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Review

Insight into the molecular evidence supporting the remarkable chemotherapeutic potential of *Hibiscus sabdariffa* L.

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ABSTRACT

Hibiscus sabdariffa or roselle tea is popular around the globe for its antioxidant capability along with various other health benefits. Besides, it has uses in Ayurvedic and Chinese herbal medicines for the treatment of several diseases. However, the investigation for the anticancer potential of the plant began roughly in the last decade that emerged with encouraging results. Both crude extracts and pure compounds of the plant were reported to induce chemoprevention, selective cytotoxicity, cell cycle arrest, apoptosis, autophagy and anti-metastasis effects in varied types of human cancer cells. The plant contains a high quantity of polyphenolic compounds and at least two of them were proven to induce potent anticancer effects. Although, the molecular mechanism underlying the anticancer activity was roughly estimated in several studies. The present review aimed to assemble all ambiguous information to report the molecular evidence establishing the potent anticancer activity of *Hibiscus sabdariffa* and its implication for cancer therapy. This study suggests that *Hibiscus sabdariffa* is an ideal candidate to investigate its role as a herbal supplement for cancer prevention and treatment. With excellent safety and tolerability record, polyphenolic compounds from the plant need better designed clinical trials.

1. Introduction

According to the World Health Organization (WHO); cancer is the second largest cause of death after cardiovascular diseases and is responsible for an estimated 9.6 million deaths in 2018 [1]. At the current rate, nearly 29.5 million people will be diagnosed with cancer by the year 2040 [2]. Cancer is the transformation of normal cells in abnormal or malignant cells which are self-sufficient to multiply and grow rapidly [3]. Cancer befalls when a mutation takes place in genes that control or regulate cell growth, due to which affected cells propagate in an unchecked way [3]. The process of cancerogenesis is aided by the loss of controlled cell division, disruption of programmed cell death or apoptosis, initiation of angiogenesis and metastasis [3]. Several other physiological conditions like hypertension [4], hyperlipidaemia [4], hypercholesterolemia [5], hyperglycaemia [6] also aids to cancer occurrence, progression and metastasis. Despite therapeutic improvements and a better understanding of the disease, the maximum number of cancer types remains untreatable [7].

The plant, *Hibiscus sabdariffa* is commonly known as roselle or sorrel belongs to the *Malvaceae* family [8]. It is believed to be native to Africa although found in several parts of the globe and is cultivated for its medicinal and economic value [8]. Roselle juice or tea is popular around the globe for its antioxidant capability along with various other health benefits [9]. Traditionally, it is used in Ayurveda and Chinese herbal medicine for the treatment of hypertension, hypercholesterolemia, hyperlipidemia, diarrhoea and many other diseases. Beside, roselle extracts exhibited powerful protective effects against neurotoxicity [10], hepatotoxicity [11], diabetes-related complexities [12] and exhibited immunomodulatory [13] and vasorelaxant effects [14] in experimental animals. However, the investigation for its anti-cancerogenesis potential started only at the beginning of the decade and encouraging results were recorded. Several researchers reported the cytotoxic [15], antiproliferative [16], antimutagenic [17], apoptotic, antiangiogenic [18] and antimetastatic [19] activity of the plant both in human cancer cells and in animal models. The plant constituents are a rich source of diverse polyphenols and other important classes of phytocompounds that attributes towards its antioxidant and anticancer activities [20]. At least two of the polyphenols, delphinidin-3-sambubioside (Del-3-sam) and protocatechuic acid (PCA) exhibited apoptosis-inducing potential in human leukaemia cells and gastric cancer cells respectively [21,22]. However, the molecular mechanism of the anticancer activity of these polyphenols in cancer cells was roughly estimated. One of the distinct features of this plant is its low toxicity which is demonstrated by an LD_{50} value above 5000 mg/kg in rats [23]. Interestingly, comparison of the LD_{50} values of the plant extracts in different human cancer cells revealed that estrogen receptor-positive (ER

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+) breast cancer cells (MCF7) are most susceptible to the selective cytotoxic effect of the plant extract [24]. Moreover, this plant is thought to possess estrogenic activity which in turn may help to investigate its role in breast cancer progression while the estrogen-like ingredient of the plant is still unknown [25].

Previously G. Riaz and R. Chopra systematically reviewed the phytochemistry and therapeutic uses of *Hibiscus sabdariffa* in detail [26] while Hui-Hsuan Lin et al. [27] discussed in detail about the molecular mechanism of action of the bioactive compounds. The present review specifically aims to report the scientific evidence supporting the potential role of *Hibiscus sabdariffa* in human cancers and its implication for cancer therapy. To record the published reports on phytochemical composition and anti-cancer activity of Roselle, specialized electronic databases like Google Scholar, PubMed and Elsevier were searched. Results of research conducted in animal models and cell cultures were recorded. Given its reported anticancer properties and relatively low toxicity, *Hibiscus sabdariffa* and its phytochemical constituents could be a source of therapeutically useful products for cancer treatment.

2. The therapeutic value of *Hibiscus sabdariffa* in traditional medicine

Hibiscus sabdariffa has been used in Indian traditional medicine or Ayurveda, Unani Medicine and Chinese Traditional Medicine (CTM) [28,29]. Different parts of the plant are being used in traditional medicine to treat several diseases including colds, toothaches, urinary tract infections [26]. In Thai traditional medicine, it is used to treat kidney and urinary bladder stones [30]. Besides, it is used as an antibacterial, antifungal, hypocholesterolemic, antispasmodic, diuretic, uricosuric, antihypertensive, antidiabetic, pyrexia and a cardioprotective agent in traditional medicine [30,31]. This plant is often claimed in West African folk medicine to be an aphrodisiac [32].

