed from the fruit and boiled in 1 L of water for 10 minutes. Then filter the juice and drink 1 cup twice daily.	(32)
Tanzania	Anaemia	Leaves or calyces	-	(33)
Uganda	HIV and AIDS	Leaves	-	(34)
	Anaemia, Paralysis, Abdominal pain, Dizziness, UTI	Leaves	Boil the leaves and use the infused water orally.	(35)
	Low appetite, Increase milk production during lactation	Leaves	Stew the leaves and take them twice daily as soup.	(36)
Jordan	Hypertension	Calyces	Boil the calyces and drink the decoction	(37)
Egypt	Hypertension and Microbial infections	Flower	Boil the flower and drink the decoction	(38)
Iran	Obesity	Calyces	Calyces are boiled and taken as juice	(39)
Trinidad and Tobago	Blood and liver purification, hypertension		-	
Brazil Mexico	Obesity	Calyces	The infused water is taken orally	(43)

cyanidin-3-glucoside and delphinidin-3-glucoside **5** (51) (Figure 3).

Flavonoids

Flavonoids are the other secondary metabolite compounds found in *H. sabdariffa* extracts. Leaves and seeds of *H. sabdariffa* are rich in flavonoid compounds (52). Another study showed that the total of flavonoid compounds are high in the *H. sabdariffa* calyx (53). A study reported that quercetin **6**, myricetin **7**, and kaempferol **8** derivatives were identified in the *H. sabdariffa* extracts (54). The following flavonoid derivatives have been described in the extracts of *H. sabdariffa*: myricetin-3-sambubioside **9**, quercetin-3-sambubioside **10**, quercetin-3-rutinoside **11**, quercetin-3-glucoside **12**, and kaempferol-3-O-sambubioside **13**. In this study, quercetin is the highest compound obtained, followed by myricetin and kaempferol. Another study reported that quercetin was a major flavonoid compound compared to myricetin and kaempferol (55). Besides, Sandra et al identified that the extracts of *H. sabdariffa* contained kaempferol 3-(p-coumarylglucoside) **14** (48) (Figure 4).

Organic and phenolic acids

Hibiscus sabdariffa extract contains organic acid compounds, including hibiscus acid **15** and a number of its derivatives (hibiscus acid hydroxyethyl ester **16**, hibiscus acid dimethyl ester **17)** as the major compounds and hydroxycitric acid **18** as the minor compound (56). A study also reported that hibiscus acid and hydroxycitric acid concentrations were 57.0 mg/g and 4.0 mg/g, respectively (57). High-performance liquid chromatography coupled to electrospray ionization time-of-flight mass spectrometry (HPLC/ESI-ToF-MS) analysis showed the presence of hibiscus acid and hydroxycitric acid (55). In addition, phenolic acid compounds also present in *H. sabdariffa* extracts. The compounds are neochlorogenic acid **19**, chlorogenic acid **20**, cryptochlorogenic acid **21**, and ethyl chlorogenate **22**. Neochlorogenic acid showed the highest concentration compared to other phenolic acid compounds in *H. sabdariffa* extracts (56). Protocatechuic acid **23** also has been isolated in *H. sabdariffa* extracts (57). Another study by Lin et al reported the presence of protocatechuic acid, catechin **24**, epigallocatechin **25**, epigallocatechin gallate **26,** and caffeic acid **27** in *H. sabdariffa* (58) (Figure 5).

Pharmacological effects of *Hibiscus sabdariffa* L.

Antioxidants

Some studies have shown that *H. sabdariffa* extracts have antioxidant activity on lipid peroxidation. The *H. sabdariffa* calyces showed a higher effect than the leaf with the percentages of inhibition lipid peroxidation were 71.3% and 69.41%, respectively (59). In addition, a study on hypercholesterolemic rats showed that *H. sabdariffa* extracts increased antioxidant activity by inhibiting the formation of thiobarbituric acid reactive substances (TBARs) that are responsible for the oxidation of low-density lipoprotein (LDL) (60). Moreover, *H. sabdariffa* possesses a hepatoprotective property in ammonium chloride-induced hyperammonemia rats by reducing the amount of TBARs and hydroperoxide (61).

