Review

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Novel Insight into the Cellular and Molecular Signalling Pathways on Cancer Preventing Effects of *Hibiscus sabdariffa*: A Review

Raihana Yasmin[1], Sangeeta Gogoi[1], Jumi Bora[1], Arijit Chakraborty[2], Susmita Dey[1], Ghazal Ghaziri[3], Surajit Bhattacharjee[4], Laishram Hemchandra Singh[5]

[1]Department of Zoology, Royal Global University, Guwahati, [2]Department of Sports Physiology and Nutrition, National Sports University, Imphal, India, [3]Department of Cell and Molecular Biology, Kharazmi University, Tehran, Iran, [4]Department of Biological Sciences, Dr. BR Ambedkar English Model School, Agartala, [5]Department of Zoology, Dhanamanjuri University, Imphal, India

A category of diseases known as cancer includes abnormal cell development and the ability to infiltrate or spread to other regions of the body, making them a major cause of mortality worldwide. Chemotherapy, radiation, the use of cytotoxic medicines, and surgery are the mainstays of cancer treatment today. Plants or products produced from them hold promise as a source of anti-cancer medications that have fewer adverse effects. Due to the presence of numerous phytochemicals that have been isolated from various parts of the *Hibiscus sabdariffa* (HS) plant, including anthocyanin, flavonoids, saponins, tannins, polyphenols, organic acids, caffeic acids, citric acids, protocatechuic acid, and others, extracts of this plant have been reported to have anti-cancer effects. These compounds have been shown to reduce cancer cell proliferation, induce apoptosis, and cause cell cycle arrest. They also increase the expression levels of the cell cycle inhibitors (p53, p21, and p27) and the pro-apoptotic proteins (BAD, Bax, caspase 3, caspase 7, caspase 8, and caspase 9). This review highlights various intracellular signalling pathways involved in cancer preventive potential of HS.

Key Words Cancer, Herbs, Plant extracts, Apoptosis, Ethnopharmacology, Cell cycle

INTRODUCTION

Cancer is a leading cause of death worldwide, characterized by abnormal growth of cells which tend to proliferate in an uncontrolled way consequently progressing from a pre-cancerous lesion to a malignant state [1]. Nearly 2.6 million new cancer cases with 1.7 million deaths per year are projected by 2030. Numerous behavioural and dietary risk factors can increase the rate of cancer occurrence: these include physical inactivity, unhealthy diet, smoking, consumption of alcohol, and exposure to certain chemicals and hazardous substances [2]. It has been found that only 5% to 10% of all cancer cases are related to genetic defects whereas 90% to 95% are due to environmental and lifestyle factors. It is also estimated that 30% of cancer in developed countries is linked to nutritional factors [3].

According to World Health Organization (WHO), the most common forms of cancer are breast cancer, colorectal cancer, lung cancer, prostate cancer, lung cancer, prostrate cancer, skin cancer, and stomach cancer [4]. Lungs, prostate, colorectal, stomach, and liver cancers are predominantly diagnosed among men whereas, breast, colorectal, lung, cervix, and stomach cancers are common in women. The most prevalent form of cancer among women is breast cancer accounting for nearly about 25% of all cancer incidence and responsible for about 15% of death worldwide [5]. It has been found that 70% of death from cancer occurs in low- and middle-income countries according to the report by WHO [2]. In 1975, the low and middle-income countries account for 51% of all cancer worldwide which increased to 55% in 2007 and is projected to reach 61% by 2050. Western industrialized countries are no longer the only one which is largely confined to cancer of the lung, prostate, breast, and colon but it became a worldwide concern [6].

Chemotherapy, surgery, radiation, and immunotherapy are the different treatment options for cancer. Any of these

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Correspondence to Arijit Chakraborty, E-mail: arijitphysiology@gmail.com, https://orcid.org/0000-0003-2975-7333

depends on the location, grade, stage as well as the general state of the patient's health. Chemotherapy and radiation therapy destroy normal cells besides killing cancerous cells [1]. Through surgery, solid tumors are removed from the patient's body but with a plethora of side effects which reduce the quality of life. Some tumours develop resistance to chemotherapeutic drugs and as a result, the patient must be treated with a variety of combinations of drugs. Oncological research is putting a lot of effort so that they can come up with efficient therapies that can alleviate critical side effects caused by conventional treatment procedures. Different technologies are currently under evaluation in clinical trials especially nano-medicines which are also contributing to the development of biocompatible materials for diagnostic and therapeutic purposes for cancer [7]. The development of newer drugs using ethno-medicinal plants to treat various types of cancer has been done [7].

To meet the demand for anti-cancer drugs with fewer side effects, plants can be a promising source from the point of health perceptive in humans [8]. In addition to these, medicinal plants are widely available, and less expensive with negligible or no toxicity as compared to artificial chemotherapeutic drugs [1]. Several medicinal plants and herbal ingredients have been reported to have anti-cancer effects (Table 1) [9-21] and some phytochemicals isolated from these plants have been shown to decrease cancer cell proliferation, induce apoptosis, retard metastasis, and inhibit angiogenesis [22]. One such plant is *Hibiscus sabdariffa* (HS) which belongs to the *Malvaceae* family ordinarily known as red sorrel and roselle. It is used as a folk remedy for the treatment of cancer, cough, fever, heart ailments, and various other disorders. This plant has been reported to have certain anticancer properties with relatively low toxicity, and its phytochemical constituents could be a source of therapeutically useful prod-

Table 1. List of some important medicinal plants having anti-carcinogenic properties

