IJPSR (2022), Volume 13, Issue 8 (Review Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 December 2021; received in revised form, 20 February 2022; accepted, 28 April 2022; published 01 August 2022

A BRIEF REVIEW ON *HIBISCUS SABDARIFFA* LINN WITH THEIR MEDICINAL USES AND PHARMACOLOGICAL ACTIVITY

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Keywords:

Hibiscus sabdariffa, Pharmacological activity, Phytoconstituent, Anticancer, Flavonoid, Nutritional value

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ABSTRACT: *Hibiscus sabdariffa* Linn is a medicinal plant commonly known as Rosella, Sorrel, Jamaica, red-Ambadi, Queensland jelly plant *etc*. belonging to the family *Malvaceae*. Since ancient times, it has played an important role in treating various diseases. It enlarges great importance for its therapeutic potential. It is native to India, tropical Africa, and many countries like Brazil, Australia, Hawaii, Florida, Philippians, Vietnam, Central America, *etc*. *H. sabdariffa* is one such plant grown as a garden plant and vegetable plant with many traditional uses. It has immense potential as a medicinal plant and also has many beneficial effects. The plant is having height of about 3.5 m tall with dark green, red steam. The leaves are 7.5 to 12.5 cm long, calyx is red colored and consists of 5 large sepals, and the fruit is a velvety capsule about 1.25-2cm long, green in color containing 5 valves each containing 3-4 seeds. The whole plant is having many pharmacological activities reported, including Antidiabetic effect, anticancer activity, hypertensive effect, hypolipidemic effect, blood pressure-lowering effect, Antihelmentic, antioxidant effect, antiseptic, astringent, demulcent, digestive, purgative properties. It is also used to treat cancer, cough, dyspepsia, hangover, heart ailment, neurosis, scurry and strangury. These activities can be attributed mainly to the presence of phytochemicals such as flavonoid, pectin, antioxidant, anthocyanin's, hibiscus acid, Vitamin C, protein, citric acid, β-carotene and so on.

INTRODUCTION: *Hibiscus sabdariffa* Linn is a medicinal plant or shrub belonging to the family Malvaceae. It is known as Roselle, Sorrel, Red Sorrel, Indian Sorrel, Guinea Sorrel, Sour Sorrel, Jamaica Sorrel, Queensland Jelly Plant, Jelly Okra *etc*. It is also called as Karkade or Carcade in North Africa and Near East.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(8).3112-27

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(8).3112-27

In India it is known as Gonguru, Lal Ambari, Batwa (Hindi), Lal- Ambadi (Marathi), Lal mista, Chukar (Bengali), Pulichchai Kerai (Tamil), Pulachakiri Pundibija (Kannada), Polenchi, Puchichai (Malyalam) and Chukiar (Assam) [1].

The plant is generally found in Countries like India, Saudi Arabia, Malaysia, Indonesia, Thailand, Philippines, Vietnam, Sudan, Egypt and Mexico (*i.e.*, it is native to India- Malaysia or Tropical Africa) [2]. Out of the mentioned above countries, some are the main producers of Roselle blossom and cultivate to be consumed. In the tropics like the Caribbean, Central America, India, Africa, Brazil, Australia, Hawaii, Florida and the Philippines, the

plant *H. sabdariffa* is widely grown as a home garden crop [1]. Other Varieties of this plant are planted or cultivated for the fibers produced by the plant and for their medicinal uses. Hibiscus has more than three hundred species distributed in tropical and subtropical regions worldwide and is used as ornamental Plants [2]. The plant has height about 3.5 m. tall and has dark green to red stems. Leaves are 7.5 – 12.5 cm long, green with reddish veins and long or short petioles. The calyx is typically red and consist of 5 large sepals with a collar (epicalyx) of 8 -12 slim and having pointed tracts around the base. The fruit is a Velvety capsule, 1.25-2 cm long, which is green in colour in its immature stage. It has 5 valves containing 3-4 seeds which are light brown in colour. There are generally 2 varieties of species *H. sabdariffa i.e. H. sabdariffa* Var. Sabdariffa and *H. sabdariffa* Var. Altissima Wester [1]. Research has shown that Roselle consists of phytochemicals like citric acid, anthocyamin, Hibiscus, vitamins, protein, and flavonoid of various substances like gossypectine, bibiseetine and sabdaretine, *etc*. the antioxidant pectin, *etc.* [3]. *H. sabdariffa* has many nutritional and pharmacological Activity including Hypolipidemic effect, Blood pressure lowering effect, Antidiabetic activity, Antihelmentic and Microbial effect, Diuretic effect, Antiseptic, *etc*. Astringent, Demulcent, Purgative. It is also used in the treatment of abscesses bilious condition, cancer, cough, dyspepsia, fever, hangover, heart ailment, hypertension, neurosis, scurry and strangury. Hibiscus tea has many nutritional values [3].

FIG. 1: WHOLE PLANT OF *HIBISCUS SABDARIFFA*

FIG. 2: CALYCES OF *H. SABDARIFFA*

Classification:

Kingdom: Plantae
Division: Tracheophyta
Class: Magnoliopsida
Order: Malvales
Family: *Malvaceae*
Genus: Hibiscus L
Species: *Hibiscus sabdariffa* L [4].

Vernacular Name:

English: Roselle, Red Sorrel
Hindi: Gonguru, Lal-Ambari, Putuja
Bengali: Lal-mista, Chukar
Marathi: Lal- Ambadi
Tamil: Pulichchai Kerai
Telugu: Yerra gogu
Kannada: Pulachakiri, Pundibija
Malayalam: Polechi, Pulichchai
Assam: Chukiar

Varieties [1]: *H. sabdariffa* Var. Altissima Wester. *Sabdariffa* Var. *Sabdariffa* [1].

Origin: There are many arguments about the origin of *Hibiscus sabdariffa* (Roselle) among different Scholars. *H. sabdariffa* is a native plant of west Africa and from there, it was carried to other parts of the words of west Africa and America, whereas in the other, it is originated from India and Saudi Arabia [2]. *Hibiscus sabdariffa* (Roselle) was domesticated in western Sudan before 4000 Bc. It was first recorded in Europe in AD 1576. The (Mesta) variety is cultivated and harvested in Maharashtra [5].

Cultivation and Collection: *H. sabdariffa* (Mesta, Roselle) is cultivated in India in an area of about 1.5 lakh hectares and the average productivity of the crop is around 11 q/ha [6]. It is also cultivated in many countries like Sudan, America, South Africa, and so on for their different uses like medicine, nutritional purpose, fiber collection and consumption as a food. Roselle is an annual event shrub that takes 5 months from planting to harvesting (i.e., perennial plant) [5].

Climate and Soil: Roselle plant requires 130 – 250 nm month rainfall in the 1st three to four months of growth; for the desired growth of plant dry weather is well deserved or well tolerated. The quantity and yield of Roselle calyces can be downgraded by the rain or high humidity at the time of harvesting and drying [5]. It grows in kharip season and cannot withstand waterlogging. It required rich loamy soil it may grow in a variety of soils, including new and old alluvium & lateritic loam. The acid soils are not suitable without proper amendment. The Sabdariffa develop chlorosis with high pH of the soil [6].

Planting: Rosella plant is very sensitive to the length changes of the day. Rather than the rainfall requirement the plant requires the planting time to be set according to the length of the day. Deep ploughing is recommended for preparing the seedbed because it is a deep-rooted crop. The seeds required for planting 1-hectare land is about 6 - 8 kg/ hectares, and sowing is about 2.5 cm deep. The seed are generally planted at the beginning of the rainy season. 60 cm^{-1} 1m distance between rows and 45 – 60 cm apart is the criteria for planting. The larger calyx is produced if the planting rate is less. The sowing is done by hand or by using a grain drill machine. Also, the corn planter is also used for sowing the Roselle seed as an alternative tool. Thinning of the plant should done by hand (manpower). There are over 100 cultivars or seed varieties of *H. Sabdariffa*; the wide commercial varieties are growing in China, Mexico, Thailand, and Africa.

