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Nutritional quality and health benefits of roselle calyces

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Abstract. Roselle calyces (*Hibiscus sabdariffa* L.) were evaluated through a critical study of existing research works on health benefits, mineral compositions, bioactive compositions, mechanisms, and possible research gaps. The use of roselle calyces as an alternative to synthetic food dyes, addressing growing global challenges of overweight, obesity, and cardiovascular diseases, was evaluated and encouraged. Studies indicate the attenuation of obesity by chlorogenic acid (the predominant phenolic compound in roselle calyx) via mechanisms associated with the UCP-1 and PGC-1α pathways, resulting in reduced blood lipid levels, reduced fat accumulation in the liver, and increased thermogenesis through fat metabolism. Minimum inhibitory concentration (MIC) of known bacteria and fungi, such as *Listeria monocytogenes, Escherichia coli, Bacillus cereus, Salmonella typhimurium, Candida tropicalis*, and *Candida krusei*, were studied. More research, however, needs to be conducted on organic acids present in roselle calyces to look into their possible applications and maximize their possible benefits.

Keywords and phrases: oxidative stress, mechanism, weight control, antimicrobial, anthocyanin

1. Introduction

Grown predominantly for its calyx, *Hibiscus sabdariffa* L. belongs to the Malvaceae family and is widely distributed around the subtropics and the tropical hemisphere due to its ability to thrive on a relatively wide range of soil conditions (*Riaz et al.*, 2021). It is a good source of ascorbic acid, anthocyanins (mostly delphinidin-3-sambubioside and cyanidin-3-sambubioside), organic acids, phenolic compounds, as well as mineral constituents such as calcium, iron,

magnesium, and potassium. Hence, it could prove effective in treating ailments resulting from mineral deficiencies such as hypocupremia, hypomagnesemia, and anaemia (*Pham et al.*, 2014; *Shruthi & Ramachandra*, 2019).

Roselle calyx possesses antioxidant, anti-hypertensive, anti-microbial, anti-proliferative, and anti-hyperglycaemic properties (*Banwo et al.*, 2022; *Puro et al.*, 2017). Its suitability in beverage, colorant, and wine production (*Alobo & Offonry*, 2009; *Reddy et al.*, 2022) has significantly contributed to increased demand for roselle globally.

Roselle extract is often seen as a drink for the economically disadvantaged, resulting in disparity between a number of consumers in rural and urban areas, particularly in developing countries, prompting population perspective of the health benefits of roselle calyx and its extract as inflated or exaggerated.

This review was designed with the aim of evaluating existing research findings on the nutritional quality, antimicrobial potency, and health benefits of roselle calyx.

2. Methodology

This review was carried out without restriction to the year of article publication. Keywords relevant to this topic were used to search for relevant articles on academic research sources such as Google Scholar, ResearchGate, PubMed, and ScienceDirect. A holistic approach was taken to carefully evaluate research findings, identify research gaps, and provide valuable suggestions.

3. Results and discussion

Nutritional and bioactive composition of roselle calyces

Data obtained on the mineral and proximate composition of roselle calyces based on multiple research findings, as shown in *tables 1–2*, highlight their nutritional benefits. 100 grams of roselle calyx would sufficiently satisfy the recommended dietary allowance (RDA) for calcium, iron, manganese, and copper.

A high concentration of iron in roselle calyces indicates that it can be used in the treatment of anaemia, a condition in which the blood lacks sufficient haemoglobin to transport oxygen to parts of the body where it is needed, resulting in reduced physical capabilities and cognitive decline in humans (*Camaschella*, 2019; *Clark*, 2008). Low crude lipids (0.46–2.01%), high protein content (4.71–8.31%), carbohydrates (68.75–69.62%), and crude fibre (4.68–11.53%) were observed in roselle calyx, the latter of which helps to ease bowel movement, keep the digestive system clean, prevent overfeeding, and flush out carcinogens (*Barber et al.*, 2020).