3. Major phytochemical constituents of *Hibiscus sabdariffa*

As *Hibiscus sabdariffa* has ethnomedicinal importance, the phytochemical composition of the plant has been widely explored for a long time [33]. Present sophisticated analytical techniques like Ultra-High-Performance Liquid Chromatography (UHPLC) and Liquid Chromatography-Mass Spectroscopy (LC–MS) helped to detect several important classes of therapeutically important phytochemicals in both alcoholic and aqueous extracts of different parts of the plant. These phytochemicals predominantly include polyphenols, anthocyanins, flavonoids, phytoestrogens and organic acids, which possibly attributes towards the therapeutic potential of this plant [22]. A detailed description of the chemical constituents and pharmacological activity of the plant was given by Ross [34]. Some important compounds with well-known anti-cancer potential are shown in Fig. 1.

3.1. Flower/ fruit

Recently, A. Piovesana et al. for the first time reported a detailed description of the carotenoid composition of acidified hydro-alcoholic (8:2, v/v) extract of hibiscus flower calyces along with its important phenolic compounds (Table 1) using high-performance liquid chromatography coupled to a diode array detector and tandem mass spectrometry (HPLC-DAD-MS/MS) [35]. Important phytocompounds from flower includes *Hydroxycitric acid*, *Hibiscus acid*, *3-Caffeoylquinic acid*, *Delphinidin 3-sambubioside*, *3-p-Coumaroylquinic acid*, *Cyanidin 3-sambubioside*, *5-Caffeoylquinic acid*, *4-Caffeoylquinic acid*, *Myricetin 3-sambubioside*, *5-p-coumaroylquinic acid*, *Quercetin 3-sambubioside*, *5-O-Caffeoylshikimic acid*, *Quercetin 3-rutinoside*, *Quercetin 3-glucoside*, *Kaempferol 3-O-rutinoside* [35,36]. Methanolic extract of the flower contains major anthocyanins- *delphinidin* (69%) followed by *cyanidin* (27%) and other minor anthocyanidins (*malvidin*, *peonidin* and *pelargonidin*) [37,38].

3.2. Calyx

Analysis of ethyl acetate extract of the calyces using High-performance liquid chromatography-UV/Pulsed Amperometric Detector-Electrospray ionization/Mass spectroscopy (HPLC-UV/PAD-ESI/MS) method reported the presence of five important acidic components (*Hibiscus acid*, *caffeic acid*, *rutin* and two isomers of *5-caffeoylquinic acid*) [39,17]. A similar study revealed the presence of a total of 32 important phytochemicals (Table 1) in acidified ethanolic extract of the calyx [40]. Also, Idolo Ifie et al reported a comparative analysis of the major phytochemical constituents of the calyx of three colour varieties of Roselle [41].

3.3. Leaf

Phytochemical characterization of aqueous extract of *Hibiscus sabdariffa* leaves using HPLC with diode array detection coupled to Electrospray ionization (ESI) and ion trap mass spectrometry resulted in separation and identification of seventeen compounds (Table 1) using two optimized gradients programs and both, negative and positive modes of ionization [42]. Another study using Liquid chromatography/quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) method reported significant quantity of six major anti-oxidant compounds viz. *neochlorogenic acid*, *chlorogenic acid*, *cryptochlorogenic acid*, *caffeic acid*, *rutin* and *isoquercitrin* in a methanolic fraction of leaf, which could be the predominant contributors to the antioxidant activity [43–45].

4. Molecular basis of anti-cancer activity of *Hibiscus sabdariffa*

4.1. Antioxidant activity

Roselle juice is a popular drink around the globe for its antioxidant potential [26]. Roselle contains a significant quantity of antioxidants that includes *Neochlorogenic acid*, *Chlorogenic acid*, *Cryptochlorogenic acid*, *Rutin*, *Isoquercitrin, Ascorbic acid*, β-*carotene* and *Lycopene* (Table 1) [26,22,51]. Ethanolic extract was found to link with a significant increase in the levels of antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and reduced glutathione (GSH) in brain tissues, suggesting significant anti-hyperammonemic and anti-oxidant activity [52]. Protocatechuic acid (PCA) was shown to significantly decrease the leakage of lactate dehydrogenase (LDH) and alanine transaminase (ALT) and the formation of malondialdehyde (MDA) induced by tert-butylhydroperoxide (t-BHP) in rat primary hepatocytes [53]. Ethanolic extract of roselle calyx inhibited xanthine oxidase activity with IC_{50} values 290.62 μg/mL *in-vitro* [54]. In similar study, polyphenol-rich extract of dried flower showed up to 93 % inhibitory effect on xanthine oxidase activity(EC_{50} = 0.742 mg/mL) [55]. Commercialized roselle juice exhibited significant antioxidant activity in breast cancer cell line (MCF-7 and MDA-MB-231), ovarian (Caov-3) and cervical (HeLa) cancer cell lines with MCF-7 being most susceptible to its antioxidant activity [56]. Besides, methanolic fruit extract exhibited strong antioxidant activity against MCF-7 cells (IC_{50} = 124.3 ± 1.89 μg/mL) in 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging assay [57]. Anthocyanin-rich extract from the plant caused a reduction in the levels of aspartate aminotransferase (AST), alanine transaminase (ALT), uric acid, myeloperoxidase (MPO) and exhibited protective effects against N-Nitrosomethylurea-induced leukaemia in rats [58].