Harvest and Storage: *H. sabdariffa* is generally harvested in the period of late November onwards; the harvest is decided according to the ripeness of the seed. The fleshy calyces are harvested after the flower is dropped but before the seed pod has dried and opened. The disease and sun cracking are more susceptible to calyx if the capsule remains for longer time on the plant after the seed begins to ripen.

The calyces ripen about three weeks after flowering, which is 100 – 160 days after the plants are transplanted outdoors. The fruit ripens from the bottom to the top of the calyces. Harvesting is carried out by using manpower, *i.e.*, by intensive hand labour the calyces being picked singly at the appropriate stage.

FIG. 3: H. *SABDARIFFA* CULTIVATION [7]

FIG. 4: *H. SABDARIFFA* COLLECTION [8]

The fruit may be harvested when fully grown but still tender when they can be easily snapped off by hand later harvesting requires clippers. In the morning, the fruit is easy to break up than in the evening or end of the day. Each fruit yields about 7–10 gm of sepals. For the prevention or preservation the food, drying is a traditional method. The two ways the Roselle drying should be done are by harvesting the fresh fruit and sun drying the calyces or leaving the fruit to partially dry on the plant and harvesting the dried fruit, keeping the crop well protected during the process. Dehydration should be done depending on the two fundamental processes of heat transfer and mass

transfer also, the fruit, fleshy calyces, is peeled off and then dried it under shade [5].

Pest Control and Weeds: The stem rot and root rot are the major diseases found in *Hibiscus sabdariffa*, which can be prevented by the different techniques of monitoring the water content in an irrigated field, by avoiding the planting of other crops that are prone to these diseases. In this plant, insect damage is minor but exit; pest includes stem borer, flea beetles, abutilon moth, cotton bollworm and cutworm, mealy bugs and leafhopper are minor concerns cotton strainer.

Plant enemies usually do not compete in a cultivated field. Weeding can increase yield and calyx size. The filled of rollete are generally weeded if necessary [5].

FIG. 5: ATTACK OF INSECT ON *H. SABDARIFFA* PLANT [9]

FIG. 6: ATTACK OF INSECT ON *H. SABDARIFFA* PLANT [10]

Weeds Classification and Their Relative Density of non-Weeded Roselle (Karkade) During the Growing Season are as-

TABLE 1: WEEDS CLASSIFICATION AND THEIR RELATIVE DENSITY OF NON-WEEDED ROSELLE (KARKADE) DURING THE GROWING SEASON [11]

Scientific Name	Classification	Common Name	Weed Density
Cenchrous biflours	Monoco	Alhuskaneet	27%
Zornia glochidiata	Dicot	Sheilini	21%
Trienemra pentanture	Dicot	Alraba	11%
Sesamum alatum	Dicot	Simsim Elgumal	4%
Ocimum basilicum	Dicot	Elryhan	0.7%
Echinocola colonum	Monocot	Aldiffera	4%
Rullia patula	Dicot	Tagtaga	7%
Corchorus olitorius	Dicot	Almlukhia	3%
Tribulus trerrestris	Dicot	Aldraisa	0.3%
Ipomea kordofana	Dicot	Eltabar	1.6%
Solanum dobium	Dicot	Aljubain	6%
Abutilon figarinum	Dicot	Alniada	7.0%
Ipomea sinensis	Dicot	Elhantoot	0.3%

Botanical Description: The genus Hibiscus *(Malvaceae)* include more than 300 species of annual or perennial herbs, shrubs or trees. The synonym of *H. sabdariffa* are *Abelmoschus crientus* (Bertol) Walp, *Furcaria sabdariffa* Ulber, *Hibiscus crentus* Bertol, *Hibiscus fraternus* L. *Hibiscus Palmatilobus* Baill and *Sabdariffa rubrakostel*. Some people believe that *H. sabdariffa* is native to India or Saudi Arabia. In contrast, Murdock showed evidence that *H. Sabdariffa* was domesticated by the black population of western Sudan (Africa). Nowadays, it is cultivated in tropical and subtropical regions, including India, Saudi Arebia, China, Malaysia, Indonesia, The Philippines, Vietnam, Sudan, Egypt, Nigeria, and Mexico. There are mainly two varieties of *Hibiscus sabdariffa* first being *H. sabdariffa* Var. Altissima Wester, cultivated for its jute-like fiber, has green red streaked inedible calyces while the second variety is *H. sabdariffa* Var. *Sabdariffa* has yellow-green edible calyces and it also yield fibre [12].

Geographical Prevalence: The *H. sabdatiffa* L. Species is possible of Indian origin. Is occur in

tropical America and Africa. Before 4000 Bc, Roselle was domesticated in western Sudan. It was first recorded in Europe in AD 1576. It seems to have been carried from America to the new world by slaves for use as a food plant. In Jamaica, the Roselle was called a Jamaican sorrel in 1707.

The use of the plant as green was known in java as early as 1658. Roselle was cultivated in Mexico, part of central America, taken from New York. The West Indies and in southern Florida, Texas and California in the late 19th century. It is grown for culinary purposes in much of the tropical world. Roselle is grown for its calyces which are exported from Sudan, China, and Thailand, and it is also grown for its calyces in Mexico. In Sudan, it is collected by goat-herding nomadic tribes.

Karkade is grown in various parts of Sudan, particularly Kordofan and Darfur. It is one of the cash crops cultivated by traditional farmers in kordofana and Darfur states under rain-fed conditions.

Where large quantities are produced both for local consumption and for export. The total area under cultivation was estimated approx 9370 – 32960 hectares in 2000/2001. The crop is grown mainly by traditional farming methods exclusively under rainfed conditions.

China and Thailand are also major producers and control much of the world's supply. Thailand has invested heavily in Roselle production, and their product is of superior quality, whereas china's practices are less reliable and reputable. Egypt, Senegal, Tanzania, Mali, and Jamaica are important suppliers, but production is mostly used domestically [5].

Ethnopharmacology: *Hibiscus sabdariffa* has had a lengthy history of use as medicine in Africa and other neighboring tropical countries. In India, Africa, and Mexico, infusion of leaves or calyces is traditionally used as a diuretic, cholerectic, febrifugal and hypotensive effect, decreasing the blood's viscosity and stimulating intestinal peristalsis. In Senegal, it is recommended as a hypotensive agent. In northern Nigeria, this plant has been used to treat constipation. The decoction of seed is traditionally used to enhance or induce lactation in cases of poor milk production, poor let-down, and maternal mortality. In Egypt, the plant is widely used to treat cardiac and nerve diseases. In Egypt and Sudan, an Infusion of Karkade calyces is used to lower body temperature. In Guaternala it is used for treating drunkenness. In Iran, drinking sour tea is a popular practice for the treatment of hypertension.

In North Africa, calyces' preparation is used to treat sore throat and cough as well as genital problems. The mucilaginous leaves are used as a topical emollient in Africa. Roselle is also used in treatment of cancer. In India, a decoction from seed is used to relieve pain in urination and indigestion. In brazil, the root is believed to have somatic and emollient properties.

Chinese folk medicine is used to treat the liver disorder and high blood pressure. The hibiscus flower is often a component of tea mixtures in western countries. To quench thirst, Thailand's people always consume Roselle juice. Karkade seed products (*i.e.*, Karkade defatted flour, protein concentrate, protein isolate) have been studied for their nutritional and functional value.