Table 1. Mineral composition of roselle calyx

Elements	Concentration (mg/100g)	RD	A (mg)	Reference
		Male	Female	
Calcium (Ca)	1,583	1000	1000	Babalola et al., 2001
	2,105.78			Riaz et al., 2021
	912.15			Abou-Arab et al., 2011
Potassium (K)	2,060	- 4700 -	4700	Babalola et al., 2001
	1,263			Riaz et al., 2021
	20.60			Abou-Arab et al., 2011
	316	400	310	Babalola et al., 2001
Magnesium (Mg)	280.12			Riaz et al., 2021
(1715)	315.21			Abou-Arab et al., 2011
Sodium (Na)	5.50	- 1500 -		Babalola et al., 2001
	7.74		1500	Riaz et al., 2021
	6.62			Abou-Arab et al., 2011
Iron (Fe)	37.80	- 8 -	18	Babalola et al., 2001
	21.11			Riaz et al., 2021
	37.80			Abou-Arab et al., 2011
Zinc (Zn)	6.5	- 11	8	Babalola et al., 2001
	5.73			Riaz et al., 2021
	6.51			Abou-Arab et al., 2011
Manganese	2.24	- 2.3	1.8	Riaz et al., 2021
(Mn)	2.39			Abou-Arab et al., 2011
Copper (Cu)	3.68	- 0.9	0.9	Riaz et al., 2021
	4.32			Abou-Arab et al., 2011

Note: RDA = recommended dietary allowance for adults.

	Value (%)	Reference	
Moisture	10.50	Balarabe, 2019	
_	7.60	Adanlawo & Ajibade, 2006	
	9.22	Cid-Ortega & Guerrero-Beltran, 2014	
Ash	5.69	Puro et al., 2017	
	11.67	Balarabe, 2019	
	12.24	Adanlawo & Ajibade, 2006	
Proteins	4.10	Balarabe, 2019	
_	7.51	Abou-Arab et al., 2011	
	4.71	Adanlawo & Ajibade, 2006	
Carbohydrate	68.75	Adanlawo & Ajibade, 2006	
_	69.62	Abou-Arab et al., 2011	
Crude fibre	11.53	Puro et al., 2017	
_	11.17	Abou-Arab et al., 2011	
	4.69	Adanlawo & Ajibade, 2006	
Crude lipid	1.0	Balarabe, 2019	
-	2.01	Adanlawo & Ajibade, 2006	
_	0.46	Abou-Arab et al., 2011	

Table 2. Proximate composition of roselle calyx

While the nutraceutical and therapeutic benefits of roselle calyces have largely been attributed to their high anthocyanin concentration (*Table 3*), several studies have proven that this is not completely true. Some research has attributed health benefits such as weight loss and blood pressure regulation to the mineral content, proximate composition, and presence of beneficial organic acids in the calyces of roselle (*Morales-Luna et al.*, 2019).

Roselle calyx was found to contain phenolic compounds such as catechin, caffeic acid, rutin, gallic acid, cinamic acid, chlorogenic acid, and benzoic acid, some of which have been proven to possess anti-inflammatory, anti-hypertensive, hypocholesterolemic, anti-diabetic, anti-hyperglycemic, and anti-microbial properties (*Banwo et al.*, 2022). Research conducted by *Olthof et al.* (2001) on 7 subjects indicates 33% and 95% absorption of chlorogenic acid and caffeic acid, respectively, consumed by the human body.

Anthocyanins in roselle calyx

The characteristic reddish/purplish coloration of roselle calyx exists due to the presence of anthocyanins such as delphinidin-3-sambubioside, cyanidine-3-sambubioside, delphinidin-3-glucoside, and cyanidine-3-glucoside (*Wu et al.*, 2018).

		5	
	Unit	Value	Reference
Total phenolic	mg GAE/g	37.42	Abou-Arab et al., 2011
	mg GAE/g	38.58	Riaz et al., 2021
	mg GAE/g	41.07	Sirag et al., 2014
Anthocyanin	mg/100g	635.86	Riaz et al., 2021
	mg/100g	565	Abdel-Moemin, 2016
	mg/100g	80.1	Puro et al., 2017
Ascorbic acid	mg/100g	63.5	Babalola et al., 2001
	mg/100g	140.13	Abou-Arab et al., 2011

Table 3. Concentration of ascorbic acid, anthocyanin, and total phenolic content of roselle calyx

This compound can be harnessed as an alternative to synthetic food dyes, the latter of which may pose negative effects such as hyperactivity, allergies, asthmatic reactions, and possibly carcinogenic effects in humans (*Abdel-Moemin*, 2016).