Name of the plant	Parts used	Major phytochemicals	Mechanism of action	References
Azadirachta indica	Seeds	Terpenoids, limonoids	Involves the modulation of cellular proliferation, differentiation, apoptosis, angiogenesis and metastasis processes in cervical cancer	[9]
	Flowers and leaves	Nimbolide, quercetin	Induce growth inhibitory activity in ovarian cancer cells, apoptosis	[10]
Moringa oleifera	Leaves	Quercetin, gallic acid, p-coumaric acid and 4-hydroxy 3 methoxy cinnamic acid	Inhibits cancer cell growth	[11]
Withania somnifera	Roots	Alkaloids (withanolides, withaferin-A, steroids, flavonoids, phenolics	Apoptosis induction	[12]
Catharanthus roseus	Leaf	Alkaloids (vincristine, vindoline, vinblastine, catharantine)	They inhibit cell proliferation by changing the molecular dynamics which induce apoptosis	[13]
Cannabis sativa	Flower or inflorescence	Cannabinoids	Induce cell cycle arrest, promote apoptosis, inhibit proliferation, migration and angiogenesis in tumor cells	[14]
Ziziphus mauritiana	Stem bark	Betulinic acid	Induced apoptosis	[15]
Centella asiatica	Leaves	Triterpenoids (asiatic acid, medacassic acid, asiaticoside)	Anti-proliferative effect inhibits metastatic behaviour of cancer cells	[16]
Curcuma longa	Rhizome	Curcumin	Anti-proliferative and apoptotic effect	[17]
Costus speciosus	Leaves, rhizome	Diosgenin, saponin, β-sitosterol-β-D-glucoside, lupeol, zurumbone, camphene	Up-regulation of cellular apoptotic molecules (p53, p27, p21, caspases, ROS generation reactive oxygen species), down-regulation of anti-apoptotic genes (Bcl-2, JAK, STAT3)	[18]
Betula alba	Outer bark	Betulinic acid	Anti-proliferative effect in a tumor cell, decreases Bcl-2 and cyclin D1 gene expression and increases BAX gene expression, induces apoptotic cell death, decreases cancer cell motility	[19]
Hibiscus rosa sinensis	Flower	Taxol, cisplatin	Induce apoptosis in breast cancer cells	[6]
Murraya koenigii	Leaves	Polyphenols, flavonoids, alkaloids (mahanine)	Induces growth arrest and apoptosis in breast cancer cell lines	[20]
Hedyotis diffusa	Entire plant	Iridoids, flavonoids, volatile oils, anthraquinones, phenolics, sterols, triterpenes, coumarins, alkaloids and cyclotides	Activation of the mitochondrial apoptotic pathway regulates tumor invasion and migration, anti-angiogenesis, and affects tumor cell autophagy	[21]

ucts for various cancer treatments. This plant is native to West and East Africa, South-East Asia, and g North-Eastern India [22].

TAXONOMICAL DESCRIPTION OF HS

Morphology

HS is an erect, mostly branched, annual shrub. It belongs to the family *Malvaceae* and genus *Hibiscus* which is commonly known as "roselle" or "red sorrel" in English, asamsusur in Malay, Kachiebpriew in Thai, and sour tea in Iran. It can grow up to 3.5 m with a deep penetrating taproot. Leaves are dark green to red in colour, alternate, glabrous having 3 to 5 lobes and 8 to 15 cm long. Flowers are red to yellow with a dark centre which is born singly in leaf axils; sepals are prominent, bright red, and fleshy (5 sepals are present and are collectively called calyx) which are usually used to make juice or jam jelly and are mainly used for the medicinal purpose. It is a native species to India, Myanmar, Nigeria, and Egypt and is exotic to Jamaica, Mexico, Spain, and the United States of America. They are widely naturalized in tropical and subtropical regions of the world. It is cultivated mainly due to its different edible parts including seeds, leaves, and roots that are consumed as food and most importantly as medicines by different communities around the world. The calyx of the plant is mainly used for the production of different popularised food products like jam, jelly, soft drinks, and tea among others. The leaves and the stem are also used as vegetables in different parts of the world including India [23]. A summarized region-specific consumption of different parts of the plant is given in Table 2 [24-27].

NUTRIENT CONSTITUTION OF HS

HS is regarded as an ornamental plant because it's a multiple-use species and is famous for its high nutritional and medicinal value as it is highly rich in vitamins and minerals mainly vitamin C and anthocyanins. It has been found that the Indian variety of the seed contains 6% to 8% moisture, 18% to 22% crude protein, 19% to 22% fat, 5.4% ash, 39% to 42% total dietary fibre, 119 to 128 mg calcium, 596 to 672 mg phosphorous, 4.0 mg zinc, 3.1 copper, 393 to 396 mg magnesium, 0.08 to 0.18 mg chromium, 0.36 to 0.51 mg riboflavin and 0.9 mg nicotinic acid. Roselle seed oil is a rich source of gamma-tocopherol (a major form of vitamin E) about 74% and contains sterols including β-sitosterol (71.9%), campesterol (13.6%), δ-5-avenosterol (5.9%), cholesterol (1.35%), and clerosterol (0.6%) [25].

PHYTOCHEMICAL ANALYSIS OF HS

With the help of different sophisticated analytical techniques like ultra-high performance liquid chromatography and liquid chromatography-mass spectroscopy, important biologically active compounds can be detected easily [28]. Different phytochemicals can be detected from the various parts of the plant including the leaf, flower/fruit, and calyx; some chemical components present in the extract such as tannins (17.0%), saponins (0.96%), phenols (1.1%), glycosides (0.13%), alkaloids (2.14%), and flavonoids (20.08%) [29]. The major flavonoids reported in the HS extract are- hibiscetrin, sabdaritin, gossypitrin, and other gossypetin glucosides, quercetin, and luteolin. Moreover, chlorogenic acid, protocatechuic acid (PCA), pelargonidin acid, eugenol, quercetin, luteolin, and sterols such as β-sitosterol and ergosterol are also found in

Table 2. Region-specific variations of *Hibiscus sabdariffa* along with their uses

Countries	Used parts	Traditional uses	Varieties	Types	References
Bangladesh, Myanmar, Mexico, Central America, Nigeria, Queensland, Australia	Fleshy red calyces	Soft drinks, tonics, wine, juice, jam, jelly	*H. rosa sinensis* *H. cannabinus* *H. denudetus* *H. sabdariffa* *H. syrias* *H. trionum* *Alyogyne huegelii*	Tropical, hardy and perennial Hibiscus	[26]
Philippines, Myanmar, Bangladesh, India	Tender stem and young leaves	Vegetables	*H. rosa sinensis* *H. cannabinus* *H. hirtus* *H. syriacus*	Tropical and perennial Hibiscus	[24]
Central and West Africa	Seeds	Seed oil, seeds roasted and grind and consumed as coffee substitutes	*H. rosa sinensis* *H. cannabinus* *H. hirtus* *H. syriacus* *H. sabdariffa*	Perennial and hardy Hibiscus	[25]
United Kingdom, Thailand, Italy	Dried calyces	Tea, syrup, readymade drinks	*H. syriacus* *H. acetosella* *H. manihot* *H. sabdariffa*	Perennial and hardy Hibiscus	[27]

HS extract. Other flavonoids such as naringenin, reported to have anti-ageing and anti-carcinogenic properties, are found abundantly in the plant. Moreover, one peptide molecule roseltidar T1 (plant knottins) was previously reported to prevent mitochondrial dysfunction. Ferulic acid one of the most common phenolic acids is found in the extract of HS plants and has anti-ageing and anti-diabetic properties [30].

Leaf

Numerous phytochemicals were isolated from the methanolic leaf extracts of the plant which include carbohydrates, proteins, alkaloids, phytosterols, flavonoids, diterpenes, saponins, phenols, chlorogenic acid, and tannins [31]. The flavonoids include- B-sitosteryl-β-D-galactoside from wet extracts and dried leaf extract. Organic acids such as hydroxy citric acid (CA), hibiscus acid, and their derivatives were profusely found in the leaf extract of the plant [32].