In many regions it is also used for antibacterial effect, vermifuge, preventive chemo effect, laxative effect. The leaves have been used like spinach. Its flower has been used in sachets and perfumes due to its fragrant. Fibre of *H. sabdariffa* has been used to fashion rope as a jute substitute [1, 2, 12].

Phytochemicals: *H. sabdariffa* (Roselle) is cultivated in various regions mainly for its calyx, which is of three types: Green, Red, and Dark Red. The most used calyces are Red and their used are characterised by their anthocyanin concentration.

The major anthocyanin present in red calyces is Delphinidin 3- Sambubioside and Cyanidin 3-Sambubioside *H. sabdariffa* (Roselle) other components including organic acid, minerals, amino acids, carotene, vitamin C and total sugar in its calyx.

Leaves and seeds at various levels depending on variety and geographical area [2]. According to many studies on Roselle, several compounds have been isolated and characterized from Roselle, including flavonoids, triterpenoids, anthocyanidins, steroids and alkaloids.

Nutritional Value: In the fresh calyces of *Hibiscus sabdariffa* the nutritional composition varies between various studies, probably due to different varieties, genetics, environment, ecology and harvest condition of the plant. Earlier studies of calyx of *H. sabdariffa* reported that its content of protein (1.9g/100g), fat (0.1g/100g), Carbohydrates (12.3g/100g) and Fibre (2.3g/100g). The calyces are reached in vitamin c (14mg /100g), β-Carotene (300ug/100g), calcium (1.72 mg/100g), and Iron (57mg/100g) [7]. The leaves contain protein (3.3g/100g), Fat (0.3g/100g), Carbohydrates (9.2g/100g), Minerals (phosphorous) (214mg /100g), Iron (4.8mg/100g), Thiamine (0.45 mg/100g), β-carotene (4135 ug/100g), Riboflavin (0.45mg/100g) and ascorbic acid (54mg /100g) [7]. The seeds contained crude fatty acid oil (21.85 %), Crude protein (27.48 %), Carbohydrates (21.25 %), Crude fibre (16.44 %) and ash (6.2 %), in terms of minerals the most prevalent is potassium (132 g +- 1.47 mg/100g), sodium (65g +- 1.58mg/100g), Calcium (647 +- 1.21 mg/100g), Phosphorus (510 +- 1.58 mg/100g) and Magnesium (442.8 +- 1.80 mg/100g). The major saturated fatty acid in the seed oil is palmitic (20.84 %) and stearic (5.88 %) acid, and the unsaturated fatty acids are linoleic (39.31 %), Oleic acids (32.06 %) [12]. The approx nutrient content of different part of *Hibiscus sabdariffa* per 100 grams are mentioned in **Table 2.**

TABLE 2: NUTRIENT CONTENT OF DIFFERENT PART OF H. SABDARIFFA PER 100 G [2]

Nutrient	Calyxes	Seeds	Leaves
Protein (g)	2	28.9	3.5
Carbohydrate (g)	10.2	25.5	8.7
Fat (g)	0.1	21.4	0.3
Vitamin A (I.E)	-	-	1000
Thiamine (mm)	0.05	0.1	0.2
Riboflavin (mm)	0.07	0.15	0.4
Niacin (mm)	0.06	1.5	1.4
Vitamin C (mm)	17	9	2.3
Calcium (mm)	150	350	240
Iron (mm)	3	9	5

Bioactive Component: *H. sabdariffa* having main constituents relevant in the context of its pharmacological are organic acid, anthocyamin, Polysaccharide and Flavonoids.

Organic Acid: *H. sabdariffa* extracts contain a high percentage of organic acids, including citric acid tartaric acids as major compound [12]. The aqueous extract of *H sabdariffa* having major organic acids is Citric acid and Malic acid.

And oxalic acid and Ascorbic acid are minor compounds. Linn (1975) detected tartaric acid, citric acid and oxalic acids by paper chromatography in flower extract of *H. sabdariffa*.

In the calyx of *Hibiscus sabdariffa* the presence of a high concentration of oxalic, malic, tartaric and succinic acids was also reported [13].

Based on previous studies, the percentage of organic acid in hibiscus flowers varies; Hibiscus acid accounts for 13- 14% citric acid, 12- 70 %, malic acid 2 -9 %, Tartaric acid 80 %, and 0.02-0.05 % of ascorbic acid (Vitamin C).

In the late 1930s, the aqueous extract of calyx contains Citric and malic acids, also in five different strains (from Egypt, Senegal, India, Thailand and central America) of *H. sabdariffa* Var. *Sabdariffa*.

In the Calyces of H sabdariffa the presence of Ascorbic acid is detected but it contains is varies with fresh and dried calyces (260 – 280 mg/100g) the amount of ascorbic acid is higher than previous reported in literature.

The differences in content observed is due to different varieties, genetics, environment, ecology, and harvest condition [12].

The five different constituents detected in the H. Sabdariffa are [13]

- Delphinidin – 3 – Sambubioside.
- Cyanidin – 3 – Sambubioside.
- Hibiscus Protocatechuic acid.
- Hibiscetin.
- Gossypectin.

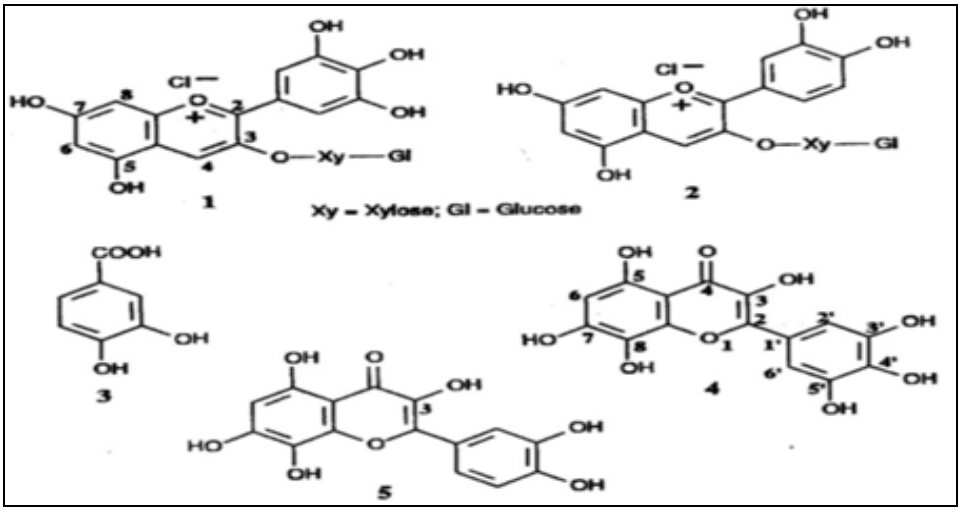


FIG. 7: SOME CONSTITUENTS OF *HIBISCUS SABDARIFFA*. (1. DELPHINIDIN-3-SAMBUBIOSIDE, 2. CYANIDIN-3-SAMBUBIOSIDE, 3. HIBISCUS PROTOCATECHUIC ACID, 4. HIBISCETIN, 5. GOSSYPECTINE.) [13]

A. Hydroxycitric Acid: *H. sabdariffa* has an additional Hydroxylic group at the second carbon of citric acid. This acid has four stereoisomers (2S, 3S), (2R, 3R), (2S, 3R) and (2R, 3S) and their lactone forms. The principle organic acid found in the calyx of *Hibiscus sabdariffa* is (2S, 3R)-Hydroxycitric acid. It is the priciple organic acid found in the calyces of *H. sabdariffa*. The (2S, 3R)-Hydroxycitric acid from Hibiscus is different from the more commonly known (2S, 3S)-Hydroxycitric acid (HCA) extracted from, *e.g.*, *Garcinia sp*. Thus, raising the question as to whether both diastereomers have identified or partially different pharmacological profiles.

