REVIEW ARTICLE

Antimicrobial, antiparasitic and anticancer properties of *Hibiscus sabdariffa* (L.) and its phytochemicals: *in vitro* and in *vivo* studies

Antimikrobiální, protiparazitické a protinádorové vlastnosti *Hibiscus sabdariffa* (L.) a jeho sloučeniny: *in vitro* a *in vivo* studie

Sherif T. S. Hassan • Kateřina Berchová • Miroslava Šudomová

Received 25 November 2015 / Accepted 16 December 2015

Summary

In the last few decades, *Hibiscus sabdariffa* L. (Malvaceae; *H. sabdariffa*) has gained much attention in research field because of its potentially useful bioactivity as well as a great safety and tolerability. For decades, microbial, parasitic and cancer diseases remain a serious threat to human health and animals as well. To treat such diseases, a search for new sources such as plants that provide various bioactive compounds useful in the treatment of several physiological conditions is urgently needed, since most of the drugs currently used in the therapy have several undesirable side effects, toxicity, and drug resistance. In this paper, we aim to present an updated overview of *in vitro* and *in vivo* studies that show the significant therapeutic properties of the crude extracts and phytochemicals derived from *H. sabdariffa* as antimicrobial, antiparasitic, and anticancer agents. The future directions of the use of *H. sabdariffa* in clinical trials will be discussed.

Key words: *Hibiscus sabdariffa* L. • antimicrobial agents • cancer preventive agents • antiparasitic drugs • natural products

Souhrn

V posledních několika desetiletích si získal *Hibiscus sabdariffa* L. (Malvaceae, *H. sabdariffa*) velkou pozornost v oblasti výzkumu kvůli svému potenciálu biologické aktivity stejně jako pro svou velkou bezpečnost a snášenlivost. Po celá desetiletí zůstávají mikrobiální, parazitární a rakovinotvorná onemocnění vážnou hrozbou pro lidi a zvířata. K léčbě těchto chorob je velmi nutné naléhavě hledat nové zdroje – rostliny, které poskytují různé biologicky aktivní sloučeniny použitelné při léčbě některých onemocnění, protože v současné době většina léků používaných při léčbě má několik nežádoucí vedlejší účinků, toxicitu a rezistenci. V tomto článku se snažíme představit aktualizovaný přehled o *in vitro* a *in vivo* studiích, které ukazují významné léčebné vlastnosti surových extraktů a fytochemikálií – odvozené z *H. sabdariffa* jako antimikrobiální, protiparazitické a proti - nádorové látky. Možnosti využití a zkoumání *H. sabdariffa* v klinických studiích budou teprve diskutovány.

Klíčová slova: *Hibiscus sabdariffa* L. • antimikrobiální látky • protinádorové látky • protiparazitické látky • přírodní látky

Ing. Sherif T. S. Hassan (✉)
Department of Natural Drugs, Faculty of Pharmacy
University of Veterinary and Pharmaceutical Sciences
Palackého tř. 1946/1, 612 42 Brno, Czech Republic
e-mail: sherif.hassan@seznam.cz

S. T. S. Hassan • K. Berchová
Department of Applied Ecology, Faculty of Environmental Sciences
Czech University of Life Sciences, Prague, Czech Republic

M. Šudomová
Department of Archeology and Museology, Faculty of Arts
Masaryk University, Brno, Czech Republic

Introduction

Hibiscus sabdariffa L. (Malvaceae; *H. sabdariffa*) is a medicinal plant which has a long history of herbal and edible uses across the world and is mainly cultivated in tropical and subtropical regions of Africa and Asia[1–3]. It is an annual or perennial plant or woody-based shrub with serrate leaves, red calyces and red stems[3, 4]. The phytochemical and pharmacological activities of various parts of *H. sabdariffa* have been evaluated including antioxidant, antidiabetic, anti-inflammatory, antimicrobial, and anticancer properties[5–7]. Nowadays, cancer, microbial, and parasitic diseases have become a global concern worldwide, since these diseases have threatened

human and animal health[8–12]. This review aims to provide a brief overview of the *in vitro* and *in vivo* studies that present the therapeutic potential of *H. sabdariffa* extract (HSE) and its bioactive substances in the treatment of cancer, bacterial, fungal, and parasitic diseases.