4.2. Anti-inflammatory effects

Different extracts have been shown effective against many inflammatory diseases including cancer. A clinical trial involving 50 patients where administration of a decoction of dried fruit (3 g/person, 3 times every day for 7 days to 1 year) was shown to produce anti-inflammatory activity (Anon, cited in Ross, 2003 [30]). Polyphenol-rich

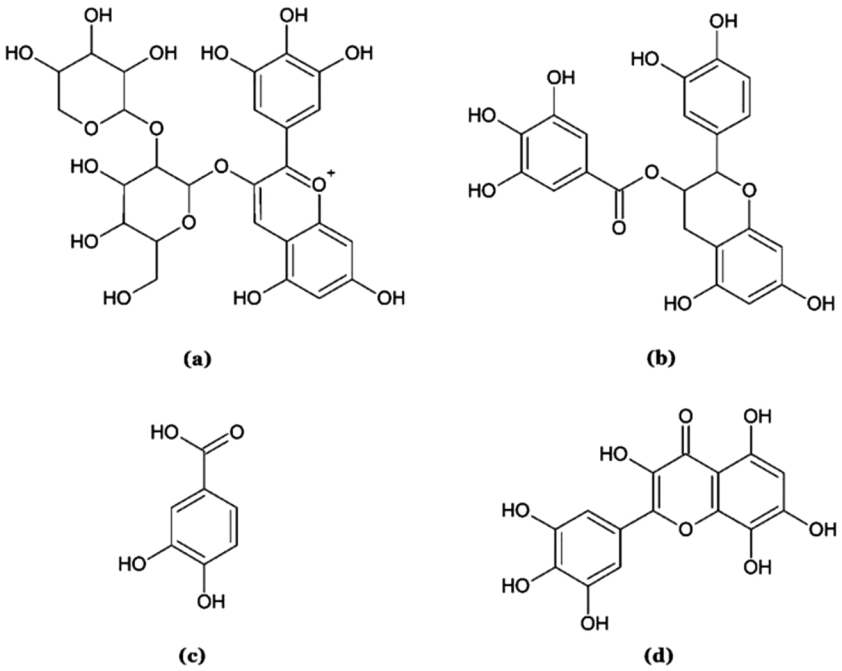

Fig. 1. Structure of major anti-cancer compounds isolated from Hibiscus sabdariffa, (a) Delphinidin 3-Sambubioside, is a potent apoptosis inducer in leukaemia cells, (b) Epicatechin gallate, induces autophagy (c) Protocatechuic acid, another antioxidant & potent apoptosis-inducing compound (d) Hibiscetin, was found to inhibit VEGFR2 expression in an in-silico investigation.

extract of dried flowers was evaluated for their anti-inflammatory capacity on nitrite and prostaglandin E2 (PGE2) in lipopolysaccharide (LPS) treated RAW264.7 cells and on Male Sprague-Dawley rats [55]. The result showed decreased nitrite and PGE2 secretions in LPS-induced cells while in rats it significantly decreased serum levels of alanine, aspartate aminotransferase and increased liver catalase activity and glutathione [55]. Additionally, the leaf extract dose-dependently inhibits nitric oxide synthetase (NOS) in RAW 264.7 murine macrophage cells at 80 lg/mL [50]. The methanol extract of *Hibiscus sabdariffa* helped to maintain the ratio of IL-1β/IL-1ra in the plasma and hippocampus of Wister rats who experienced overtraining by lowering the level of pro-inflammatory cytokine IL-1β [59]. Further work is required to study the effects of fractions and isolated compounds in experimental anti-inflammatory diseases and their possible mechanism(s) of action.

4.3. Apoptosis-inducing activity

Both crude alcoholic extracts and pure polyphenolic fractions of *Hibiscus sabdariffa* were reported to induce apoptotic cell death *in-vitro* and *in-vivo* human cancers types including cancer of gastric cavity [60], prostate [61], mammary tissue [24], leukaemia [62] and many more. Protocatechuic acid (PCA) and Delphinidin-3-sambubioside (Del-3-sam) are the two most potent apoptosis inducer isolated from *Hibiscus sabdariffa* whereas (-) epicatechin gallate (ECG) was found to inhibit apoptosis but trigger autophagy. PCA inhibited the survival of human promyelocytic leukaemia cells (HL-60) in a concentration- and time-dependent manner by reduction of retinoblastoma (RB) phosphorylation, Bcl-2 expression and increased Bax protein expression [62]. PCA was also reported for its ability to inhibit the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced promotion in skin tumours

Table 1
Reported major phytochemical composition of both alcoholic and aqueous extracts of Hibiscus sabdariffa using HPLC-based methods.