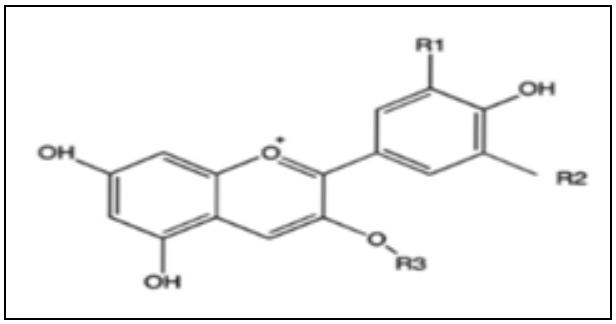
B. Hibiscus Acid: Is the lactone form of (+) – allo – hydroxycitric acid. It comprises a citric acid moiety with an additional hydroxy group at the second carbon and has two diastereomers due to the existence of two chiral centers in the molecule. Hydroxylic acid, Hibiscus acid, is derivative as the major organic acid and calyces extract of *H. sabdariffa*.

Anthocyanins: Anthocyanin's are a group of flavonoid derivatives and naturals pigments present in the dried flowers of *H. sabdariffa,* and their colour varies with pH [12]. In the *Hibiscus sabdariffa* flower extract, most of the chemical investigated has been directed towards characterization of their pigments [13]. Delphinidin and Cyanidin-based anthocyanin's, include Delphinidin – 3 – Sambubioside (hibiscin), Cyanidin – 3 – Sambubioside (gossypicyanidin) and other [7]. Yamamoto and Oshima (1932) isolated an anthocyanin from the calyx of H. Sabdariffa are, "hiviscin" also known as "hibiscin", to which later they assigned the name Delphinidin – 3 – Sambubioside and assigned the structure Cyanidin – 3 – glucoside this was later changed to Delphinidin – pentoside – glucoside [12, 13]. This pigment is further isolated by DU and Francis (1973), Three different anthocyanin's were isolated from the pigment of calyces of *H. sabdariffa i.e.*, Delphinidin – 3 – Sambubioside (hibiscin), Delphinidin – 3 – glucoside and Cyanidin – 3 – glucoside (chrysanthenin) using material from Taiwan and Trinidad [12]. In addition, they characterized Cyanidin – 3 – Sambubioside as the second most abundant anthocyanin in the extract [13].

Shiberta and Furukawa (1969) studied the pigment of Taiwanese Roselle and reported the presence of Delphinidin – 3 – Sambubioside and a small amount of Delphinidin 3 – monoglucoside, Cyanidin – 3 – monoglucoside and Delphinidin [13].

Later, Cyanidin – 3 – 5 – diglucoside and Cyanidin – 3 – (2G – glucosylrytinoside) in the flower pigment of H. *sabdariffa* Var. *Altissima* was reported. A study was conducted with 5 different strains of *H. sabdariffa* Var. *Sabdariffa* reported Cyanidin – 3 -Sambubioside and Cyanidin – 3 – glucoside as major compounds present in the plant. In the case of the strains (Senegalese), Delphinidin glycosides were absent. In the study, the anthocyanin content reached 1.7 % to 2.5 % of the dry weight of calyces in terms of Delphinidin – 3 – Sambubioside [12]. More recently, Anthocyanin's in *H. sabdariffa* has been qualified with HPLC and

their relative percentage determined Delphinidin – 3 – Sambubioside (56%), Delphinidin – 3 - glucoside (4%), Cyanidin – 3 – Sambubioside (33%) and Cyanidin – 3 – glucoside (3%) [13].



Cyanidin-3-Sambubioside (R1 = OH; R2 = H; R3 = Sambubioside Delphinidin-3Sambubioside (R1 = OH; R2 = OH; R3 = Sambubioside) Cyanidin-3- glucoside (R1 = OH; R2 = H; R3 = Glucose) Delphinidin-3-glucoside (R1 = OH; R2 = OH; R3 = Glucose)

FIG. 8: CHEMICAL STRUCTURE OF MAIN ANTHOCYANIN [12]

Flavonoid's: In the last few decades, there has been great interest in plant polyphenolic flavonoids and phenolic acids due to their antioxidant activity and protective effect against the development of cardiovascular diseases and cancer [13]. In simple or polymerized form, the polyphenol and flavonol types are present in *H. sabdariffa*. The following flavonoid has been described in H. Sabdariffa extracts Hibiscetin (Hibiscetin – 3 – glucoside), quercetin and lute Olin, as well as chlorogenic acid, Protocatechuic acid, pelargonidic acid, eugenol, the sterol β-sitosterol and ergosterol.

The flower of *H. sabdariffa* was recorded to contain 3 – monoglucoside of Hibiscetin (Hibiscitrin) 7 – glucoside of gossypectine (gossypitrin) and Sabdaritrin, which on acid hydrolysis yielded an hydroxy flavone named sabdaretine. These flavonol glycosides were low, with Hibiscitrin being the major compound followed by gossypitrin and Sabdaritrin, in 1961 gossypetin – 3 – glucoside (gossytrin) was isolated. The petals of *H. safdariffa* Var. Altissima also contain gossypetin – 8 – glucoside (0.4%) and gossypetin – 7 – glucoside. In the leaves of *H. sabdariffa* the presence of β -Sitosterol – β – D - galactoside and from the seeds ergosterol were reported, in *H. sabdariffa* extract. The quercetin lute Olin and its glycoside are contained in the methanolic extract of the *H. safdariffa* flower. Quercetin has already been identified in *H. safdariffa*. One study reported that the amount of quercetin in water extract of calyces of *H. safdariffa* was 3.2 mg/g while rutin was 2.1 mg/g. Quercetin and its conjugated glycosides (quercetin – 3 – glycoside) and rutin (quercetin – 3 – rutinoside) were frequently identified in water extract of calyces of *H. sabdariffa*, along with Kaempferol. The water extract of the dried leaves showed the presence of catechin (4.25 %) and ellagic acid (28.20 %), while the water extract of Calyces of *H. sabdariffa* showed the presence of protocatechuic acid (19.85 %), gallocatechin gallate (27.98 %), Huang and Co-workers reported a similar result. Phenolic acid Protocatechuic acid (PCA) is an important phenolic acid present in *H. sabdariffa* extract. It was isolated from the dried flowers of *H. sabdariffa* and assigned the structure of 3 – 4 -dihydrobenzoic acid. Another phenolic acid present in the leaf and calyces of *H. sabdariffa* is the cholinergic acid, belonging to the family of esters formed between certain trans-cinnamic acids (caffeic acid, ferulic acid, and p-coumaric acid) and quinic acid. In one study, the amount of chlorogenic acid in the extract was reported to be 2.7 mg/g [12].