Calyx

Phytochemical analysis of HS calyces reveals the presence of a relatively high amount of anthocyanin as compared to phenolic acid [33]. In the aqueous extract, the phytochemicals found were anthocyanins-delphinidin-3-sambubioside, cyanidin-3-sambubioside, chlorogenic acid, chlorogenic acid isomer I, chlorogenic acid isomer II, 5-O-caffeoyl-shikimic acid, 3-caffeoylquinic acid, 4-caffeoylquinic acid, quercetin, quercetin-3-sambubioside, quercetin-3-rutinoside, quercetin-3-glucoside, quercetin-pentosylhexoside and myricetin-pentosylhexoside along with organic acids such as hibiscus acid, hydroxyl-CA, CA, malic acid, tartaric acid and ascorbic acid [34]. The anti-carcinogenic effects of the plant are provided in Table 3 [6,35-44].

Flower

Major phytochemicals isolated from flowers were PCA, polysaccharides (mannose, sucrose, xylose, glucose, galactose, rhamnose, glucuronic acid, and rhamnose), and phenolic acids. The major flavonoids found were cyanidin-3-glucoside, cyanidin-3-sambubioside, delphinidin, delphinidin-3-glucoside, and delphinidin-3-sambubioside [26]. The chief phenolic compounds isolated from the petals were sabdaretin, gossypetin, quercetin, astragalin, nicotiflorin, luteolin, gossytrin, and chlorogenic acid [45].

Seeds

Various studies suggest that starch, cholesterol, cellulose, campesterol, ergosterol, propionic acid, pentosans, pelargonic acid, palmitoleic acid, isopropyl alcohol, ethanol, 3-methyl-1-butanol, caprylic acid, formic acid, linoleic acid, palmitic acid, malvalic acid, methanol, myristic acid, and oleic acid are abundant molecules present in seeds of the plant. Some of the bioactive compounds present in seeds were alkaloids, flavonoids, saponins, triterpenes, sterols, tannins, and phenolic compounds among others [45].

Roots

Tartaric acid and saponins were isolated along with the major alkaloid acid called cardiac glycosides from the root extract of HS [45].

EFFECTS OF HS ON SYSTEM PHYSIOLOGY

Anti-microbial effect

Numerous scientific studies revealed that the phytochemicals found in different parts of HS plants have antibacterial properties against different pathogens including methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* among others. The compounds like flavonoids, phenolic compounds, saponins, and alkaloids present in the different parts of the plant especially in the calyces have antibacterial properties against different invading microorganisms [27]. It has been also found that the roselle seed oil has some antibacterial effects against *Bacillus anthracis* and *S. albusin* [27]. It has been reported that the methanolic extract of the HS calyces has antibacterial activity against MDR strains of *Acinetobacter baumanni* and also inhibits pedestal induction by entero-pathogenic *Escherichia coli* and promotes bacterial filamentation [46].

Anti-inflammatory effect

The phytochemicals present in HS extract effectively act as an anti-inflammatory substance that can reduce pain and inflammation due to ascorbic acid present in the calyces of the plant acts as a decent anti-inflammatory agent [47]. For this reason, in West Africa, this plant is cultivated mainly for the treatment of pain and inflammation by local application of boiling leaves in the affected area. The methanolic extract of the plant has been found to have some therapeutic properties for the treatment of inflammation [47].

Antioxidant effect

It has been shown that the free radicals present in living organisms are responsible for causing oxidative damage to different biological molecules such as nucleic acid, lipids, and proteins present in our body which may result in degenerative diseases [48]. Antioxidants like poly-phenolic acid, flavonoid acid, and anthocyanins play a major role which inhibits the oxidation of aforementioned molecules. In calyces of HS, compounds such as anthocyanins have antioxidant properties and there is a positive correlation between the weight of the petals and the antioxidant capacity of the plant extract [33].

Anti-hypertensive effect

The tea made from the HS leaves effectively lowers the blood pressure level in patients because of the presence of phytochemicals that induce systemic vasodilation along with lowering the low-density lipoprotein level thus improving the blood lipid profile of the patients [49].

Table 3. Summated findings of *Hibiscus sabdariffa* (roselle) in the prevention and treatment of cancer

Sl. No.	References	Roselle component	Route of administration	Study type	Treatment dose and duration	Human equivalent dose	Technique for preparation	Study model	Methods	Statistical analysis	Salient findings
1.	[38]	Flower extract	Oral	In vivo	175, 250, 500 mg/kg b.w.; 21 wk	~14.06 mg/kg b.w.	Roselle juice is prepared from the dried calyces by boiling, filtered and dried	Pre-pubertal Wister rats and induction of breast cancer by 7,12-dimethybenz(a) anthracene (DMBA)	Haematological analysis, ELISA, histological analysis	ANOVA, Dunnett's post hoc test	*Hibiscus* juice reduced tumor incidence, tumor burden and decreased tumor volume in experimental models significantly increasing antioxidant activity and thus having anti-breast tumour properties.
2.	[39]	Flower extract	Cell culture medium	In vitro	1.5, 3.05, 6.25, 12.5, 25, 50, 100 μg/mL; 24 h	NA	Flowers are macerated in three different solvents- ethyl acetate, ethanol and n-hexane	A549 lung cancer cell line-eight variety	MTT assay, ELISA	SD, linear equation	The leaf extract showed anticancer activity which can be further developed as a source of natural anticancer from plants.
3.	[6]	Flower extract	Cell culture medium	In vitro	4 mg/mL; 48 h	NA	Flowers are ground into a fine powder, boiled, and filtered via gravity filtration followed by vacuum filtration. It was the freeze using a lyophilizer in ddH_20 and obtaining a final stock which was then passed through a filter	Breast cancer cell line MCF-7, breast cancer cell line MDA-MB-231 and normal human skin fibroblast cell line	Annexin V binding assay and propidium iodide staining, tail image-based cytometer, fluorescent images of cells	ANOVA, Dunnett's post hoc test and standardised software	HS flower extract shows an ability to enhance apoptotic induction by chemotherapy treatments through an increase in ROS generation and mitochondrial membrane collapse on both triple-negative and ER-positive breast cancer cell lines.
4.	[35]	Calyces	Cell culture medium	In vivo	400 mg/kg b.w.; 15 d	32.52 mg/kg b.w.	An infusion was prepared with dried calyces, filtered, evaporates and applied	Adult male Wister rats	HPLC, micronucleus test (MNT)	Chi-square test	The study shows that the plant has potential chemoprotective properties against DNA damage and fights against free radicals, and genotoxic substances thus preventing carcinogenesis.