Part of Plant	Type of Fraction	Major Phytochemicals	Reference
Flower/Fruit	Acidified Methanol–Water (8:2, v/v)	Hydroxycitric acid, Hibiscus acid, 3-Caffeoylquinic acid, Delphinidin 3-sambubioside, 3-p-Coumaroylquinic acid, Cyanidin 3-sambubioside, 5-Caffeoylquinic acid, 4-Caffeoylquinic acid, Myricetin 3-sambubioside, 5-p-coumaroylquinic acid, Quercetin 3-sambubioside, 5-O-Caffeoylshikimic acid, Quercetin 3-rutinoside, Quercetin 3-glucoside, Kaempferol 3-O-rutinoside, malvidin, peonidin, pelargonidin	[35,37]
	Ethanol	Ascorbic acid, β-carotene, Lycopene, Delphinidin-3-sambubioside, Delphinidin-3-glucoside, Cyanidin-3-sambubioside	[46]
Calyx	Acidified Ethanol	Hydroxycitric acid, Hibiscus acid, Hibiscus acid hydroxyethylesther, Delphinidin-3-sambubioside, Chlorogenic acid, Cryptochlorogenic acid, Methyl digallate, 2-O-trans-caffeoyl-hydroxicitric acid, 1-O-caffeoylquinic acid, Myricetin-3-arabinogalactoside, Coumaroylquinic acid, Hibiscus acid hydroxyethyldimethylesther, Quercetin-3-sambubioside, 5-O-caffeoylshikimic acid, 2-O-trans-feruloyl-hydroxicitric acid, Quercetin-3-rutinoside, Kaempferol-3-O-sambubioside, Quercetin-3-glucoside, Kaempferol-3-O-rutinoside, Ethylchlorogenate, Methylepigallocatechin, Myricetin, N-feruloyltyramine, Quercetin, Kaempferol.	[39,40,47]
	Aqueous	Protocatechuic acid, Rutin, Hydroxycitric acid, Hibiscicus acid, Chlorogenic acid (isomer I), Chlorogenic acid, Chlorogenic acid (isomer II), Myricetin-3-arabinogalactose, Quercetin-3-sambubioside, 5-O-Caffeoylshikimic acid, Quercetin-3-rutinoside, Quercetin-3-glucoside, Kaempferol-3-O-rutinoside, N-Feruloyltyramine, Kaempferol-3-(p-coumarylglucoside), Quercetin, 7-Hydroxycoumarin, Delphinidin-3-sambubioside, Cyanidin-3 sambubioside	[48,49,17]
Leaf	Aqueous-Methanol	Neochlorogenic acid, Chlorogenic acid, Cryptochlorogenic acid, Rutin, Isoquercitin, Kaempferol-3-o-rutinoside, Kaempferol-3-o-glucoside, Quercetin, Kaempferol, 5-(hydroxymethyl)furfural, Caffeoylshikimic acid, Epigallocatechin, Epigallocatechin gallate.	[43,45,50]
	Aqueous	Hydroxycitric acid, Hibiscus acid, Chlorogenic acid and its isomers, Myricetin 3-arabinogalactoside, Quercetin 3-sambubioside, Quercetin 3-rutinoside, Quercetin 3-glucoside, 5-O-Caffeoylshikimic acid, Kaempferol 3-O-rutinoside, N-Feruloyltyramine, Kaempferol 3-(p-coumarylglucoside), Quercetin, Delphinidin 3-sambubioside, Cyanidin 3-sambubioside, 7-Hydroxycoumarin, Epigallocatechin, Epigallocatechin gallate.	[42]

of female CD-1 mice [63]. Another polyphenol, Del-3-sam isolated from dried calyces was shown to induce apoptosis in leukaemia cells (HL-60), through elevation of intracellular reactive oxygen species (ROS) resulting in activation of caspase cascade and reduction of poly(ADP)ribose polymerase (PARP) [21]. In human gastric carcinoma cells (AGS), polyphenolic extract induced apoptosis via two major signalling pathways that include phosphorylation of p53 leading to activation of apoptotic Bax protein and activation of p38 which triggered phosphorylation of c-jun, activation of FasL and downstream Fas-mediated signalling leading to apoptotic cell death [60]. A similar study on AGS cells proposed chemopreventive effect by induction of apoptosis via JNK/p38 signalling leading to Fas/FasL mediated caspase cascade [64]. Breast adenocarcinoma cell line (MCF7) was shown to be most vulnerable to the apoptogenic effect of aqueous extract of the calyx (IC50 = 0.5 mg/mL), assayed using DNA fragmentation method [24]. Similarly, leaf extract was found to be most potent apoptosis-inducer against androgen-dependent human prostate cancer (LNCaP) cells and in xenograft nude mice demonstrated by reduced expression of Bcl-2 and Mcl-1, mitochondrial translocation of Bax, cytochrome C release and increased expression of FasL [61]. Beside, Ellagic acid (25–100 μM) found in methanolic leaf extract was observed to induce apoptosis in prostate cancer cells by up-regulating the formation of Fas/ FasL complex in LNCaP cells [61]. Recently, a flowcytometric analysis found that ethanolic extract of calyx could play a protective role against doxorubicin-induced damage in cardiomyoblast (H9c2) cells by inducing apoptosis through DNA fragmentation [65]. Besides, methanolic extract was also reported to induce a potent cytotoxic effect on hepatocellular carcinoma cells (Hep 3B), while further investigation is required to understand the mechanism [66]. Flavonoids from calyx were reported to induce apoptotic morphological changes with elevated gene expression of p53 in Ehrlich ascites carcinoma cells [67]. Total extract of the plant was also reported to have impaired cell growth, exerted a reversible cytostatic effect, and reduced cell motility and invasiveness in Multiple Myeloma (RPMI 8226) cells and Oral Squamous Cell Carcinoma (SCC-25) cells via activation of p38 and modulation of Extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) [68]. Flower anthocyanins caused Human leukaemia (HL-60) cells apoptosis in a dose- and time-dependent manner, which is mediated via increased phosphorylation in p38 and c-Jun, cytochrome c release, and expression of t-Bid, Fas, and FasL [69]. This observation suggests that *Hibiscus* anthocyanins induced apoptosis via the p38-FasL and Bid pathway [69].