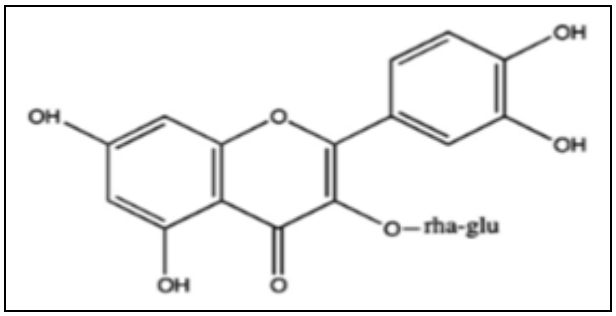


FIG. 9: QUERCETIN – 3 – RUTINOSIDE

Mucilage, Pectin and Carbohydrates (Polysaccharide): The petals of *H. sabdariffa* were reported to yield 65% (dry weight of mucilage, and this yielded galactose, galacturonic acid, and rhamnose on hydrolysis [13]. In the water extract of calyces of *Hibiscus sabdariffa*, polysaccharides are another key group of compounds present in large quantities. In one study, ethanol precipitate water extract yielded 10 % of the reddish polysaccharide. In two different fractions, the following compound are identified i.e., arabinose, galactose, glucose, rhamnose and smaller amounts of galacturonic acid, glucoronic acid, manose and xylose, the similar result obtained in two other studies.

TABLE 3: THE GENERAL COMPOSITION OF FRESH LEAF AND FRUIT OF *HIBISCUS SABDARIFFA*

Component	Amount (% fresh leaf weight)	Amount (% fresh fruit weight)
Water	85.6	84.5
Protein	3.3	1.9
Fat	0.3	0.1
Total carbohydrate	9.2	12.3
Fibre	1.6	2.3
Ash	1.6	1.2
Calcium	0.213	0.0017
Phosphorus	0.093	0.057
Iron	0.0048	0.0029
β-carotene equivalent	0.0041	0.0003
Ascorbic Acid	0.054	0.014
Thiamine	0.00017	-
Riboflavin	0.00045	-
Niacin	0.0012	-

In the calyces of five strains of *H. sabdariffa* Var. Sabdariffa the mucilage contained was reaching 24 – 28 %. This amount was only reached at a later stage of development in the strain from Senegal and Thailand. In these five strains, the pectin content is only accounted 2-4 %, while the sugar reached a maximum of 3-5%, mucilage and pectin consisted of 60-80% anhydrouronic acid. The petals of *H. sabdariffa* yielded about y5 % of the dry weight of mucilage, which is on hydrolysis produced galactose, galacturonic acid and rhamnose while the leaves only yield 10% [12].

Lipidcontent: Salama and Ibrahim in 1979 studied the sterols of seed oil of *Hibiscus sabdariffa* and reported the presence of cholesterol, campasterol, stigmosterol, β-sitosterol, α-spinasterol and ergosterol. They also reported that the seed oil is a good source of lipid-soluble antioxidant, α-tocopherol 25%, β-tocopherol 74.5% and γ-tocopherol 0.5%. while the component acid of the seed lipid where, myristic 2.1%, palmitic 35.2%, palmitoleic 2.0%, stearic 3.4%, oleic 34%, linoleic 14.4 % and 3 unusual HBr reaching fatty acids (cis-12, 13- epoxy – cis – 9 – octadecenoic (12- 13 – epoxoleic) 4-5%, sterculic 2.9 % and malvalic 1.3%) [13].

Volatile Compound: The volatile compound is responsible for aroma in *H. Sabdariffa*. In the study conducted in 1992, more than twenty-five volatile compounds (accounting for less than 8 % of total *H. sabdariffa* seed composition) were reported in seed of *H. sabdariffa*. They were mainly unsaturated hydrocarbons, alcohols and aldehyde from C8 to C13. Subsequently, thirty-seven volatile compounds are characterized by the five different groups from the water extract of *H. sabdariffa* calyces. These compounds included fatty acid derivatives (such as 2 – ethyl furan and hexanal), sugar derivatives (furfural and 5 – methyl – 2 – furaldehyde), phenolic derivatives (eugenol) terpenes (such as 1, 4 – cineole, limonene) and miscellaneous compound (*e.g.,* Acetic acid).

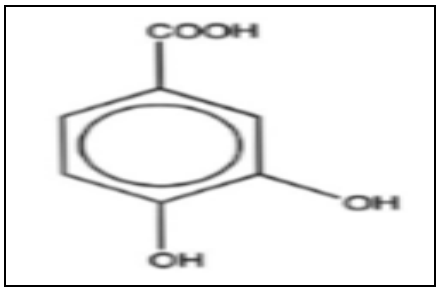


FIG. 10: PROTOCATECHUIC ACID

In another study, the volatile profile was examined in four aqueous extracts from fresh and dried calyx using GC-MS's two different, time – temperature extraction conditions. A total of thirty-two compounds were identified and could be divided into five chemical groups, *i.e.,* aldehyde (fourteen compounds), terpenes (two compounds) and acid (one compound). A total of seven aromatic volatile were common to all four-sample tested (Hexagonal, 3 – octanone, octanal, 1 – octen – 3 – one, nonanal, 2 – 4 nonadienal (E, E) and geranylacetone. While researching the Pharma-cological action of *H. sabdariffa* extract, it is important to keep in mind that the polyphenol content of the extract was for some activities reported as the one responsible for the effect. Nevertheless, the polyphenol content is a very general and often poorly defined term as it includes

a complex mixture of anthocyanin's, organic acids, phenolic acids and flavonoid compound [12].

Uses of *H. sabdariffa*:

Domestic Application: The uses of a different part of *H. sabdariffa* (Roselle) are many and varied both in food and in traditional medicine. All parts of *H. sabdariffa*, including leaves, fruit, seed and roots, are used as food in different parts of the world. For the production of soft drinks and tonics without alcohol like wine, juice, jam jelly, syrup, dried and brewed into tea and spice, the fleshy red calyces are commonly used. These are rich in carotene, riboflavin, anthocyanin's, ascorbic acid, niacin, calcium, iron, and Vitamin C [2]. The young leaves and tender stems of Roselle are consumed raw as green vegetables; the seeds of Roselle are a good source of protein, fat, and total sugar and are widely used in the diet in many African countries [2].

Medicinal and Industrial Application: The Roselle plants have many medicinal applications developed worldwide. In China, it is used to treat hypertension, pyrexia, liver damage and Ayurvedic medicine. Recently for the effective treatment of leukemia the sepals extract has been used due to its high content in polyphenols, particularly protocatechuic acid. Roselle seed, which has no commercial application, is a source of vegetable oil that is low in cholesterol and rich in other phytosterols and tocopherol, particularly β-sitosterol and γ-tocopherol. The overall characteristics of Roselle seed oil allow for important industrial application and represent added value for its cultivation. *H. sabdariffa* has certain therapeutic properties *i.e.*, reported benefit of taking it internally in the form of herbal tea.

Include soothing cold, clearing a block nose, clearing mucous, as an astringent, promoting kidney function, aiding digestion as general tonic, as diuretic and helping to reduce fever, taken as a drink made from the calyx, it is a mild diuretic and purgative, among the many other effects. The drink is said to be a folk remedy for cancer. Restored Roselle drink has no bacterial isolation.

***Hibiscus* Tea:** Hibiscus tea is an herbal tea free from caffeine from a special type of Hibiscus called *Hibiscus sabdariffa*. Specifically, the tea is made out of dried fruit part of Roselle called calyx. It is red in colour and taste like berries. It is also commonly sold in domestic markets.

FIG. 11: DRIED CALYCES OF *H. SABDARIFFA* [6]

FIG. 12: HIBISCUS TEA BAG OF *H. SABDARIFFA* [5]

Steps of Preparation of Hibiscus Tea: First, collect the hibiscus fruits and wash them clean and air dry or dry them in an oven at 70 degrees C for 3days. Peel off the calyx and store them in air-tight containers.

To make tea, simply take 2grams of the dried calyx, and crush them into small pieces using a wooden roller. Put them in a teabag or a net, bring out your favorite mug, add 8 oz. of boiling water, steep it for 2-4 min, add sugar if desired, or add other flavours of your choice few drops of lemon juice. You can also refrigerate it and make *Hibiscus* iced tea [5].