Table 3. Continued

Sl. No.	References	Roselle component	Route of administration	Study type	Treatment dose and duration	Human equivalent dose	Technique for preparation	Study model	Methods	Statistical analysis	Salient findings
5.	[40]	Flower	Cell culture medium	In vitro	250 μg/mL; 24 h	NA	Fresh flowers were extracted in 90% ethanol, concentrated in a vacuum, fractionated with ethyl acetate and the dried powder was dissolved in DMSO solvent	HepG2 liver cancer cell lines	MTT assay	ANOVA, Student's *t*-test	The solid obtained from the ethyl acetate fraction has moderate anticancer activity. Further, an increase in the concentration of the extract can induce apoptosis in human cancer cell lines.
6.	[41]	Leaf extract	Cell culture medium	In vivo	50 mg/mL; 42 d	~15 mg/kg b.w.	The dried leaves were macerated with hot water, evaporated, filtered and lyophilized	Athymic nude mice, CaP cell line	Folin-Ciocalteau method, spectrophotometer, HPLC, MTT assay, western blot, gelatin-zymogram protease assays, SDS PAGE, real-time qRT-PCR	ANOVA, Dunnett's test	Inhibited the cell invasion of LNCaP cells via downregulation of PI3K/Akt signalling and by inactivation of NF-κβ. The leaf extract inhibited the growth of prostate tumor xenograft in athymic nude mice.
7.	[42]	Dried calyces	Cell culture medium	In vivo	5%, 10% (meal), 2.5%, 5% (juice); 38 wk	NA	Calyx juice was prepared with ethanolic extract	3-week-old male Fisher 344 rats. Induction of tumor development by azoxymethane followed by standard protocols	Cellular and biochemical tests	ANOVA, Tukey's post hoc test	Dietary administration of roselle as a meal and juice reduced the development of colon tumors in the Fisher 344 male rats.
8.	[37]	Calyces	Cell culture medium	In vitro	0.05, 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL; 24, 48, 72 h	NA	Dried powder used for extraction by maceration method, filtered, evaporated and sterilized	Human breast adenocarcinoma (MCF-7, NCBI, C135) and normal human fetal foreskin fibroblast (HFFF, NCBI, C170) cell lines	MTT assay, spectrophotometer, agarose gel electrophoresis	ANOVA, Tukey's post hoc test	Plant extract significantly inhibited the growth of breast cancer cells. HS extract proved to be apoptogenic for MCF-7 breast carcinoma however the exact mechanism of aqueous extract on cancer is not known.

Table 3. Continued

Sl. No.	References	Roselle component	Route of administration	Study type	Treatment dose and duration	Human equivalent dose	Technique for preparation	Study model	Methods	Statistical analysis	Salient findings
9.	[43]	Flower	Cell culture medium	In vitro	0.1%-10%; 72 h	NA	Roselle juice was prepared by adding distilled water to the crushed roselle flower in a ratio of 30:70. Three samples are taken of three different stored periods (one week, one month, one year)	Estrogen-receptor positive and estrogen-receptor-negative human breast cancer (MCF-7 and MDA-MB-231), human cervical cancer (HeLa cells, human ovarian cancer [Caov-3])	MTT cell proliferation assay, ELISA, spectrophotometer, DPPH-free radical scavenging assay	ANOVA, Duncan's multiple-range test	Roselle juice exhibited the strongest anti-proliferative potency towards MCF-7 human breast cancer cells in comparison to Caov-3, Hela and MDA-MB-231 cells. The storage period of the juice did not decrease the anti-proliferative effect on cell lines and the scavenging effect of juice samples was found to be concentration dependent.
10.	[44]	Leaf extract	Cell culture medium	In vitro	0.2 mg mL; 24 h	NA	Dried leaves powdered dissolved in methanol, chloroform, ethanol and ethyl-acetate, placed in a shaking incubator, filtered and evaporate the solvent	Hepatocellular carcinoma Hep 3B cell culture.	MTT assay, spectrophotometer	ANOVA	Methanol was predicted to be the suitable solvent for the extraction of anticancer compounds from the leaves and it thus the potent anticancer activity against the Hep 3B cell lines.
11.	[35]	Dried flower	Cell culture medium	In vitro	0.1-10 mg/mL; 24 h	NA	The dried flower was macerated with hot water, evaporated, filtered and lyophilized to obtain the extract	AGS cell line and Chang liver cell lines	MTT assay, spectrophotometer, DAPI staining, electrophoresis, flow cytometric analysis, SDS-PAGE, immuno-blotting	ANOVA with post hoc test	Dried flower extracts of the plant appeared to have a stronger apoptotic effect with the phosphorylation of c-Jun via JNK/P38 signalling triggering apoptosis. It also induced the translocation of cyt-c from the mitochondria to the cytosol and caspase cascade activation.

Table 3. Continued

Sl. No.	References	Roselle component	Route of administration	Study type	Treatment dose and duration	Human equivalent dose	Technique for preparation	Study model	Methods	Statistical analysis	Salient findings
12.	[36]	Dried flower	Cell culture medium	In vitro	0-4 mg/mL; 24 h	~19.96 mg/kg b.w.	The dried flowers are mixed with methanol containing 1% HCl for 24 h subsequently filtered and reacted on an Amberlife Diaion HP-20 resin column, cleaned, eluted with methanol and then lyophilized	HL-60 (human Caucasian promyelocytic leukaemia) cell line	HPLC, MTT assay, flow-cytometric analysis, RT-PCR, immuno-blotting	ANOVA, Duncan's multiple-range test	Flower extracts induce apoptosis via activating p38 MAPK and activating the p38-FasL and Bid pathway

b.w., body weight; NA, not applicable; HS, *Hibiscus sabdariffa*; ROS, reactive oxygen species; ER, estrogen receptor; HPLC, high performance liquid chromatography; DMSO, dissolved in dimethyl sulfoxide; qRT, quantitative reverse transcription; DPPH, 2,2 diphenylpicryl-hydrazyl; MAPK, mitogen-activated protein kinase.

Anti-diabetic effect

The phytochemicals present in the calyces extract of HS can reduce the blood sugar level in diabetic patients. Experimentally, the ethanol extract is found to be very effective in lowering the blood sugar level of diabetic mice [50]. The two enzymes called intestinal α-glycosidase and pancreatic α-amylase help in the digestion of complex carbohydrates and play roles in postprandial hyperglycemia. Inhibition of these two enzymes by the plant extract of HS will control postprandial hyperglycemia. Roselle tea is also found to be very effective in treating high blood sugar levels as studies reveal that extracts at a dose of 200 mg/kg have anti-insulin resistance properties and a reduction in hyperglycemia and hyper-insulinemia [51]. It has been also shown that hibiscus acid (hydroxy CA) acts as a potent inhibitor of pancreatic α-amylase and intestinal α-glucosidase activity. It has been reported that roselle extracts act as an effective inhibitor of pancreatic α-amylase [52].

Hypo-lipidemic effect

It has been found that the ethanolic extract of the roselle calyces and ethanolic extract of leaves have significant anti-hyperlipidaemic activity when rats are fed with HS extract, hibiscus acid could inhibit lipogenesis and promote reverse cholesterol transport; this may prove beneficial in lipid disorders and can also serve as a cardio-protective factor to prevent the onset of atherosclerotic processes [35].