Phytochemical profile

H. sabdariffa has a long tradition as it contains a rich bioactive profile responsible for its therapeutic efficacy such as anthocyanins, flavonoids, polysaccharides, and organic acids including malic, ascorbic, hydroxycitric, and *Hibiscus* acids[13–15]. Furthermore, HSE is rich in minerals such as iron and calcium with a low content of glucose, and 18 volatile compounds were identified via GC and GC-MS analyses[16]. It has been reported that *H. sabdariffa* seeds contain a large amount of polyunsaturated fatty acids, tocopherol, and the major fatty acids of seeds were found to be oleic 37.92%, linoleic 35.01% and palmitic 19.65% acids[16, 17]. Another studies have explored that 1g of aqueous extract of *H. sabdariffa* contains anthocyanins (56.5 mg/g delphinidin-3-*O*-sambubioside and 20.8 mg/g cyanidin--3-*O*-sambubioside), 3.2 mg/g quercetin, 2.1 mg/g rutin and 2.7 mg/g chlorogenic acid, while ethanol was revealed as the best solvent for the extraction of anthocyanins (ranged from 17.3 to 32.2 mg of cyanidin--3-glucoside/g dry weight in the pigmented varieties)[18, 19].

Antimicrobial and antiparasitic properties

Numerous studies have described the potential use of HSE and its phytochemicals as significant antimicrobial and antiparasitic agents in the treatment of various infections.

Antibacterial activities

Recently, Alshami and Alharbi[20] explored the effective potential of HSE to prevent recurrent urinary tract infections (UTIs). HSE was found to exhibit bacteriostatic effect with potent inhibition of the growth of six *Escherichia coli* (*E. coli*) and two *Klebsiella pneumoniae* isolates (collected from patients with recurrent UTIs) and remarkable inhibition of biofilm production of all isolates. MIC (Minimum Inhibitory Concentration) values ranged from 0.5 to 4 mg/mL, and MBC (Minimum Bactericidal Concentration) ranged from 8 to 64 mg/mL. The effectiveness of aqueous extracts of *H. sabdariffa* were investigated for antimicrobial activity against *E. coli* and *Staphylococcus aureus* (*S. aureus*) strains in a microbiological medium and ultrahigh-temperature-processed milk with various fat percentages. The results showed that extracts treated by heat revealed higher antimicrobial activity than in microbiological medium[21]. A methanol extract of *H. sabdariffa* was found to inhibit effectively the growth of *E. coli* O157:H7 (at a concentration of 10%) isolates from food, veterinary, and clinical samples, as determined by disk diffusion method[22]. Liu et al. reported that aqueous extract of *H. sabdariffa* and *H. sabdariffa* protocatechuic acid (PCA) at a concentration of 5 mg/mL inhibited notably the growth of methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. In addition, PCA (in a dose-dependent manner) exerted higher antibacterial activity against tested pathogens in broth than in human plasma. Moreover, the antibacterial effect was independent from temperature, when treated by a heat[23]. The antibacterial effects of aqueous and ethanol extracts, and PCA of *H. sabdariffa* were examined against food spoilage bacteria *Bacillus cereus*, *Salmonella typhimurium* DT104, *E. coli* O157:H7, *Listeria monocytogenes*, and *S. aureus*. MICs of aqueous, ethanol extracts, and PCA against tested bacteria were in the range of 112–144, 72–96, and 24–44 µg/mL, respectively. The results revealed that ethanol extract exhibited greater antibacterial effects than aqueous extract[24]. Jung et al.[16] evaluated the antimicrobial properties of aqueous and ethanol extracts of *H. sabdariffa* against *Bacillus subtilis*, *S. aureus*, and *E. coli*. The results showed that ethanol extract against *Bacillus subtilis* and *S. aureus* explored higher activity than that of aqueous extract, while aqueous extract at concentrations of 25 and 50 mg/mL inhibited potently the growth of *E. coli* via the paper disc method. Olaleye[25] investigated *in vitro* the inhibitory effect of aqueous-methanol extract of dried *H. sabdariffa* calyx against nine bacterial pathogens such as *Clostridium sporogenes*, *S. aureus*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *Serratia mascences*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas fluorescens*, and *Bacillus cereus*. The inhibitory effect was observed potently against all tested pathogens. Several studies have also demonstrated that HSE exerted antibacterial activity against bacteria causing oral cavity infections such as. *Streptococcus mutans* with MIC value of 2.5 mg/mL, while at concentrations ranging from 96–152 µg/mL exhibited also significant inhibitory activity against *Campylobacter coli* and *Campylobacter fetus*, which contaminate beef, pork, and chicken meat[26, 27]. The crude extracts of *H. sabdariffa* seeds were tested for inhibitory activity against three types of Gram-negative bacteria species *Enterobacter*, *Salmonella*, and *Shigella*. The higher antibacterial activity was observed against *Salmonella* sp. at a concentration of 200 mg/L[28].