Pharmacological Activity:

Effect on Smooth Muscle: Many studies show that the Hibiscus sabdariffa has a relaxation effect on the smooth muscle. This has been proposed to be partially responsible for its hypotensive action [13]. The alcoholic extract of *H. sabdariffa* flower had an antiseptic effect by relaxing the uterus and intestine strips *in-vivo*. This was also observed in rabbit aortic smooth muscle. The extract of

Hibiscus calyces inhibited the tone of various isolated muscle preparation that included rabbit and rat aortic strips and ideal rat strips. The extract also rhythmically contracted rat uterus, Guinea pig tracheal chain, and rat diaphragm. The same extract stimulated quiescent rat uterus and frog rectus abdominus muscle [12, 13]. The tonic effect on rat uterus were partially reduced by hydrocortisone and indomethacin. The overall effect is a direct relaxation of smooth muscle [13].

More recently, the *H. sabdariffa* water extract (1 – 100 mg/kg) was found to inhibit rat bladder and uterine contractibility in a dose-dependent manner but via a mechanical unrelated to a local or remote autonomic receptor or calcium channel, as previously suggested by Salah [12]. The relaxation response was related to endothelium-dependent and endothelium-independent mechanisms or mediated through calcium channels possibly generated by constituents such as quercetin and eugenol [13]. Later, Salah showed that crude extract mainly induced the endothelium-dependent relaxation effect in the isolated thoracic aorta of rats *via* stimulation of the Nos enzyme by the Pi3–k/Akt pathway. It was suggested that this was due to polyphenols. The non-endothelium-dependent relaxation is a direct smooth muscle activation and results in the activation of smooth muscle potassium channels.

Antibacterial, Antifungal and Antiparasitic Activity: The water extract of calyces of *Hibiscus sabdariffa* and protocatechuic acid (5mg/ml) inhibited the growth of methicillin, resistant *Staphylococcus aureus, Klebsiella, pneumoniae, pseudomonas, aeruginosa* and *Actinobacter baumannii*. Moreover, protocatechuic acid (in a dose-dependent manner) showed greater antimicrobial activity against these pathogens in broth than in human plasma. The study is also shown that the antimicrobials effect was independent of temperature, as shown by heat treatment; hibiscus extract also demonstrated an antibacterial effect against streptococcus mutants, cariogenic bacteria from the oral cavity with a minimum inhibitory concentration of 2.5 mg/ml, and campylobacter species (campylobacter jejune, campylobacter coli and campylobacter fetus) that contaminates meat like poultry, beef and pork at a concentration range of 96 – 152 ug/ml.

The aqueous methanolic extract of dried calyces of *H. sabdariffa* showed an *in-vitro* inhibitory effect against several bacterial strains, such as *S. aureus, Bacillus strearothermopilus, micrococcus luteus*, serrate mascences, *Clostridium sporogenes, Escherichia coli, K. pneumoniae, Bacillus cereus* and *Pseudomonas* fluorescence, but did not affect the growth the fungus *Candida albicans*. The fresh calyces water extract of *Hibiscus sabdariffa*, ethanol extract and protocatechuic acid (20mg/ml) effectively inhibited the growth of food spoilage bacteria such as salmonella typhimurium DT104. *E. coli* O157:H7 listeria monocytogenes, *S. aureus* and *B. cereus*. Again, the antimicrobial effect was not affected by heat treatment and ethanolic extract. The ethanolic extract shows a greater antimicrobial effect than the aqueous extract. A methanol-water extract of *Hibiscus sabdariffa* was effective against E. coli O157.H7 isolated from food, veterinary and clinical samples, with the high concentration (10%) being the most effective. The crude extract of *H. sabdariffa* seed (200mg/l) shows antimicrobial effect against three types of gram-negative bacteria. The extract exhibited higher activity against salmonella, followed by shigella and Enterobacter.

Neuroprotective Activity: Two studies were reported on the nephroprotective activity of H. *Safdariffa* extracts on diabetic nephropathy in streptozotocin-induced type 1 diabetic rats. Nephropathy may progress to end-stage renal disease. A study was conducted to investigate the effect of the polyphenol extract of *H. safdariffa* (100 and 200 mg/kg/day) in streptozotocin-induced diabetic nephropathy in rats. The extract revealed beneficial effects as the kidney mass was reduced and the hydropic change of renal proximal convoluted tubules was improved; it reduced serum triglyceride, total cholesterol, and LDL and increased the activity of catalase and glutathione and reduced lipid peroxidation in the kidney. It was found that the extracts reduced kidney mass and improved hydropic change of renal proximal convoluted tubules in this rat model. The positive effect shown by the extracts might be via improving the oxidative status and regulating Akt / Bad / 14-3-3c signaling (anti-apoptotic mechanisms). Another *in-vivo* study also revealed that its nephroprotective effect results from the protection of the kidney from oxidative stress.

Antipyretic, Antinociceptic, Anti-inflammatory Activity: Many studies show that Hibiscus sabdariffa effectively relieves pyrexia in popular medicines. Also, in the extract of calyces of *H. sabdariffa* the antipyretic and anti-inflammatory potential were studied *in-vivo*. The ethanol extract (more potent) and water extract showed antipyretic effects by significantly reversing yeast-induced fever in rats. The mechanism differs from the one of aspirin, a prostaglandin inhibitor. Nevertheless, fever entails enhanced formation of cytokines such as interleukins (IL), interferons and tumour necrosis factor-α (TNF-α), The calyces extract may be involved in the inhibition of some of these substances, also resulting in an anti-inflammatory effect [12].

Dafallah, in their studies, suggested that the flavonoid, polysaccharide, and organic acid might be the compound responsible for the pharmacological activity. In a more recent study, the ethanolic extract from the calyces also showed antinociciptive effect in a rat model [12, 13]. The two factors of the crude aqueous ethanolic extract of the dried calyces of *H. sabdariffa* exhibit impressive immunological activity by production of TNF-α were studied *in-vivo*. Another mechanism in which the polyphenol extract exhibits its anti-inflammatory activity is by impairing cyclooxygenase-2-induction by downregulating SNK and P38 MAPK.

Antioxidant Activity: Some studies, both in vitro and *in vivo*, have shown that extract of *H. sabdariff*a has a potent antioxidant effect. This activity is due to a strong scavenging effect on reactive oxygen and free radicals and inhibition of xanthene oxidase activity. By lipid peroxidation, the protective action against tert-butyl hydro peroxide-induced oxidative damage and protection of cell damage were studied.

Inhibition in cu2+ mediated oxidation of LDL and the formation of thiobarbituric acid reactive substances (TBSRs), inhibition of the formation of malondialdehyde content (100-300 mg/kg). Reduction of glutathione depletion, increase of the liver and decrease in blood activity of superoxide dismutase and catalase, while in the liver it increases superoxide dismutase, catalase and glutathione and decreases malondialdehyde. The effect was observed for both water and ethanolic extract from flowers of *H. sabdariffa* as well as from the seeds or leaves [12].