Other pharmacological effects

It has been reported that roselle is a possible anti-obesity agent [26]. The seed extract of roselle enhanced the serum prolactin level of lactating female albino rats which confirms that HS has lactogenic properties [53].

CELL CYCLE MEDIATED ANTI-PROLIFERATIVE POTENTIAL OF HS IN CANCEROUS CELLS

Abnormal cell growth and proliferation are fundamental hallmarks of cancer that contribute to the development of tumor and cancer cell metastasis. Cell proliferation is tightly regulated in normal healthy cells by cyclins, cyclin-dependent kinases (CDKs), CDK inhibitors (CDKIs), cell cycle checkpoints, and various allied transcription factors. Uncontrolled cellular proliferation associated with cancer mostly depends on the structural and functional abrogation of checkpoint pathways [54]. The active phytochemicals of HS are involved in the regulation of cell proliferation, and restoration of checkpoint pathways by preventing over-dividing of cells with damaged genomes or inducing these cells to commit suicide through the classical pathway of apoptosis [54]. It has been noted that flower extracts of the plant containing cyanidin-3-glucoside and anthocyanin significantly inhibit the growth of HeLa cervical cancer cells and dried leaf extract of the plant containing saponins, phenols showed proliferation inhibitory effects in

prostate cancer cells. In addition, HS leaf polyphenols (HLP) inhibit abnormal vascular smooth muscle cell migration and proliferation in vitro and in vivo [55]. It has been experimentally demonstrated that noncytotoxic doses of HLPs abolished the TNF-α-induced matrix metalloproteinase-9 (MMP-9) expression by inhibiting the protein kinase B, inhibiting cancer cell progression. HLP also mediates the G0/G1 cell cycle arrest of cancerous cells by inducing the expression of p53 and its downstream factors that in turn suppress cyclin E/cdk2 activity [56]. The effects of HS or its constituents especially saponin, caffeic acid, and flavonoids on the cell cycle and proliferation of tumor cells have led to the identification of multiple target proteins involved in cancer [56]. A thorough analysis of cell cycle status in saponin-treated hepatic carcinoma cells revealed that their constituents activate cell extracellular signal-regulated kinase (ERK) together with its downstream machinery leading to the inhibition of human hepatoma (Hep G2) cell lines. The saponins also inhibited the proliferation of malondialdehyde (MDA)-MB-231 and MCF-7 cells in a dose-dependent manner which was associated with an increase in the sub-G1 population of the cell cycle leading to cell cycle arrest [57]. Caffeic acid, a component of HS extract induced cell cycle arrest and thus inhibited the growth and proliferation of human cervical cancer [58], colon cancer and oral cancer cells via regulation of SKP2, p53, p21Cip1, and KIP1 protein signalling pathways [59].

NF-κB SIGNALLING MEDIATED ANTI-PROLIFERATIVE EFFECTS OF HS

HS has also a pronounced effect on nuclear factor-κβ (NF-κβ) and regulates the expression of the genes involved in cellular proliferation, survival, migration, inflammation, angiogenesis and a variety of other processes. Increased activity or constitutive activation of NF-κβ signalling is observed in most types of cancer and plays an important role in the progression of carcinogenesis. Studies have revealed that HS extract or its components suppress NF-κβ activity through regulation of the fine balance between the inhibitor of NF-κβ and its expression as administration of HS leaf extract suppressed NF-κβ DNA binding activity in prostate cancer cells lines (LN-Cap) [60]. The inhibitory effect of HS leaf extract on the motility and invasiveness of LN-Cap cells was primarily achieved via the initial inactivation of NF-κβ followed by suppression of MMP-9 expressions. Since NF-κβ is a positive regulator of tumorigenic protein expression, its suppression is associated with decreased transcription of Bcl-2, Bcl-xL, IAP-1, IAP-2, survivin, MCI-1, Cyclin D1, MMP-9, and VEGF collectively impacted various cellular functions, such as proliferation, apoptosis, cell migration and angiogenesis reducing cancer cell progression [61]. Caffeic acid, an active phytochemical present in HS extract is proven to be down-regulating NF-κβ, thereby controlling abnormal cell proliferation [37]. In addition, the novel hetero-polysaccharides (HSP-I, HSP-II, HSP-III, HSP-III) have been extracted from the flower buds of the plant, and these components were found to possess immune-modulatory activity through mitogen-activated protein kinases (MAPKs) and NF-κβ pathways in RAW264.7 cells. Phytochemicals present in the plant like quercetin potentially inhibit keloid fibroblast proliferation in a dose-dependent manner. It has been found that the basal level of insulin-like growth factor-I receptor (IGF-IR) β, C-Raf, phospho-Raf-1, phospho-MAPK were significantly reduced when these cells were exposed to quercetin. Prostate cancer often expresses high levels of serum TNF-α that promotes cell proliferation and the presence of terpenoids from flower extract of HS plant inhibits cancer cell growth by suppressing the expression of TNF-α [62].

THE ANTI-PROLIFERATIVE POTENTIAL OF HS ON CANCER CELLS IS ASSOCIATED WITH NEWER GENE EXPRESSION AND TRANSCRIPTION-MEDIATED ACTIONS

Cancer can be described as a disease of altered gene expression as a result of a mutation in some critical pathways that specifically control cell proliferation, cell death, or the repair of damaged DNA. Alterations of expression in genes such as TP53, EGFR, and KRAS are common in lung cancers; MHSH2, and MLH1 in colon cancer along with the most studied tumor suppressor gene p53 which is mutated in many cancer cell types. HS phytochemical extracts may provide unique clinical benefits to a greater extent than artificial chemotherapeutics drugs that rely on changes in gene regulation (transcription, translation, and post-translation). Anthocyanin extract from HS has been shown to up-regulate p53 in colon cancer and prostate cancer cells. Anthocyanin has the potential to initiate the transcription of p21 and p27, a broad-spectrum inhibitor of CDK, the cell cycle arrest of cancer cells [63]. It has also been found that anthocyanin-rich extract of HS inhibited the proliferation of Caco-2 cells by up-regulating the expression of p21, arresting the cell cycle progression and further inducing apoptosis by caspase-3 activation [64]. Delphinidin, a flavonoid component of HS extract can also down-regulate the gene expression of CDK-1 and CDK-2, inhibit the expression of cyclin B, cyclin A, and cyclin E, and promote the expression of CDKIs and induce cancer cell cycle arrest at the G0/G1 and G2/M stages [65]. Triterpene, another bioactive component of HS extract has been shown to inhibit the growth of HepG2 liver cancer cells by increasing the expression of the p53 gene. It has also been found that curcumin contained in the HS leaf extract inhibits the proliferation of human glioblastoma cells by up-regulating the expression of the p53 gene. Caffeic acid isolated from HS plant extracts also showed anti-proliferative effects through overexpression of AKt1 and c-Myc and decreased expression of cyclinD1, cyclin E, SKP2, and AKt2 in prostate cancer [66].