Antifungal activities

The anticandidal activity of methanol extract of *H. sabdariffa* fruit (at a concentration of 10 mg/mL) was evaluated against six pathogenic *Candida* species such as *C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*. The inhibitory activity was detected only against *C. albicans*[29]. El-Nagerabi et al.[30] examined the inhibitory effect of aqueous extract of *H. sabdariffa* calyx at concentrations of 5, 7.5, 10 and 12.5 g/100 mL on the growth and aflatoxin B1 production by two fungal strains *Aspergillus parasiticus* (CBS 921.7) and *Aspergillus flavus* (SQU 21). The results indicated that no inhibitory effect was observed on the growth of both fungal strains, while the inhibition of aflatoxin B1 production by the different concentrations of *H. sabdariffa* calyx ranged between 91.5–97.9% and 87.1–93.3% for *A. flavus* and *A. parasiticus* strains, respectively. In addition, the study confirmed the metabolic effect of aqueous extract of *H. sabdariffa* calyx

on aflatoxin biosynthesis pathway of both *Aspergillus* species, and a beneficial use in food industry as an effective biocontrol and non-toxic biopreservative agent.

Antiparasitic activities

Human lymphatic filariasis, a vector-borne disease, is distributed in tropical, subtropical regions, causing a public health problem. Saxena et al.[31] determined antifilarial activity of ethanolic extract of *H. sabdariffa* leaves by *in vitro* motility and MTT methods. The results showed that the extract affected both the adult worms and microfilariae of *Brugia malayi*. The butanol fraction exhibited remarkable inhibitory effect, which was related to anthocyanin-glycosides. Animal trypanosomosis, a parasitic disease is still the main factor of decreasing the growth of livestock in Africa. Umar and colleagues[32] investigated *in vivo* the effect of aqueous extracts of *H. sabdariffa* calyces on the heamatological profile and organ pathological changes in *Trypanasoma congolense*-infected rats. The results showed that consumption of the extract (9.94 mg/100g/day) enhanced the pathological changes in blood and organs of *T. congolense*-infected rats.