Hepatoprotective Activity: Water extract of calyces of *Hibiscus sabdariffa* (100-800 mg/kg) showed a hepatoprotective effect in a range of models based on toxin-induced hepatitis including tert-butyl hydroperoxide, lipopolysaccharide, azathioprine, carbon tetrachloride, cadmium, ammonium chloride, acetaminophen and irradiation *in-vivo* and *in-vitro*. This effect is due to a strong antioxidant activity that reduces cellular damage by reducing oxidative stress and attenuating mitochondrial dysfunction by decreasing Bax and tBid expression in the liver. The activity of superoxide dismylase (SOD), catalase (CAT), glutathione peroxidase (Gpx) and aminolevulinate dehydratase (d-ALA-D) enzyme increases by the extract. While decreased lipid peroxidation in induced models of liver damage and decreased liver marker enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) in experimental hyperammonemia. *Hibiscus* anthocyanin extract also induced phase 2 drug detoxifying enzymes, such as glutathione s- transferase, NAD (H) quinone oxidoreductase and uridyl diphosphoglucuronosyl transferase in an induced liver damage model (ccl4-mediated toxicity model) [12].

Renal Effect / Diuretic Effect: The renal effect of *H. sabdariffa* extract has been characterized pharmacologically in clinical trials and preclinical experiments in rats. In Thailand, a two-phase study was carried out with thirty-six healthy men to evaluate the changes in urine after consumption of *H. safdariffa* juice (16g/day and 24 g/ day) to determine its effect on the treatment and prevention of renal stone.

Despite the fact that the consumption of H sabdariffa causes a decrease in creatinine, uric acid, citrate tartrate, calcium, sodium, potassium, and phosphate. It did not affect the concentration of oxalate in urinary excretion. The water extract of calyces (250, 500 & 750 mg/kg body weight) also effectively prevents the development of urolithiasis (stone- disorder) in male albino rats. A decrease in renal ca2+ mg2+ ATPase activity and unaltered

renal calcium handling un rats after administration of water extract of calyces at 25 and 50 mg/kg was shown. By reduction of serum urea and creatinine levels, renal function was enhanced. In another preclinical study in rats, at the dose range of 500-2500, mg/kg b.w of water extract of calyces produced diuretic and natriuretic effects with a potassium-sparing effect [13].

Lactating Activity: The ethanolic seed extract of *H. safdariffa* (200–1600 mg/kg) increased the serum prolactin level ($p< 0.01$) when compared to the controlling a dose-dependent manner in lactating Albino Wistar rats.

Anti-obesity Activity: Pre-clinical data from Brazil indicates a potential role in the control of certain conditions associated with obesity, such as hyperlipidaemia. However, further studies were suggested A report showed that a standardized (33.64 mg of total anthocyanins per each 120 mg) water extract of calyces of *H. sabdariffa* could reduce weight gain in obese mice while at the same time it increases the liquid intake in healthy and obese mice. This effect is probably achieved through the modulation ofPI3-K/Akt and ERK pathway, which play pivotal roles during adipogenesis. *In-vitro* and *in-vivo* studies showed that Hibiscus extract (or tea) inhibited amylase activity, blocking sugars and starch absorption, which may assist in weight loss. A study conducted in Mexico using an ethanol extract of *H. sabdariffa* concluded the extract could be considered as a possible anti-obesity agent due to its effects on fat absorption-excretion and body weight of rats. The therapeutic use of the extract, possibly due to polyphenols, was also evaluated in patients with metabolic syndrome, an obesity-associated collection of disorders. Meanwhile, a study showed that the aqueous extract was more efficient in inhibiting triglyceride accumulation when devoid of fibre and polysaccharides. Still, when polyphenols were fractionated and isolated, the benefits of the whole extract was greater than the sum of its parts.

Delayed Puberty Activity: A few studies with rats have shown that consumption of *H. safdariffa* water extract during pregnancy and lactation resulted in increased postnatal weight gain, delayed onset of puberty, and elevated body mass index at the onset of puberty in the female offspring. The consumption of the extract during pregnancy and lactation caused a decrease of maternal fluid and food intake with increased plasma Na+ and corticosterone concentration, while the accelerated growth and delayed puberty observed in the offspring could be due to increased corticosterone and decreased leptin delivery through breast milk. However, these studies require confirmation as no observations have been reported in the literature up to date pointing to the presence of respective effects in humans [12].

Antihypertensive: Aqueous extract of petals exhibited antihypertensive and cardioprotective effects in rats. Infusion is also significantly lower both systolic and diastolic pressure in spontaneously hypertensive and normotensive rats. Tea of calyces showed an 11.2% reduction in systolic blood pressure and 10.7% decrease in diastolic pressure. The effectiveness and tolerability of a standardized extract were studied in patients with mild to moderate hypertension, which revealed a reduction in systolic and diastolic blood pressure by more than 10 percent. The aqueous extracts of the calyx showed a dose-dependent decrease in the mean arterial pressure of the rats. The extract has a vasodilator effect in the isolated aortic rings of hypertensive rats. These effects are probably mediated through the endothelium-derived nitric oxide-cGMP-relaxant pathway and inhibition of calcium influx into vascular smooth muscle cells. Daily consumption of tea lowers blood pressure in pre and mildly hypertensive adults. It may prove an effective component of the dietary changes recommended for people at risk of developing hypertension [1].

Scavenging of ROS: *Hibiscus sabdariffa* extracts and their constituents, Protocatechuic acid, anthocyanins demonstrated the ability to scavenge the 1, 1- diphenyl-2- picrylhydrazyl (DPPH) and 2, 2-azino-bis (3-ethylbenzthiazoline-6-sufonic acid) (ABTS) free radicals using a cell-free system. *Hibiscus sabdariffa* extracts and their constituents have also been observed to scavenge the t-butyl hydro peroxide radical and prevent oxidative damage in rat primary hepatocytes. The extracts have been shown to scavenge hydroxyl radical (OH•) and Hydrogen peroxide (H_2O_2). The extracts also showed a strong inhibitory effect on xanthine

oxidase activity and superoxide ($\bullet O_2^-$) radical. Hibiscus protocatechuic acid isolated from *Hibiscus sabdariffa* inhibits lipopolysaccharide-induced rat hepatic damage and inhibits oxidation of low-density lipoprotein induced by either copper or a nitric acid donor. *Hibiscus sabdariffa* anthocyanins were effective in significantly mitigating the path toxicity induced by paracetamol in mice, it also protects against DNA damage induced by tert-butyl hydroperoxide in rats' smooth muscle and hepatoma cells. The strong antioxidant and anti-lipid peroxidation actions of *Hibiscus sabdariffa* extracts and the compounds they contain anthocyanins and Hibiscus protocatechuic acid may potentially be useful in ameliorating or preventing these diseases and conditions [13].

Anti-diabetic Activity: Diabetes mellitus can be defined as an endocrine and metabolic disorder characterized by chronic hyperglycemia, dyslipidemia, and protein metabolism resulting from defects in insulin secretion regulations and/or insulin action. The protective effect of a polyphenol extract of *H. sabdariffa* was studied in a type II diabetic rat model (high-fat diet model). At a 200 mg/kg dose, the extract demonstrated anti-insulin resistance properties as it reduced hyperglycemia and hyperinsulinemia. It decreased serum triacylglycerol, cholesterol, and the ratio of low-density lipoprotein/high-density protein (LDL/HDL) and reduced the plasma advanced glycation end products (AGE) formation and lipid peroxidation. The currently accepted therapeutic strategy for postprandial hyperglycemia control is based on the inhibition of a-glucosidase and amylase. This results in an aggressive delay of carbohydrate digestion to absorbable monosaccharides. With this in mind, a study was conducted to determine the effect of Hs extraction intestinal glucosidase and pancreatic amylase activity *in-vitro*. As a result, *H. Sabdariffa* extract was a potent pancreatic-amylase inhibitor. Similar results were found for hibiscus acid (hibiscus-type (2S,3R)-hydroxy citric acid lactone), which inhibited pancreatic-amylase and intestinal-glucosidase enzyme.