POST-TRANSLATIONAL MODIFICATIONS MEDIATED ANTI-PROLIFERATIVE EFFECTS OF HS

The expression of the altered protein dramatically modifies the cell function and contributes to the progression of cancer. Caffeic acid has been shown to significantly reduce the phosphorylation of ER-α and Akt in PC3 [67]. Flavonoids such as epigallocatechin-3-gallate decreased phosphorylation of Akt and FoxO3a, which correlated with an increase in FoxO-responsive proteins including BTM and p27/kip1 in prostate cancer cells leading to cancer cell cycle arrest [68]. The mevalonate pathway plays a vital role in prenylation and consequently membrane anchorage of growth-related proteins including Ras, and the prenylated proteins have been found to collectively support the proliferation and growth of cancer cells. Isoprenoids trigger post-translational events to regulate HMG Co-A reductase expression. Depleting melvonate-derived intermediates such as FPP, GGPP, isoprenoid, and terpenoid can interrupt the prenylation and modification of critical signalling proteins like Ras, lamin B, and other growth and proliferation factors of cancer cells [69].

EFFECT OF HS ON EPIGENETICS OF CANCER

Epigenetic alterations are as important as a genetic mutation in neoplastic transformation as a mechanism of silencing tumor suppressor and activation of oncogenes; these include CpG island methylation, histone modification, and dysregulation of DNA binding proteins [70]. Quercetin, a fairly abundant flavonoid in the polyphenolic fraction of HS extract, inhibited the histone acetyl-transferase activity in the promoter region of tumor-associated genes [71]. HS polyphenolic constituents are capable of regulating microRNA expression in cancer cells. Some polyphenols and isoflavones of HS extract mediated inhibitory responses which were attributed to their inhibition of histone deacetylases (HDACs), specifically HDAC1 and HDAC3. This effect the hyper-acetylation of histone H3 on the p21 promoter of class 1 HDAC1 protein expression, thereby increasing the acetylation of KU70 and resulting in apoptosis in prostate cancer cells [72].

EFFECT OF HS PHYTOCHEMICALS ON CELLULAR AND MOLECULAR EVENTS IN CANCEROUS CELLS

Effect on DNA damage and repair pathways

DNA repair-damage pathways enable tumor cells to survive DNA damage that is often caused as a result of chemotherapeutic treatments. Tumor development associated with perturbed DNA damage response (DDR) repair pathway results in genetic instability in tumor cells [73]. The TP53 and WAF1/cip1 encoding for p21 are essential genes in the DNA damage pathway [69]. HS calyx extracts inhibited DNA damage in a dose-dependent manner by activating DNA damage preventive protein signalling pathways [74]. It has been noted that anthocyanin-treated cancer cells activate the ATM/chk2/p53 cascade pathway in glioma cancer cells. The bioactive components activate DDR which in turn activated the sensor ATM, ATR, and DNA-PK multiple downstream substrates including effector kinases chk1 and chk2; this resulted in cell cycle checkpoint initiation and apoptosis of the tumor cells [75].

Effects of HS components on cancer cell death or apoptosis

Besides inhibiting the proliferation of cancer cells, HS components exert an anticancer effect by induction of apoptosis as well as initiating the self-digestive cellular mechanism of autophagy [73].

Apoptosis may occur through the intrinsic mitochondrial pathway or the extrinsic pathway mediated by membrane-associated death receptors. HS extract induces apoptosis through the intrinsic pathway in prostate, cervical, and hepatoma cells which is accompanied by an elevated release of cytochrome c (Cytc) from mitochondria. It has been found that Cytc release is tightly regulated by pro-apoptotic (Bax and Bad) and anti-apoptotic (Bcl-2 and Bcl-xL) markers, thus these proteins are important targets for exerting the anticancer effect of HS extract in cancer cells. PCA, the component in HS extract, inhibited the survival of human promyelocytic leukaemia cells (HL-60) in a dose and time-dependent manner by reducing the retinoblastoma phosphorylation, Bcl-2 and increasing the Bax expression levels [76]. PCA was also reported to inhibit the 12-O-tetradecanoylphorbol-13 acetate induced promotion in skin tumors of female CD-1 mice inducing similar mechanisms. In addition, apoptotic effects of PCA have also been reported on human breast cancer MCF 7, lung cancer A549, HepG2, cervix HeLa, and prostate cancer LNcap cells. These findings showed that PCA dependently decreased cell viability, increased lactate dehydrogenase (LDH) leakage, enhanced DNA fragmentation, reduced mitochondrial membrane potential, and lowered sodium-potassium ATPase activity along with elevated caspase-3 activities in these cancer cells [36].

Among the most common forms of breast cancer, triple negative type accounts for approximately 15% to 20% of all breast cancer and is characterized by negative expression of estrogen and progesterone receptors as well as HER2 protein [77]. The extract of HS was able to selectively induce apoptosis in both triples–negative MDA-MB-231 and estrogen–receptor-positive MCF-7 breast cancer cells in a dosage-dependent manner. The extract enhanced the induction of apoptosis by chemotherapeutic treatment (taxol and cisplatin) in MDA-MB- 231 as well as ER+ breast cancer cells when compared to treatment alone [73]. HS anthocyanin extract induced apoptotic cell death in human promyelocytic

leukaemia cells (HL-60) in the dose and time-dependent manners by increasing phosphorylation of p38 and expression of t- Bid, Fas, and FasL [78].

Gallic acid (GA), an active compound isolated from HS extract has significant apoptotic activity against melanoma, lymphoma, and lung cancer cells. Besides inducing apoptosis, it also enhances the anticancer effect of cisplatin in the human lung cancer cell line H446 via the ROS-mediated mitochondria apoptotic pathway. Both GA and cisplatin altered cellular morphology inhibited growth and induced apoptosis through the generation of ROS leading to disruption of matrix metalloproteinase (MMP), down-regulation of XIAP expression, and up-regulation of Bax, Apaf 1, and p53. Flavonoids isolated from the calyx of the plant were also reported to induce morphological apoptotic features with an elevated cellular expression of p53 in Ehrlich ascites carcinoma (EAC) cells [37]. The aqueous extract of the calyx on breast adenocarcinoma cell line MCF7 induced apoptosis, as evidenced by the formation of DNA fragmentation, membrane cell blebbing, and chromatin condensation [79].