Anticancer activity

The crude extracts and isolated substances from *H. sabdariffa* were found to be potential cancer chemopreventive agents. For instance, *Hibiscus* anthocyanins (HAs) exhibited an ability to promote cancer cell apoptosis, particularly in gastric cancer and leukemia, while PCA was found to suppress the carcinogenic action of various substances in different tissues of rat models *in vivo*[33, 34]. Tsai and co-workers[35] have recently evaluated the protective effect of HAs on N-nitrosomethylurea (NMU)-induced leukemia of rats *in vivo*. The results indicated that oral administration of HAs (0.2%) significantly suppressed progression of NMU--induced leukemia by approximately 33.3% in rats. Several studies have also evaluated the effectiveness of HAs including delphinidin-3-sambubioside on human leukemia cells. Interestingly, HAs effectively induced apoptotic cell death in human promyelocytic leukemia cells via the p38-FasL and Bid pathway, and ROS--mediated the mitochondrial dysfunction pathway[36, 37]. Lo and colleagues[38] also reported that HAs induced apoptosis of the proliferating smooth muscle cell via activation of P38 MAPK and p53 pathway. PCA, a phenolic acid was found to exert *in vitro* protective effects against cytotoxicity and genotoxicity of hepatocytes induced by tert-butylhydroperoxide (t-BHP). Mechanism of PCA's protective effect may be related to its ability to inhibit DNA repair synthesis caused by t-BHP and by scavenging free radicals as well[39]. Tseng et al.[40, 41] presented in two studies that PCA exhibited remarkable inhibition of 12-*O*-tetradecanolyphorbol-13--acetate (TPA)-induced skin tumor formation in female CD1-mice and the survival of human promyelocytic leukemia HL-60 cells. In their studies, they revealed that the mechanism by which PCA utilized anticancer activities is due to its ability to induce antitumor activities through DNA fragmentation, GI arrest, apoptosis, and decreasing reactive oxygen species (ROS). The apoptosis-inducing activity was implicated with the phosphorylation and degradation of RB and the suppression of Bcl-2 protein. Lin and colleagues[42] studied the apoptotic effect of PCA on human gastric carcinoma (AGS) cells. The results suggested that the apoptotic effect may be mediated via p53 signaling and p38 MAPK/FasL cascade pathway. Olvera-Garcia and co-workers[43] reported that PCA inhibited the mutagenicity of 1-nitropyrene and checked the proliferation of HeLa cells, both in a dose-response manner in human stomach adenocarcinoma AGS cells. In addition, the effect of *Hibiscus sabdariffa* extract induced cytotoxicity and apoptosis of the cancer cells in dose-dependent manner through JNK/p38 signaling cascade-mediated apoptosis. Saeed at al.[44] reported that ethanolic extract of *H. sabdariffa* exerted moderate proliferative activity in cell-culture using estrogen-responsive breast cancer cell lines (MCF-7). Moreover, the results revealed that *H. sabdariffa* extract was found to be the richest in quercetin and daidzein as phytoestrogens among the other tested plants used in the study. Lin et al.[45] studied the anticancer properties of *H. sabdariffa* leaf extracts against various human prostate cancer (CaP) cells *in vitro* and *in vivo*. The study explored that anti-apoptotic activity was mediated via both intrinsic (Bax/cytochrome c-mediated caspase 9) and extrinsic (Fas-mediated caspase 8/t-Bid) pathways and by inhibiting the growth of prostate tumor xenograft in athymic nude mice as well. The results suggested that leaf extracts contained higher amounts of polyphenolic compounds than extracts from calyces and hence, *Hibiscus* polyphenolic compounds provide effective anticancer agents. Chiu and co-workers[46] evaluated *in vitro* anticancer activity of *Hibiscus* leaf polyphenolic (HLP) extract in melanoma cells. It has been found that HLP is rich in epicatechin gallate (ECG) and other polyphenols. The results explored that anticancer effect of HLP was associated with ECG by inducing the caspases cleavages, Bcl-2 family proteins regulation, and Fas/FasL activation in A375 cells. The apoptotic activity was determined by DAPI stain, cell-cycle analysis, and acidic vascular organelle (AVO) stain. Eventually, the study suggested that HLP could be a potential antimelanoma agent.

Conclusion and future directions

In summary, *H. sabdariffa* exerted various beneficial activities with no remarkable genotoxic effects as well as great tolerability. *Hibiscus sabdariffa* was found to be a great target as a source of many chemotherapeutic agents useful in food industry and drug discovery development. The most bioactive compounds in HSE that have been found to have significant therapeutic properties against microbial, parasitic and cancer diseases were PCA, HAs, and polyphenols. In this review, we summarized exclusively the potential use of HSE and its phytochemicals in the treatment of the most serious diseases which affect human and animal health such as cancer, bacterial, fungal, and parasitic diseases. *H. sabdariffa* has been examined both in *in vitro* and *in vivo* studies but more robust, randomized, and controlled

clinical trials with well-characterized HSE preparations would be needed in future research to confirm the therapeutic potential.

Conflict of interest: none.

This study was funded by Internal Grant Agency (IGA) of the Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Czech Republic. Project No. 20154247/2015.