Based on the effects on the regulation of different proteins and genes in the cancer signalling pathway, a proposed model for the molecular mechanism of apoptosis-induction by *Hibiscus sabdariffa* is shown in Fig. 2 and a detailed description of all assays are given in Tables 2 and 3.

4.4. The autophagy-inducing activity

Hibiscus sabdariffa leaf polyphenols (HLP) and one of the major constituent, (−)-epicatechin gallate (ECG) was accessed for its protective role against oxidized low-density lipoprotein (ox-LDL) induced injury of human endothelial cells. The result indicated that HLP and ECG triggered autophagic flux via class III phosphoinositide 3-kinase (PI3K)/Beclin-1 and Phosphatase and tensin homolog (PTEN)/class I PI3K/Akt cascade signaling [71]. HLP was also found to induce autophagic cell death in human melanoma cell (A375) by increasing the expressions of autophagy-related proteins autophagy-related gene 5 (ATG5), Beclin1, and light chain 3-II (LC3-II) [70]. *Hibiscus sabdariffa* anthocyanin, Delphinidin, prepared from dried flowers was found to trigger both autophagy and necrosis cell death in breast cancer cells MCF7, where autophagy was initiated via LC3 (light chain 3) activation and AMPK (AMP-activated kinase) phosphorylation [37].

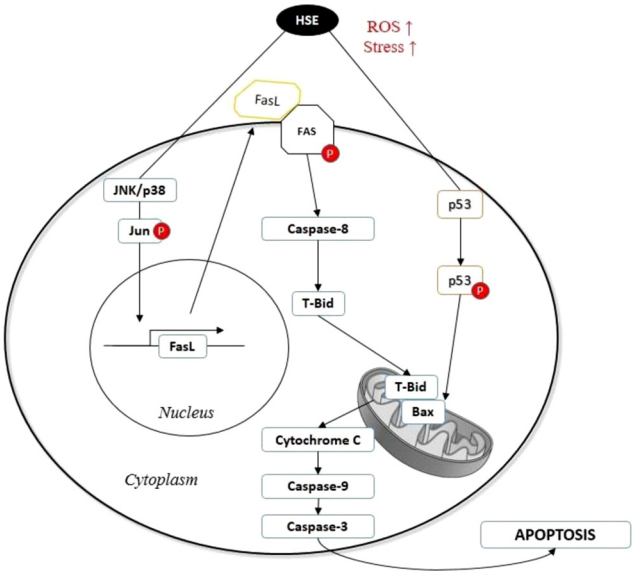

Fig. 2. A proposed model for the molecular mechanism of Hibiscus sabdariffa -mediated apoptosis in cancer cells.

4.5. Effects on angiogenesis and metastasis

Anthocyanins extracted from dried calyx showed an anti-angiogenic effect in time and concentration-dependent manner when injected in chick embryo [18]. *In-silico* analysis depicted that this effect might be mediated by hibiscus anthocyanin, *hibiscetin* which binds to vascular endothelial growth factor receptor 2 (VEGFR2) hindering its activity [18].

Polyphenolic isolates of *Hibiscus sabdariffa* dose- and time-dependently inhibited high-glucose-stimulated cell proliferation and migration in vascular smooth muscle cell (VSMC) by suppressing the proliferating cell nuclear antigen (PCNA) level, matrix metalloproteinase (MMP)-2 activation, connective tissue growth factor (CTGF) and receptor of the advanced glycation end product (RAGE) [72]. Matrix metalloproteinase-9 (MMP-9) plays a key role in cell migration and invasion in many types of human cancers [73]. In human prostate cancer cells, LNCaP, leaf polyphenols of *Hibiscus sabdariffa* dose-dependently suppressed the migration and invasion of cells under non-cytotoxic concentrations by inhibiting the activity of matrix metalloproteinase-9 (MMP-9) (Table 4) [74]. This observation suggests that inhibition of MMP-9 expression by polyphenols is a consequence of inactivation of Akt/NF-κB/MMP-9 cascade pathway confirmed by the transfection of Akt1 overexpression vector [74]. In a recent study, Ching-Chuan Su et. al. reported that daily oral administration of *Hibiscus* anthocyanins (HAs) efficiently reduced blood vessel development, lung metastasis, tumour size, haemoglobin (Hb) content as well as CD31 expression of the tumour (Table 4) [75]. This study was conducted on melanoma cells B16-F1, where *Hibiscus sabdariffa* anthocyanins suppressed cell migration and tube formation via inhibition of PI3K/Akt and Ras/MAPK pathways, resulting in reduced expression of vascular endothelial growth factor (VEGF) and MMP-2/-9 proteins. Particularly, protocatechuic acid (PCA) was found to inhibit cell migration and invasion at non-cytotoxic concentrations in AGS cells and B16/F10 melanoma cell injected mice via down-regulation of Ras/Akt/NF-kB pathway and MMP-2 production [76]. Recently, the polyphenol-enriched extract was reported to possess an anti-metastatic effect on colon carcinoma (DLD1) cells via altering cytoskeletal organisation, reduced MMP-2, MMP-9, and urokinase-type plasminogen activator (uPA) expression, increased metallopeptidase inhibitor 2 (TIMP2) and suppressed focal adhesion kinase (FAK) and CD44/ tyrosine-protein kinase Met (c-MET) signalling (Table 4) [77]. Furthermore, the study

Table 2
Molecular basis of apoptotic activity of different extracts of Hibiscus sabdariffa in various types of human cancers in different studies.