Diabetes mellitus is a risk factor for coronary heart diseases and atherosclerosis. An ethnobotanical study conducted in the Caribbean for urinary problems and diabetes mellitus revealed that *H. safdariffa* is traditionally used to 'clean' the liver and blood within a group of plants used for cooling, high cholesterol, and urinary problems. When the respondents were asked which medicinal plants we reused for high blood pressure, diabetes and jaundice, *H. safdariffa* was referred to as hypertension. A study in alloxan-induced diabetic rats showed that an ethanolic extract of H. *Safdariffa* flowers (200 mg/kg) had a strong hypolipidemic as well as antioxidant effect. Thus, *H. safdariffa* extract showed therapeutic promise in decreasing and preventing the development of atherosclerosis and possible related cardiovascular pathologies linked with diabetes. The authors suggest that this activity might be related to polyphenolic compounds and dihydrobenzoic acids, like protocatechuic acids, but further identification of the active compounds is warranted. A similar effect was reported with the extract suppressing the high-glucose-induced migration in a vascular smooth muscle cell model [12].

Effect on Blood Pressure: Intravenous injection of aqueous extracts of *Hibiscus sabdariffa* calyx to anesthetized cats and anesthetized rats lowered blood pressure dose-dependent. More recently, the antihypertensive action of *Hibiscus sabdariffa* has been confirmed in rats with experimental hypertension and in spontaneously hypertensive rats given the aqueous extracts at doses of 250–1000 mg/kg for up to 14 weeks. Dietary supplementation with *Hibiscus sabdariffa* has reduced blood pressure in patients with moderate essential hypertension. This hypotensive action of *Hibiscus sabdariffa* extracts was due to inhibition of the angiotensin-converting enzyme. The angiotensin-converting-enzyme inhibition has also been demonstrated in vitro with a crude hydro ethanol extract of *Hibiscus sabdariffa* calyces and was ascribed to flavones present in the extract. In addition, a beneficial cardioprotective effect of this extract was shown in vivo and was attributed to flavonoids and anthocyanins.

Lipid-lowering Effects: Blood lipids and lipoproteins circulating in the blood in the form of LDL are decreased in response to treatment with *Hibiscus sabdariffa*. Ethanol extract of *Hibiscus sabdariffa* has been shown to reduce cholesterol, VLDL-cholesterol, and LDL-cholesterol in

alloxan–diabetic rats. Dietary supplementation with *Hibiscus sabdariffa* effectively lowered serum concentrations of triglycerides, total cholesterol, and LDL-cholesterol in hypercholesterolemic rabbits and hypercholesterolemia rats.

In addition, thiobarbituric acid reactive substances (TBARs) and conjugated dienes formed during oxidation of LDL by $CuSO_4$, CCl_4 were reduced. Similar study using Hibiscus anthocyanin's (HAs) extracts shown the extracts decrease the relative electrophoretic mobility of oxLD, inhibit fragmentation of Apo B, reduced TBARS formation in the Cu2+-mediated oxidize LDL and scavenge over 95% of free DPPH radicals. Lipid fractions in plasma, heart, brain, kidney and liver were lowered in hypercholesterolemia rats fed with *Hibiscus sabdariffa* calyx (5% or 10%) for 9 weeks [13].

Anticancer Effect: *In-vitro* studies have shown that *Hibiscus sabdariffa* extracts can induce apoptosis in cancer cells. Hibiscus polyphenol-rich extracts (HPE) induce cell death in human gastric carcinoma (AGS) in a concentration-dependent manner; this effect of HPE on AGS cells was mediated via p53 signalling and p38 MAPK/FasL cascade pathway.

Also, Hibiscus anthocyanin's extract (a group of natural pigments existing in the dried calyx of *Hibiscus sabdariffa* L.) caused cancer cell apoptosis, in HL-60 cells, similarly, Delphinidin 3-Sambubioside (Dp3-Sam), isolated from the dried calices of *Hibiscus sabdariffa* L. induce apoptosis in human leukaemia cells (HL-60).

Anticlastogenic effects of *Hibiscus sabdariffa* extract has been demonstrated against sodium arsenite-induced micronuclei formation in erythrocytesin mouse bone marrow. Various studies on Hibiscus protocatechuic acid have demonstrated its ability to inhibit the carcinogenic action of various chemicals in different tissues of the rat, including diethylnitrosamine in the liver, 4-

nitroquinoline-1-oxide in the oral cavity, azoxymethane in the colon, N-methyl-N-nitrosourea in glandular stomach tissue and N butyl-N-(4-hydroxybutyl) nitrosamine in the bladder. Tseng *et al.* (2000) also demonstrated that Hibiscus protocatechuic acid inhibits the survival of human promyelocytic HL-60 cells in a concentration- and time-dependent manner.

The data presented by Tseng *et al*. (2000) suggest that the compound is an apoptosis inducer in human leukaemia cells and that RB phosphorylation and Bcl-2 protein may play a crucial role in the early stage [13].

Anti-Anaemic Effect: A preliminary study on the use of *H. sabdariffa* decoctions as an alternative source of iron to treat anaemia and some other mineral deficiency diseases was conducted and showed that dry fermented calyces of Hibiscus exhibited a very low pH value which enhanced mineral availability. Another reason for enhancing mineral (iron, zinc, calcium and magnesium) bioavailability is the high concentration of ascorbic acid

Toxicological Effect: The extract of *Hibiscus sabdariffa* was found to be relatively and virtually non-toxic, with LD_{50} in rats being above 5000 mg/kg [8]. In bioassay for screening plant extracts for their biological activity, the lethal dose (LD_{50}) of three different types of *H. safdariffa* extract was assessed in the brine shrimp toxicity assay.

Aqueous *H. safdariffa* extract (*i.e.*, infusion) produced an LD_{50} of 9.59lg/ml, while the dichloromethane extract was 24.51lg/ml 4.75lg/ml for the ethanolic extract. Given the very limited value of the brine shrimp assay for complex mixtures like plant extracts and the incomplete information on the mode of preparation of the extracts, this work is mentioned here for completeness only.

The LD_{50} in mice (b. w. 30 g) was reported to be about 0.4–0.6 ml on intraperitoneal administration of a 30% aqueous *H. safdariffa* decoction (20 min in distilled water).

The same authors observed a lowered blood pressure in dogs (b. w.: 7 kg) with no side effects after administering (i.p.) 10 ml of a 10% solution of the *H. safdariffa* decoction [12].

CONCLUSION: The information from *in vitro* and *in-vivo* studies shows a wide range of potential new health applications and therapeutic targets for *Hibiscus sabdariffa. Hibiscus sabdariffa* is

relatively safe and virtually non-toxic. Many pharmacological properties of *H. sabdariffa* may be attributed to a plethora of phytochemicals in the plant. The potent antioxidant activity of *Hibiscus sabdariffa* may be linked to the presence of different antioxidants compounds with different sites and mechanisms of action, which may act alone or in concert with one another.

Therefore, dietary supplementation of *Hibiscus sabdariffa* plant extract may be beneficial in reducing the risk of developing various pathological conditions such as cardiovascular disease, cancer, neurological disorders and diabetes.

ACKNOWLEDGMENT: I wish to acknowledge the contribution of Dr. G. M. Chavan, for his assistance in refractive data collection and for all his support. Also, I want to thank all cited literature owners for their valuable or quality work from which I completed my review.

CONFLICT OF INTEREST: No conflict of interest

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How to cite this article:
Himanshu CC and Chavan GM: A brief review on *Hibiscus sabdariffa* Linn with their medicinal uses and pharmacological activity. Int J Pharm Sci & Res 2022; 13(8): 3112-27. doi: 10.13040/IJPSR.0975-8232.13(8).3112-27.