References

1. **de Arruda A., Cardoso C. A., Vieira M. D., Arena A. C.** Safety assessment of Hibiscus sabdariffa after maternal exposure on male reproductive parameters in rats. Drug Chem Toxicol. 2015; 16, 1–6.
2. **Wang J., Cao X., Jiang H., Qi Y., Chin K. L., Yue Y.** Antioxidant activity of leaf extracts from different Hibiscus sabdariffa accessions and simultaneous determination five major antioxidant compounds by LC-Q-TOF-MS. Molecules 2014; 19(12), 21226–21238.
3. **Nyam K. L., Leao S. Y., Tan C. P., Long K.** Functional properties of roselle (Hibiscus sabdariffa L.) seed and its application as bakery product. J Food Sci Technol. 2014; 51(12), 3830–3837.
4. **Ramírez-Martínez D., Alvarado-Méndez E., Trejo-Durán M., Vázquez-Guevara M. A.** Nonlocal nonlinear refraction in Hibiscus Sabdariffa with large phase shifts. Opt Express 2014; 22(21), 25161–25170.
5. **Mihaljev Z., Zivkov-Balos M., Cupić Z., Jaksić S.** Levels of some microelements and essential heavy metals in herbal teas in Serbia. Acta Pol Pharm. 2014; 71(3), 385–391.
6. **Pérez-Ramírez I. F., Castaño-Tostado E., Ramírez-de León J. A., Rocha-Guzmán N. E., Reynoso-Camacho R.** Effect of stevia and citric acid on the stability of phenolic compounds and in vitro antioxidant and antidiabetic capacity of a roselle (Hibiscus sabdariffa L.) beverage. Food Chem. 2015; 172, 885–892.
7. **Ademiluyi A. O., Oboh G.** Aqueous extracts of Roselle (Hibiscus sabdariffa Linn.) varieties inhibit -amylase and -glucosidase activities in vitro. J Med Food. 2013; 16(1), 88–93.
8. **Chen Z., Lu W.** Roles of Ubiquitination and SUMOylation on Prostate Cancer: Mechanisms and Clinical Implications. Int J Mol Sci. 2015; 16(3), 4560–4580.
9. **Hassan S. T. S., Masarčíková R., Berchová K.** Bioactive natural products with anti-herpes simplex virus properties. J Pharm Pharmacol. 2015; 67(10), 1325–1336.
10. **Giannini G., Battistuzzi G., Vignola D.** Hydroxamic acid based histone deacetylase inhibitors with confirmed activity against the malaria parasite. Bioorg Med Chem Lett. 2015; 25(3), 459–461.
11. **Da-Costa-Rocha I., Bonnlaender B., Sievers H., Pischel I., Heinrich M.** Hibiscus sabdariffa L. – a phytochemical and pharmacological review. Food Chem. 2014; 165, 424–443.
12. **Sogo T., Terahara N., Hisanaga A., Kumamoto T., Yamashiro T., Wu S., Sakao K., Hou D. X.** Anti-inflammatory activity and molecular mechanism of delphinidin 3-sambubioside, a Hibiscus anthocyanin. Biofactors 2015; 41(1), 58–65.
13. **Gurrola-Díaz C. M., García-López P. M., Sánchez-Enríquez S., Troyo-Sanromán R., Andrade-González I., Gómez-Leyva J. F.** Effects of Hibiscus sabdariffa extract powder and preventive treatment (diet) on the lipid profiles of patients with metabolic syndrome (MeSy). Phytomedicine 2010; 17(7), 500–505.
14. **Pérez-Torres I., Ruiz-Ramírez A., Baños G., El-Hafidi M.** Hibiscus sabdariffa Linnaeus (Malvaceae), curcumin and resveratrol as alternative medicinal agents against metabolic syndrome. Cardiovasc Hematol Agents Med Chem. 2013; 11(1), 25–37.
15. **Mohamed R., Fernández J., Pineda M., Aguilar M.** Roselle (Hibiscus sabdariffa) seed oil is a rich source of gamma-tocopherol. J Food Sci. 2007; 72(3), S207–211.
16. **Jung E., Kim Y., Joo N.** Physicochemical properties and antimicrobial activity of Roselle (Hibiscus sabdariffa L.). J Sci Food Agric. 2013; 93(15), 3769–3776.
17. **Akinoso R., Suleiman A.** Heat treatment effects on extraction of roselle (Hibiscus sabdariffa L) seed oil. Eur J Lipid Sci Technol. 2011; 113, 1527–1532.
18. **Alarcón-Alonso J., Zamilpa A., Aguilar F. A., Herrera-Ruiz M., Tortoriello J., Jimenez-Ferrer E.** Pharmacological characterization of the diuretic effect of Hibiscus sabdariffa Linn (Malvaceae) extract. J Ethnopharmacol. 2012; 139(3), 751–756.