Type of extract	Cancer type	Cell Line/ Animal Used	Dose/Duration	IC_{50} Value	Main Activity	Molecular mechanism	Reference
Aq. Calyx	Breast Cancer	MCF-7	0.5,0.4,0.3,0.2,0.1 & 0.05 mg/mL	0.5 mg/mL	Apoptosis	–	[24]
Aq. Flower	Gastric Cancer	AGS	0, 1, 2, 3 mg/mL for 24/48 h	2.5 mg/mL	Cell cycle arrest, apoptosis	p38↑, JNK↑, c-Jun↑, Fas/FasL↑, Bax↑, Bid↑, Cytochrome c↑	[64]
Met. Polyphenol	Gastric Cancer	AGS	0, 0.1, 0.5, 1, 1.5, 2, 2.5, 3 mg/mL for 24 h.	0.95 mg/mL	Cell cycle arrest, apoptosis	p53↑, p38 MAPK/ FasL↑	[60]
Met. Calyx	Ehrlich Ascites carcinoma	EAC/ Swiss albino mice	5 and 10 mg/kg/day	28.16 μg/mL	Antineoplastic, apoptosis	p53↑	[67]
Total Extract	Squamous Cell Carcinoma	SCC-25 RPMI 8226	0.5, 1, 3, 5 mg/mL for 24/48/72 h.	5 mg/mL	Apoptosis, cytostatic, anti-invasion	p38↑, ERK1/2 modulation	[68]
Met. Leaf Polyphenol	Melanoma	A375	100, 250 μg/mL for 24 h.	250 μg/mL (0.25 mg/mL)	Apoptosis, Autophagy	Fas/FasL↑, Caspase activity, Bcl-2↓, ATG5↑, LC3-II↑, Beclin1↑	[70]
Aq. Leaf	Prostate cancer	LNCaP/ Athymic nude mice	0.1–10.0 mg/mL for 24 h.	2.5 mg/mL	Cell cycle arrest, apoptosis	Caspase activity, Bcl-2↓, mitochondrial translocation of Bax/t-Bid, Fas/ FasL↑, TNFα↑	[61]
Met. Flower	Leukaemia	HL-60	0, 0.05, 0.1, 0.2, 0.5, 1, 3, 4 mg/mL for 24 h.	2.49 mg/mL	Cell cycle arrest, apoptosis	p38↑, c-Jun↑, Fas/FasL↑, caspase activity, Bid↑, Bcl-2↓	[69]

Table 3
Apoptotic activity of pure compounds isolated from Hibiscus sabdariffa.

Compound	Cancer type	Cell Line Used	Dose/Duration	IC_{50} Value	Main Activity	Mode of Action	Reference
Delphinidin-3- sambubioside from calyx	Leukaemia	HL-60	0, 25, 50, 75, 100, 125 μM for 24 h.	75μM	Apoptosis, cell cycle arrest	ROS↑ Caspase activity, PARP↓, Bid transduction, Cytochrome c↑	[21]
Protocatechuic acid from flower	Leukaemia	HL-60	0, 0.2, 0.5, 1, 2 mM for 24/48 h.	2 mM	Apoptosis, cell cycle arrest	Reduction of Retinoblastoma Phosphorylation, Bcl-2↓, Bax↑	[62]

Table 4
Molecular basis of Inhibitory effects of Hibiscus sabdariffa against metastasis and angiogenesis in different cancerous cells in different studies.

Type of extract	Cancer type	Cell Line Used	Dose/Duration	IC_{50} Value	Main Activity	Mode of Action	Reference
Met. Flower polyphenols	Colorectal cancer	DLD-1, Balb/c-nude mice	0−5 mg/mL for 24 h.	–	Inhibition of metastasis	Decreased phosphorylation of FAK at Tyr397, CD44/c-MET↓, MMP-2↓, MMP-9↓, uPA↓, TIMP2↑	[77]
Met. Flower anthocyanins	Melanoma	B16-F1, C57BL/6 male mice	0, 1, 3, 5, 7, 9 mg/mL for 24 h.	5.23 mg/mL	Inhibition of metastasis & angiogenesis	VEGF↓, MMP-2/-9↓, CD31↓, PI3K/Akt↓, NF-κB/p65↓, Ras/MAPK↓	[75]
Aq. Leaf	Prostate Cancer	LNCaP, Athymic nude mice	0–20 mg/mL for 24/48/72 h.	3.0 mg/mL	Inhibition of metastasis	MMP-9/2↓, NF-κB↓, PI3K/Akt↓	[74]

also found that *Hibiscus sabdariffa* Extract (HSE) inhibited lung metastasis of DLD1 cells in xenograft animal model [77].