Effect of HS extracts on autophagy inducing activity

The general modulatory effects of HS extract on the elimination of cancer cells are also highlighted by their capability to induce other forms of cell death such as caspase-independent apoptosis and autophagy. Autophagy deficiency promotes malignant transformation and carcinogenesis on the other hand; autophagy can restrict the necrosis and inflammation of a tumor, thereby relieving chromosome injury and the metabolic stress response in tumor cells [80]. Autophagy is another form of programmed cell death that plays an important role in normal cell growth. Autophagy plays a cytoprotective role against cancer by removing misfolded proteins, damaged cells, and free radicals resulting in limited genomic damage by checking mutation [80]. It has been reported that damaged cellular machinery of autophagy is linked with genomic instability, tumorigenesis, and malignant transformation In addition, animal models lacking Beclin 1 developed spontaneous tumors more than their control counterparts. Autophagy-related gene 5 and 7 (ATG5 and ATG7) plays a crucial role in tumor suppression, as their reduced expression caused mitochondrial damage and oxidative stress (OS) culminating in hepatic tumor development in mice [81]. Dysregulation of the PI3K/AKT pathway and impairment of its key protein associate like phosphatase and tensin homolog reduces autophagy in malignant cells and backs tumorigenesis conversely; the AKT/mTOR signaling is known to suppress autophagy. HLP was also found to induce autophagic cell death in human melanoma cells (A375) by increasing the expression of autophagy-related proteins, ATG5, Beclin 1, and light chain 3-II (LC3-II) [78]. It has also been found that the human hepatocellular carcinoma (HCC) cell line when treated with anthocyanins resulted in the expression of downstream BCK-2 family and rapamycin target proteins were down-regulated while the expressions of eukaryotic initiation factor 2α and autophagy-related gene LC3-II were up-regulated which all suggests that anthocyanin extracts of HS can induce autophagy of human HCC cells [80].

Anti-inflammatory and antioxidant activity of HS

HS extract of different types has been shown effective against many inflammatory diseases including cancer [82]. The extract and mucilage from HS have anti-inflammatory activity by inhibiting Cox-2 and 5-Lox enzymes. A unique sterol of glycoside and six phenolic compounds such as neochlorogenic acid, caffeic acid, rutin, isoquercitrin, quercetin, and kaempferol have been found in HS extract and possess more significant anti-inflammatory activity compared to its mucilage [83]. The essential oil extracts from HS calyx exhibited excellent anti-inflammatory activity via inhibiting the activation of NF-$\kappa\beta$ and MAPK (JNK and ERK1/2) pathways leading to a decrease production of nitric oxide and pro-inflammatory cytokines (IL-1, IL-6, TNF-L, Cox-2 and iNOS) production. Another study on RAW-264.7 cells demonstrated that the HS extract (flower, leaf, roots, cortexes, and seeds) decreased nitric oxide production and also reduced mRNA expression of the cytokines IL-6/1β and the iNOS/cycloxygenase-2 resulted in the overall regulation of cellular inflammation [84]. Furthermore, HS extract inhibited the production of pro-inflammatory cytokines such as TNF-α and IL-6/12 in macrophages with inhibition of the TLR4/MD2-mediated NF-$\kappa\beta$ signalling pathway. HS extract also decreased NF-$\kappa\beta$ and CYP2E1 in liver tissue with an increase in the effector apoptotic marker caspase-3, resulting in the restoration of the altered hepatic architecture with an increase in the activity of superoxide dismutase (SOD) and glutathione enzyme profiles [84] establishing its anti-inflammatory roles. In addition, bioactive compounds of the plant mainly phenolic acid and organic acids have been found to possess potent anti-inflammatory effects [85].

Reactive oxygen species (ROS) are highly toxic in high concentrations and culminate in OS, which can damage cellular biomolecules, viz protein, lipid, and DNA. Increased ROS production beyond the physiological levels can also be caused by altered metabolism and mitochondrial dysfunction associated with ageing and diseases like cancer. Oxidative damage can be prevented by natural enzymes like SOD, catalase (CAT), and glutathione peroxidase (GPx) as well as by topical antioxidants. Ethanolic extract of the HS substantially increases the levels of the antioxidants CAT, SOD, GPx and reduced glutathione (GSH) in brain tissue, thus possessing significant antioxidant activity. Anthocyanin in the extract of HS acts on the anti-oxidant system, and scavenges free radicals thus reducing damage to genomes of regular cells and the mutations, thus stopping tumor formation [86]. Anthocyanin interacts with antioxidant response element through the Keap1-Nrf2 pathway inhibiting the activity of cysteinyl aspartate specific proteinase-3 (caspase 3) via regulation

of phase II antioxidants (glutathione reductase, GPx, and quinone oxidoreductase) [80]. Anthocyanin also reduced the levels of aspartate transaminase, alanine transaminase (ALT), uric acid and myeloperoxidase; exhibited protective effects against N-nitrosomethyl urea-induced leukaemia. PCA, from HS extract was shown to significantly decrease the leakage of LDH, ALT, and the formation of MDA induced by terbutylthydroperoxide in rat primary hepatocytes. The methanolic fruit extracts of HS exhibited strong antioxidant activity against MCF-7 cells in 2,2 diphenylpicryl-hydrazyl (DPPH) radical scavenging assay, thus indicating a potential anti-oxidant role of the HS that may potentially mitigate cancer [87].

The CA has important antioxidant effects and decreases lipid peroxidation, inflammation of brain lipids, liver damage, and DNA fragmentation [40,88]. The anti-oxidant potential of methanolic extract of the HS roots was evaluated with the EAC cell line. Polyphenolics and flavonoids in the methanolic extract are capable of antioxidant defence from ROS, scavenging; restoring redox homeostasis and anti-neoplastic efficacy [89].

Effects of HS on the angiogenesis and metastasis of cancer cells

Tumor angiogenesis is regulated by the production of angiogenic stimulators which include fibroblast growth factors (β-FGF), transforming growth factor (TGF-X), and vascular endothelial growth factor (VEGF) to promote tumor growth and metastasis. The phytochemicals from HS extracts have been shown to possess anti-angiogenic effect and inhibit tumor invasion in various cancer therapeutic studies. Delphinidin and cyanidin (class of anthocyanin) both strongly inhibit the expression of VEGFR-3 in vascular smooth muscle cells by blocking the P38 MAPK and JNK Pathways [90]. Cyanidin could inhibit the invasion and metastasis of breast cancer cells (BT474, MDA-MB231, and MCF-7) by blocking the ERb B2/CSrc/FAK pathway. Delphinidin could reduce the membrane translocation of PKC-α and the phosphorylation of Stat3 in MCF- 10A cell lines, inhibiting the invasion of cells. Anthocyanin-rich extract of HS inhibited B16-F1 cell migration and suppressed HUVECS tube formation attenuating melanoma cancer metastasis. Under the influence of these extracts, the inhibitory effect could be mediated by the P13K/Akt and Ras/Mark cascade pathways and their downstream effectors VEGF and MMP-2/9 [91]. In silico analysis revealed the role of HS anthocyanin, hibiscetin which binds to the active site of VEGFR2 and inhibits its activity. The polyphenol extract of HS inhibited high glucose-stimulated cell proliferation and migration in vascular smooth muscle cells (VSMC) by suppressing the proliferating cell nuclear antigen (PCNA) level, MMP-2 activation, connective tissue growth factor (CTGF) and receptor of the advanced glycation. Flower extract of HS contains cyanidin-3-glucoside, and cyanide-3 rutimoside which inhibited the migration and invasion of metastatic A549 human lung carcinoma cells by inhibiting the activation of c-junction and NF-κβ [90]. PCA found in extract of HS inhibited cell migration and invasion to non-cytotoxic cells via down-regulation of the Ras/Akt/NF-κβ pathway and MMP-2 production [92].