19. **Camelo-Méndez G. A., Ragazzo-Sánchez J. A., Jiménez-Aparicio A. R., Vanegas-Espinoza P. E., Paredes-López O., Del Villar-Martínez A. A.** Comparative study of anthocyanin and volatile compounds content of four varieties of Mexican roselle (Hibiscus sabdariffa L.) by multivariable analysis. Plant Foods Hum Nutr. 2013; 68(3), 229–234.
20. **Alshami I., Alharbi A. E.** Antimicrobial activity of Hibiscus sabdariffa extract against uropathogenic strains isolated from recurrent urinary tract infections. Asian Pac J Trop Dis. 2014; 4(4), 317–322.
21. **Higginbotham K. L., Burris K. P., Zivanovic S., Davidson P. M., Stewart C. N. Jr.** Antimicrobial activity of Hibiscus sabdariffa aqueous extracts against Escherichia coli O157:H7 and Staphylococcus aureus in a microbiological medium and milk of various fat concentrations. J Food Prot. 2014; 77(2), 262–268.
22. **Fullerton M., Khatiwada J., Johnson J. U., Davis S, Williams L. L.** Determination of antimicrobial activity of sorrel (Hibiscus sabdariffa) on Escherichia coli O157:H7 isolated from food, veterinary, and clinical samples. J Med Food 2011; 14(9), 950–956.
23. **Liu K. S., Tsao S. M., Yin M. C.** In vitro antibacterial activity of roselle calyx and protocatechuic acid. Phytother Res. 2005; 19(11), 942–945.
24. **Chao C. Y., Yin M. C.** Antibacterial effects of roselle calyx extracts and protocatechuic acid in ground beef and apple juice. Foodborne Pathog Dis. 2009; 6(2), 201–206.
25. **Olaleye M. T.** Cytotoxicity and antibacterial activity of Methanolic extract of Hibiscus sabdariffa. J. Med. Plants Res. 2007; 1(1), 9–13.
26. **Afolabi O. C., Ogunsola F. T., Coker A. O.** Susceptibility of cariogenic Streptococcus mutans to extracts of Garcinia kola, Hibiscus sabdariffa, and Solanum americanum. West Afr J Med. 2008; 27(4), 230–233.
27. **Yin M. C., Chao C. Y.** Anti-Campylobacter, anti-aerobic, and anti-oxidative effects of roselle calyx extract and protocatechuic acid in ground beef. Int J Food Microbiol. 2008; 127(1–2), 73–77.
28. **Nwaiwu N. E., Mshelia F, Raufu I. A.** Antimicrobial activities of crude extract of Moringa Oleifera, Hibiscus sabdariffa and Hibiscus esculentus seeds against some enterobacteria. J. Appl. Phytotechnol. Environ. Sanit. 2012; 1(1), 11–16.
29. **Rukayadi Y., Shim J. S., Hwang J. K.** Screening of Thai medicinal plants for anticandidal activity. Mycoses 2008; 51(4), 308–312.
30. **El-Nagerabi S. A. F., Al-Bahry S. N., Elshafie A. E., AlHilali S.** Effect of Hibiscus sabdariffa extract and Nigella sativa oil on the growth and aflatoxin B_1 production of Aspergillus flavus and Aspergillus parasiticus strains. Food Control 2012; 25, 59–63.
31. **Saxena K., Dube V., Kushwaha V., Gupta V., Lakshmi M., Mishra S., et al.** Antifilarial efficacy of Hibiscus sabdariffa on lymphatic filarial parasite Brugia malayi. MedChem Res. 2011; 20, 1594–1602.
32. **Umar I. A., Maryoms N. G., Daikwo E., Gidado A., Buratai L. B., Igbokwe I. O., Ibrahim M. A.** The effect of aqueous extracts of Hibiscus sabdariffa (Sorrel) calyces on heamatological profile and organ pathological changes in Trypanasoma congolense – infected rats. Afr J Tradit Complement Altern Med. 2009; 6(4), 585–591.
33. **Patel S.** Hibiscus sabdariffa: An ideal yet under-exploited candidate for nutraceutical applications. Biomedicine & Preventive Nutrition 2014; 4, 23–27.
34. **Kakkar S., Bais S.** A review on protocatechuic Acid and its pharmacological potential. ISRN Pharmacol. 2014; 2014, 952943.
35. **Tsai T. C., Huang H. P., Chang Y. C., Wang C. J.** An anthocyanin-rich extract from Hibiscus sabdariffa linnaeus inhibits N-nitrosomethylurea-induced leukemia in rats. J Agric Food Chem. 2014; 62(7), 1572–1580.