Antihyperlipidemic and anti-obesity

Some studies have shown the ability of *H. sabdariffa* to reduce lipid levels. Inhibition of adipocyte differentiation through the PI3-K and MAPK pathway affects adipogenesis activity by reducing the lipid levels (62). In addition, on obese Sprague-Dawley rats, *H. sabdariffa* showed anti-obesity effects by suppressing the appetite leading to reduced food intake. Weight reduction can be obtained after the administration of high doses of *H. sabdariffa* extracts (63). Moreover, ethanolic extracts of *H. sabdariffa* flower showed a reduction in lipid profile

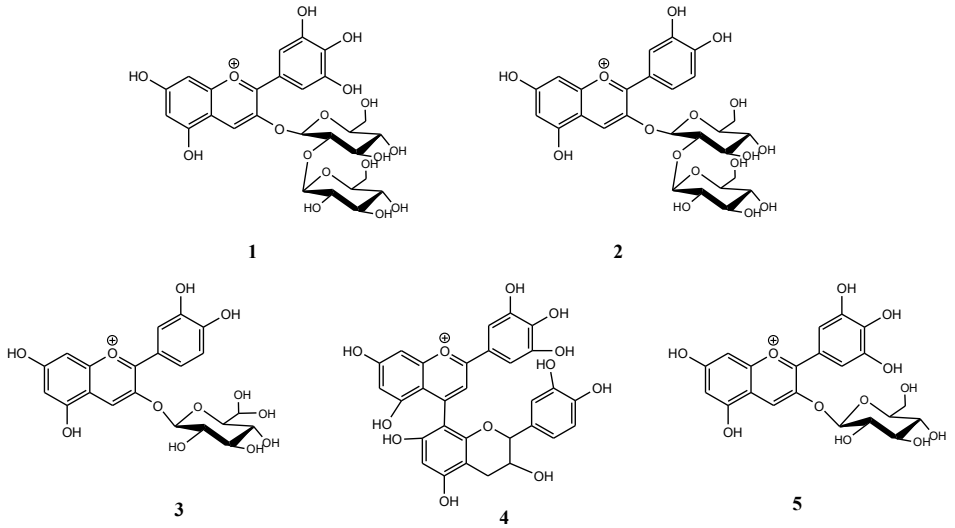

Figure 3. Chemical structure of anthocyanin compounds.

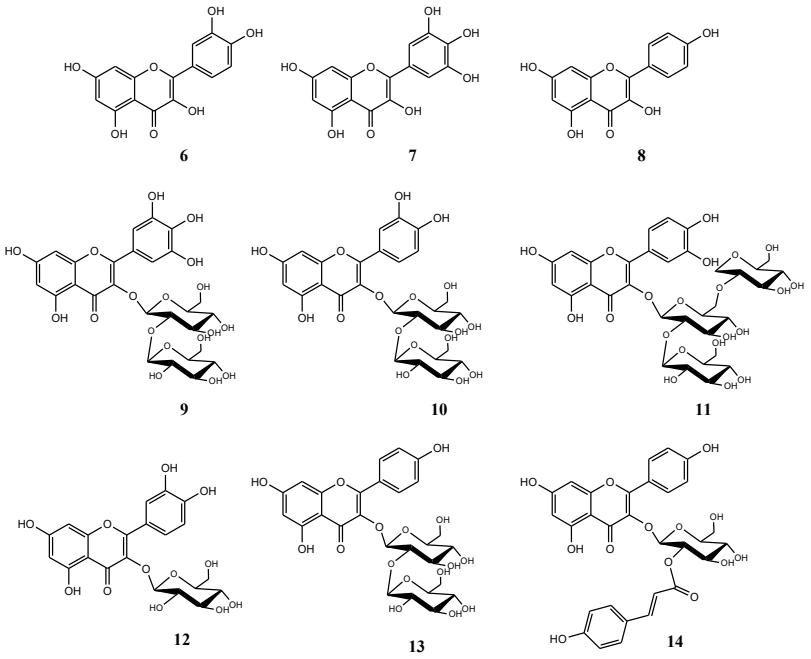

Figure 4. Chemical structure of flavonoid compounds.

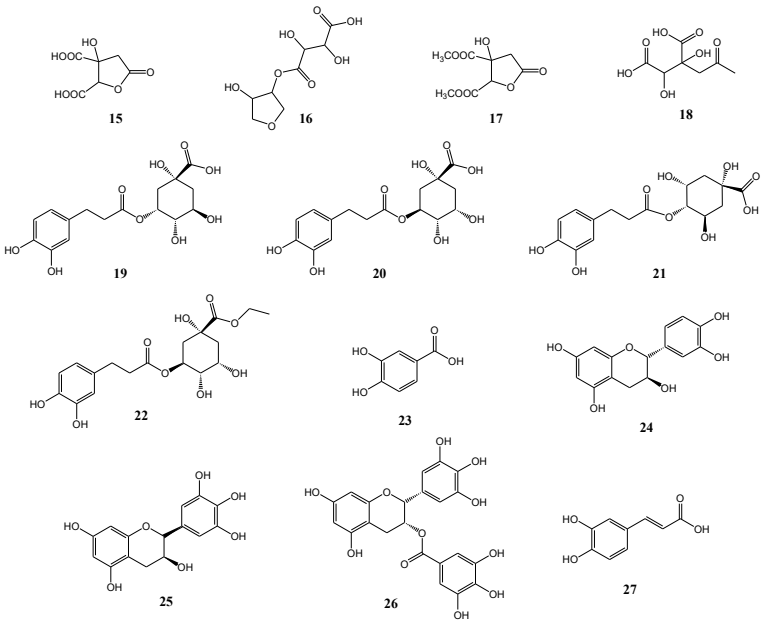

Figure 5. Chemical structures of organic and phenolic acid compounds.

in male Sprague-Dawley rats that were employed with dietary fat (64).