5. The estrogenic activity and implication for breast cancer

Estrogen receptor (ER) positive breast cancer is the most common subtype of breast cancer accounting for nearly 75 % of all breast cancer cases [78]. Significant efforts have been made toward blocking the estrogen receptor activity for treatment and chemoprevention of breast cancer [79]. Rapid advances in developing selective estrogen receptor modulators (SERM) like tamoxifen and raloxifene lead to falling in breast cancer incidences by 38 % in the last decade [80]. The binding of SERM to ER changes the receptor conformation, that prevents the estrogen binding and its downstream activity [81]. Though, these strategies help with consistent improvements in disease-free survival but associated with certain short-term as well as long-term side effects including cardiotoxicity [82], venous thrombosis [83], osteoporosis/osteopenia [84] and development of secondary cancer [85]. Currently, several studies have focused on naturally occurring phytoestrogens and their growth-inhibiting effect on breast cancer by inhibition of local oestrogen synthesis [86]. Some studies have indicated an association between higher consumption of dietary phytoestrogens and reduced incidences of breast cancer [87]. Epidemiologic evidence suggests that consumption of phytoestrogen-rich soy products possibly decreases the risk of breast cancer by 30 % among Asian populations [88,89]. *Hibiscus sabdariffa* is a rich source of phytoestrogens like quercetin and daidzein [90] and was reported to have mild estrogen-like effects on the uteri of immature female rats [91]. Other phytoestrogenic constituents of this plant include β-sitosterol, stigmasterol [92] α-spinasterol, campesterol and ergosterol [93]. Presently, a single report is available that claims the deleterious effect of *Hibiscus sabdariffa* on estrogen receptor-mediated growth signalling [94]. This study reported the inhibitory effects of ethyl acetate extract of *Hibiscus sabdariffa* (7.5 and 3.5 mg/mL) on the growth and invasiveness of MCF-7 cells by affecting the cellular level of ERα receptor where the cytosolic localization of ERα increases in comparison to untreated cells [94]. Additionally, the aqueous extract of *Hibiscus sabdariffa* was reported to significantly effects the circulating levels Follicle-stimulating hormone(FSH), Prolactin, Estradiol and Testosterone in male Wistar rats [95].

6. Discussion

Despite the advances in cancer therapeutic research, the search for an effective anticancer agent continues. The association between diet and cancer risk is epidemiologically well-established and thus edible plants are increasingly being considered as sources of anticancer drugs. *Hibiscus sabdariffa* or roselle is a popular medicinal herb around the globe for its widespread disease-healing capacity including cancer. Polyphenols, flavonoid and anthocyanin compounds known for oxidizing activities indicated an important role among the contents of the plant extracts, which recently gained attention as chemopreventive agents. Among several human cancer cells, gastric carcinoma and mammary carcinoma cells were most susceptible to the cytotoxic effect of *Hibiscus sabdariffa* extracts with IC_{50} value ranging near 0.95 and 0.5 mg/mL respectively. From selective cytotoxicity, apoptosis induction to inhibiting metastasis and angiogenesis, the plant showed a wide range of anti-cancer activity (Fig. 3) by regulating several important pro-oncogenic and anti-oncogenic pathways.

Our study suggests that *Hibiscus sabdariffa* extract (HSE) induced apoptosis might be stimulated by stress and a rise in cellular reactive oxygen species (ROS) level. These stimuli are further mediated via both the extrinsic and intrinsic pathway of apoptosis. HSE showed strong potential to up-regulate proapoptotic proteins like Bid, Bax and mediated caspase cascade death signalling. On the other side, it hindered the activity of antiapoptotic protein family Bcl-2, which is an ideal condition for achieving apoptosis in cancer cells. *Delphinidin-3-sambubioside*

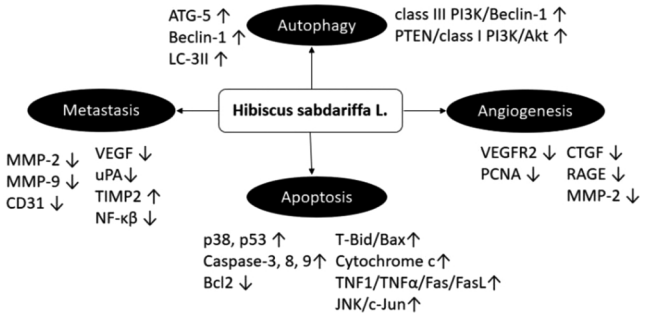

Fig. 3. Indicating the crucial anticancer activities showed by Hibiscus sabdariffa along with the regulation of different important proteins and genes network by various extracts of Hibiscus sabdariffa L.

and *protocatechuic acid* are the two potent apoptosis-inducing compound from the plant that showed strong activity against leukaemia cells, however, proper clinical trials are necessary to investigate their role in common human cancers. Most importantly, the addition of HSE was found to enhance the induction of apoptosis by chemotherapeutic drugs (taxol and cisplatin) by increasing oxidative stress and decreasing mitochondrial membrane potential in triple-negative breast cancer cells when compared to individual treatment. Additionally, HSE was also found to play a protective role against doxorubicin-induced cardiotoxicity in H9c2 cardiomyoblast cells [65]. This observation suggests that HSE could potentially be combined with chemotherapeutic treatments in adjuvant therapy as a herbal supplement to enhance drug action and reduce chemotherapy-associated side effects.

Autophagy plays a cytoprotective role against cancer by removing misfolded proteins, damaged cells and free radicals resulting in limited genomic damage by limiting mutations [96]. Like apoptosis, damaged autophagy machinery is linked with genomic instability, tumorigenesis, and malignant transformation [96,97]. For instance, mutation or absence of autophagy-related gene *beclin1* was observed in 40–75 % of breast, ovarian, and prostate cancers [98,99]. Besides, mice lacking *beclin1* was seen to have developed spontaneous tumours [96]. Alongside, autophagy-related gene 5 and 7 (ATG5 and ATG7) plays a crucial role in tumour suppression, reduced expression of which causes mitochondrial damage and oxidative stress resulting in hepatic tumour development in mice [100]. Dysregulation of the PI3K/Akt pathway and impairment of its key protein associates like Phosphatase and tensin homolog (PTEN) reduces autophagy in malignant cells and backs tumorigenesis [101]. Conversely, the Akt/mTOR pathway is known to suppress autophagy [102]. Polyphenols from *Hibiscus sabdariffa* leaf (HLP) were reported have an up-regulatory effect on the expression of autophagy-related proteins, including PI3K class III/Beclin1, ATGs, LC3, PTEN and p62 in human melanoma cells (A375) [70]. Interestingly, the same study reported that HLP reduced the expressions of autophagy- inhibitory proteins, p-Akt and p-mTOR [70]. Similar results were observed in breast cancer cells (MCF7) treated with the major anthocyanin of *Hibiscus sabdariffa*, delphinidin [37].