Effect of HS on cancer stem cells

Cancer stem cells (CSC) are a subpopulation of tumor cells that can initiate tumors and cause recurrences. During the transformation process, oncogenes are overexpressed and tumor suppressors such as the p53 gene are inactivated, resulting in cell proliferation and the acquisition of stem cell characteristics by transformed cells. The phytochemical extracts of HS inhibited the growth, angiogenesis, migration, invasion and intracellular hypoxia-inducible factor-1 α (HIF1-α) of HCC; an example of CSC. The extracts have increased GSH levels and SOD activity without modifying the level of ROS. Important findings from this review show that HS phytochemicals, which include flavonoids, polyphenols, caffeic acid, catechins, saponins, polysaccharides, triterpenoids, alkaloids, glycosides, phenols (quercetin and luteolin), kaempferol and luteolin glycosides, can inhibit tumour cell proliferation and induce apoptosis on different cancer cells [93].

CONCLUSION

This review summarizes various intrinsic cellular and molecular mechanisms underlying cancer preventive and therapeutic properties of the extracts of HS (Fig. 1). The antioxidant properties reduce free radicals, DNA oxidation, and apoptosis by promoting cell cycle arrest and decreasing PI3K, P-Akt protein, MMP expression, anti-apoptotic Bcl-2, Bcl-xL proteins, and PCNA, cyclin A, D1, B1, and E. In broad terms, this paper revealed that HS extracts might be able to stop cancer cells from growing and spreading. But before these results can be used in clinical applications, they need to be confirmed by more studies. Future new therapeutic methods, such as the purification of herbal compounds and proving their effectiveness, may lead to new and effective ways to control and treat different types of cancer.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

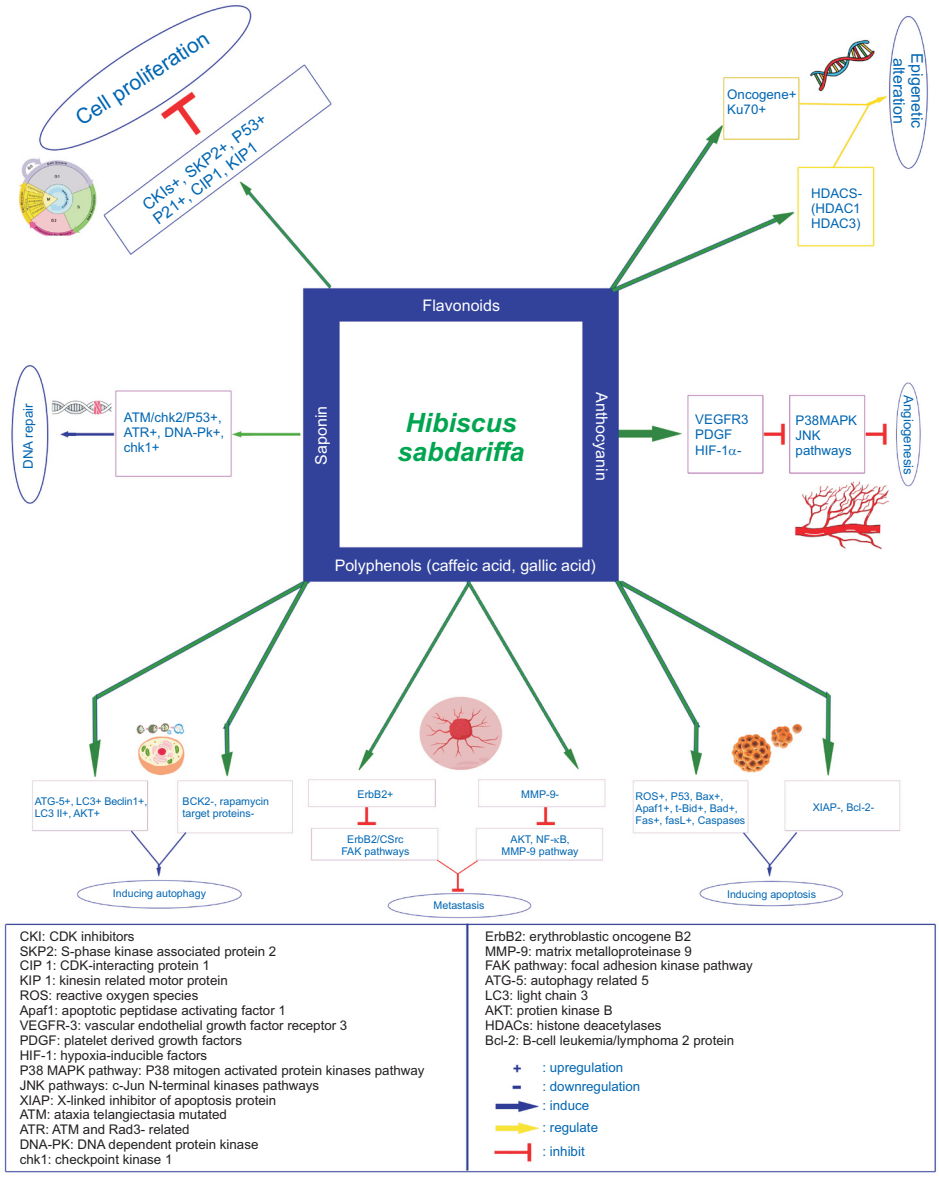

Figure 1. Cancer preventive and therapeutic potential of *Hibiscus sabdariffa* (HS).

ORCID

Raihana Yasmin, https://orcid.org/0000-0002-9074-6871
Sangeeta Gogoi, https://orcid.org/0000-0002-9355-1172
Jumi Bora, https://orcid.org/0009-0005-2561-0184
Arijit Chakraborty, https://orcid.org/0000-0003-2975-7333
Susmita Dey, https://orcid.org/0000-0002-7795-7906
Ghazal Ghaziri, https://orcid.org/0000-0001-9070-6514
Surajit Bhattacharjee, https://orcid.org/0000-0003-3877-3044
Laishram Hemchandra Singh,
https://orcid.org/0009-0009-3482-0318

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