36. **Chang Y. C., Huang H.P., Hsu J. D., Yang S. F., Wang C. J.** Hibiscus anthocyanins rich extract-induced apoptotic cell death in human promyelocytic leukemia cells. Toxicol Appl Pharmacol. 2005; 205(3), 201–212.
37. **Hou D. X., Tong X., Terahara N., Luo D., Fujii M.** Delphinidin 3-sambubioside, a Hibiscus anthocyanin, induces apoptosis in human leukemia cells through reactive oxygen species-mediated mitochondrial pathway. Arch Biochem Biophys. 2005; 440(1), 101–109.
38. **Lo C. W., Huang H. P., Lin H. M., Chien C. T., Wang C. J.** Effect of Hibiscus anthocyanins-rich extract induces apoptosis of proliferating smooth muscle cell via activation of P38 MAPK and p53 pathway. Mol Nutr Food Res. 2007; 51(12), 1452–1460.
39. **Tseng T. H., Wang C. J., Kao E. S., Chu H. Y.** Hibiscus protocatechuic acid protects against oxidative damage induced by tert-butylhydroperoxide in rat primary hepatocytes. Chem Biol Interact. 1996; 101(2), 137–148.
40. **Tseng T. H., Hsu J. D., Lo M. H., Chu C. Y., Chou F. P., Huang C. L., Wang C. J.** Inhibitory effect of Hibiscus protocatechuic acid on tumor promotion in mouse skin. Cancer Lett. 1998; 126(2), 199–207.
41. **Tseng T. H., Kao T. W., Chu C. Y., Chou F. P., Lin W. L., Wang C. J.** Induction of apoptosis by hibiscus protocatechuic acid in human leukemia cells via reduction of retinoblastoma (RB) phosphorylation and Bcl-2 expression. Biochem Pharmacol. 2000; 60(3), 307–315.
42. **Lin H. H., Huang H. P., Huang C. C., Chen J. H., Wang C. J.** Hibiscus polyphenol-rich extract induces apoptosis in human gastric carcinoma cells via p53 phosphorylation and p38 MAPK/FasL cascade pathway. Mol Carcinog. 2005; 43(2), 86–99.
43. **Olvera-García V., Castaño-Tostado E., Rezendiz-Lopez R. I., Reynoso-Camacho R., González de Mejía E., Elizondo G., Loarca-Piña G.** Hibiscus sabdariffa L. extracts inhibit the mutagenicity in microsuspension assay and the proliferation of HeLa cells. J Food Sci. 2008; 73(5), T75–81.
44. **Saeed I. A., Ali L., Jabeen A., Khasawneh M., Rizvi T. A., Ashraf S. S.** Estrogenic activities of ten medicinal herbs from the Middle East. J Chromatogr Sci. 2013; 51(1), 33–39.
45. **Lin H. H., Chan K. C., Sheu J. Y., Hsuan S. W., Wang C. J., Cheng J. H.** Hibiscus sabdariffa leaf induces apoptosis of human prostate cancer cells in vitro and in vivo. Food Chemistry 2012; 132(2), 880–891.
46. **Chiu C. T., Hsuan S. W., Lin H. H., Hsu C. C., Chou F. P., Chen J. H.** Hibiscus sabdariffa Leaf Polyphenolic Extract Induces Human Melanoma Cell Death, Apoptosis, and Autophagy. J Food Sci. 2015. doi:10.1111/1750-3841.12790.