Majority of the presently available anticancer drugs causes irreversible damage to healthy cells along with the cancerous cells that results in low therapeutic index of those drugs [103]. To overcome this issue with conventional cancer treatment, present-day searches are mainly focused on natural products that selectively inhibits the growth of cancer cells. Surprisingly, HSE was found to induce selective cytotoxicity especially in breast cancer cell lines- ER + MCF-7 and triple-negative MDA-MB-231, when tested along with normal cells. A western blot and immunofluorescence analysis showed that following the HSE treatment in MCF7 cell, ERα receptor, which shows greater expression in nuclear and cytoplasmic location became more cytoplasmic suggesting that HSE can alter ERα expression in MCF7 cells [94]. This interpretations advocates for breast cancer being a better model for investigating the chemotherapeutic potential of *Hibiscus sabdariffa*.

In addition to hyperproliferation, metastasis is another important capability of cancer cells that causes an approximate 90 % of all cancer deaths [104]. The degradation of basement membranes and the stromal extracellular matrix (ECM) are crucial steps for tumour invasion and metastasis [105]. The matrix metalloproteinases (MMPs) family is responsible for the degradation of the ECM [105]. Among them, MMP-2 and MMP-9 efficiently degrade native collagen types IV and V, fibronectin, and elastin [106]. The expression of the MMPs gene is primarily regulated at the transcriptional (through activator protein-1 (AP-1) or nuclear factor-κB (NF-κB) via mitogen-activated protein kinase (MAPK) or phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB, also known as Akt) pathways) and posttranscriptional levels, and at the protein level via their activators or inhibitors, and their cell surface localization [106]. MMPs and their regulatory pathways have been considered promising targets for anticancer drugs and chemotherapeutic agents [107]. Different extracts of *Hibiscus sabdariffa* were reported for their inhibitory effects on MMPs, especially MMP9 and MMP2 expression at the transcriptional level [72]. Furthermore, HSE also marked reduction of nuclear NF-κB activity by decreasing the DNA-binding ability of NF-κB and subsequently led to a reduction in MMP-9 expression. HSE-induced down-regulation of MMP9 activity might be a consequence of the suppression of the PI3K/Akt/NF-κB signalling pathway, which in turn led to the reduced invasiveness of the cancer cells. Alongside, polyphenolic isolated of *Hibiscus sabdariffa* was reported to have suppressed the expression of Proliferating cell nuclear antigen (PNCA), which is significantly elevated in metastatic tumours and hence it acts as the marker of proliferation [72,108]. The cysteine-rich secreted peptide connective tissue growth factor (CTGF) was reported to be involved in fibroblast proliferation, migration, attachment, and ECM formation in various cell lines, frequently by elevating matrix metalloproteinases(MMPs) and PNCA expression [72,109] and suppressing TIMP2 expression [110]. The polyphenolic isolated of *Hibiscus sabdariffa* blocked the expression of CTGF and the downstream ECM protein accumulation along with increased TIMP2 expression in proliferating vascular smooth muscle cell and colon carcinoma cells [72,77]. Platelet endothelial cell adhesion molecule-1 (PECAM-1) or CD31 and CD44 have been demonstrated to be highly expressed in many human cancers and regulates metastasis mostly by inducing epithelial-mesenchymal transition (EMT), proving them as important markers of metastatic cancer and chemotherapeutic targets [111–113]. Especially, CD44 plays a central role in stimulating cell motility by activating highly expressive oncoprotein tyrosine kinase receptors such as c-MET subsequently lead to FAK phosphorylation and the downstream signalling cascade that promotes tumour proliferation, migration, and invasion [114]. Polyphenolic and anthocyanin ingredients of *Hibiscus sabdariffa* was found to considerably reduce the expression of both CD31 and CD44 in melanoma cells(B16-F1) and colon carcinoma cells (DLD1) mostly by downregulating FAK signalling cascades [75,77]. However, the exact polyphenolic component of *Hibiscus sabdariffa* with anti-metastatic potential is still unknown.

Historically, *Hibiscus sabdariffa* is used in the treatment of hypercholesterolemia and hyperlipidaemia [115], two conditions well-linked with cancer progression and metastasis [116,117]. Thus the effectiveness of the plant constituents in lowering the serum cholesterol and serum lipid level of cancer patients needs an investigation. A better understanding of the mechanism of anti-cancer activity of the extracts and isolating novel fractions might help to find the potential of the plant to be used as a herbal supplement along with chemotherapeutics.

7. Conclusion

Hibiscus sabdariffa and its phytochemicals are potent anticancer agents, yet an under-exploited candidate for chemotherapeutic applications. The compounds like Delphinidin-3-sambubioside and protocatechuic acid needs a proper clinical trial to understand its chemotherapeutic potential as well as the synergistic activity with

chemotherapeutic drugs. Several chemotherapeutic features of this plant suggest that cancer of the breast and gastric cavity could be a better model to exploit its anticancer property. However, no significant investigation has been made to exploit the anticancer effect of plant in those cancer types. With low toxicity and tolerability record, a proper clinical setup might help to recommend *Hibiscus sabdariffa* as a herbal supplement for cancer patients.

Declaration of Competing Interest

All the authors declare no conflict of interest concerning this manuscript.

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