Antihypertensive activity

The rat aorta is subjected to assess the vasorelaxant effect. The relaxation effect is achieved after the administration of *H. sabdariffa* extract. It blocks the voltage-dependent calcium channels in smooth muscle and leads to inhibition of the influx of extracellular calcium. This may reduce the blood pressure level (65). In addition, *H. sabdariffa* extracts have an antihypertensive effect in the condition of salt-induced hypertension and hypertension due to chronic nitric oxide synthase (NOS) inhibition (66).

Antimicrobial effect

Methanol extracts of *H. sabdariffa* presented an antimicrobial effect on multidrug-resistant bacteria, *Acinetobacter baumannii,* in hospitalized settings with

the minimum inhibitory concentration range from 11.3 to 13.6 mm (67). Calyx of *H. sabdariffa* has shown to have inhibitory effects against gram-positive and gram-negative bacteria. It showed the highest inhibitory effect on *Klebsiella pneumonia* (gram-positive) and *Staphylococcus aureus* (gram-negative) with minimum inhibitory concentration of 17.5 mm and 18.5 mm, respectively (68). Extraction of calyx and leaf of *H. sabdariffa* showed antibacterial activity against *Escherichia coli, Salmonella enterica,* and *Listeria monocytogenes.* Leaf extracts showed a significant effect on *Listeria monocytogenes* and *E. coli,* while calyx extracts of *H. sabdariffa* showed a strong inhibitory effect on *S. enterica,* with no detectable bacteria at all times observed (69).

Diuretic and anti-hyperuricemic activities

Hibiscus sabdariffa extract has been shown to influence oxonic acid (OA)-induced hyperuricemia in rats. It reduces the serum uric acid levels by affecting the uricase activity in the liver and blood of rats. Uricase is important in the conversion of uric acid to allantoin, which reduces the serum uric acid level in the body (70). Increased sodium excretion in the urine has been shown due to the natriuretic effect of *H. sabdariffa* extract (71).

Anti-tumour

Hibiscus sabdariffa exhibited antitumor activity in human gastric carcinoma cells by activating JNK or p38 MAPK kinase, which induced apoptosis in human gastric carcinoma cells (72). Furthermore, in humans with promyelocytic leukaemia cells, *H. sabdariffa* inhibited the growth of HL-60 cells by inducing apoptosis (73). *H. sabdariffa* possessed antimetastatic activity in mice tumour models. The antimetastatic activity was developed during the migration and angiogenesis of B16-F1 cells. The migration of tumor cells was reduced by inhibiting the PI3k pathways and reducing the expression of Rho proteins and MAPKs (74).

Anti-inflammatory activity

Hibiscus sabdariffa plays a role as an anti-inflammatory agent. A study reported reducing inflammation in the renal tract by inhibiting lipopolysaccharide (LPS)-induced IL-1β production, NF-κB activity, and inflammatory cell infiltration in the kidney (75). Additionally, consumption of the aqueous extract of *H. sabdariffa* in healthy adults was shown to have low inflammatory mediators like IL-6 and IL-8, which are responsible for the inflammatory process (76). Furthermore, the leaf extract of *H. sabdariffa* demonstrated anti-inflammatory activity in LPS-induced inflammatory response with the inhibition of NOS (77).

Conclusion

In summary, *H. sabdariffa* shows great ethnomedicinal properties accounting for various pharmacological activities. In India, Africa, and Jordan, it is used to treat hypertension, microbial infection, and other diseases. It is also used in food preparations, such as herbal tea, sauces, and jellies.

The plant has high amounts of anthocyanin compounds followed by flavonoids, organic, and phenolic acids. Anthocyanins significantly contribute to its antioxidant property, which prevents lipid peroxidation of LDL cholesterol. Animal studies have consistently shown that the consumption of *H. sabdariffa* extracts reduces lipid levels by affecting adipogenesis activity. The plant has also been shown to significantly reduce hypertension and uricemia; however, its mechanism of action should be investigated.

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Authors' contributions

All authors have equally contributed to the literature survey and collected the data from the various published articles to be included in the manuscript. NIMS conceived of the presented idea, developed the article, wrote, and prepared the manuscript; NM supervised the research and critical revision of the article. All authors read the manuscript and confirmed the publication of the final version.

Conflict of interests

The authors declare there is no conflict of interest concerning this study.

Ethical considerations

Ethical issues regarding authorship, data acquisition, review, and analysis have been carefully observed by the authors.

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