Research conducted by *Hernández-Nava et al.* (2023) and *Abdel-Moemin* (2016) on the use of roselle calyx in the production of biscuits and cupcakes, respectively, reported on colour difference resulting from the incorporation of roselle calyx. Both products obtained good sensory evaluation and were proven to have significantly improved the anthocyanin content and antioxidant quality of the products as compared to the respective control samples, thereby making for a healthier diet. Anthocyanin at a concentration of 3 mg/mL exhibited cytotoxicity towards leukaemia HL-60 cells in a dose- and time-dependent manner through the activation of c-Jun and P38 MAP kinases, triggering Bcl-2 activation, thereby resulting in the induced apoptosis of HL-60 cells (*Chang et al.*, 2005).

Antimicrobial properties of roselle calyx

Antimicrobial resistance has increasingly become a threat to development, global health, and food security. Reduced effectiveness of antibiotics on infections ranging from mild to life-threatening occurs naturally or from the inappropriate use of antibiotics, resulting in increased medical costs and mortality rates (*WHO*, 2020). This mounting challenge has prompted researchers to explore alternative pathways for combating bacterial infections.

Microbes exist in our environment under various conditions and can contaminate foods at any stage of production, resulting in reduced shelf life of agricultural produce and, in some cases, can have adverse effects on the health of consumers (*Gonelimali et al.*, 2018). Research findings, as indicated in *Table 4*, highlight the

inhibitory ability of roselle calyx extract against bacteria associated with food contamination and spoilage such as *B. cereus, E. coli, S. typhimurium,* and *P. aeruginosa*.

Table 4. Minimum inhibitory concentration (MIC) of roselle calyx extract against bacteria and fungi

	Species	Calyx extract	MIC	Reference
Bacteria	S. aureus	Aqueous	2.342 mg/mL	Hamrita et al., 2022
		Methanol	2.342 mg/mL	Hamrita et al., 2022
		Aqueous	112 μg/mL	Chao & Yin, 2009
		Ethanol	72 μg/mL	Chao & Yin, 2009
	L. monocytogenes	Aqueous	136 μg/mL	Chao & Yin, 2009
		Ethanol	84 μg/mL	Chao & Yin, 2009
	P. aeruginosa	Aqueous	9.375 mg/mL	Hamrita et al., 2022
		Methanol	2.342 mg/mL	Hamrita et al., 2022
	E. coli	Aqueous	128 μg/mL	Chao & Yin, 2009
		Ethanol	72 μg/mL	Chao & Yin, 2009
	B. cereus	Aqueous	144 μg/mL	Chao & Yin, 2009
		Ethanol	96 μg/mL	Chao & Yin, 2009
	S. typhimurium	Aqueous	120 μg/mL	Chao & Yin, 2009
		Ethanol	80 μg/mL	Chao & Yin, 2009
Fungi	Candida tropicalis	Aqueous	9.375 mg/mL	Hamrita et al., 2022
		Methanol	9.375 mg/mL	Hamrita et al., 2022
	Candida krusei	Aqueous	9.375 mg/mL	Hamrita et al., 2022
		Methanol	9.375 mg/mL	Hamrita et al., 2022

Note: MIC = minimum inhibitory concentration.

Anti-hypertensive properties of roselle calyx

Hypertension has greatly affected morbidity and mortality rates globally, with a prevalence of 20–30% observed in developing countries, especially among the older generation (*Holm et al.*, 2006).

A study by Herrera-Arellano et al. (2004) on the use of roselle calyx as a remedy for hypertension indicated a significant decrease in systolic (139.05 to 123.73 mmHg) and diastolic (90.81 to 79.52 mmHg) blood pressure in hypertensive test subjects (30–80 years) orally administered 10 g/0.5L of Hibiscus sabdariffa extract

daily as treatment for a period of four (4) weeks after abstaining from other forms of hypertensive treatment four (4) weeks prior to the study.

Aliyu et al. (2014) obtained similar results: orally administered 15 mg/kg of Hibiscus sabdariffa extract effected the attenuation of the sympathetic nervous system. Anti-hypertensive properties exhibited by roselle calyx can also be attributed to mechanisms associated with the inhibition of angiotensin-converting enzymes (ACE) by anthocyanin, resulting in reduced serum sodium concentration without compromising potassium levels in a dose-dependent manner (*Ojeda et al.*, 2010). Anthocyanin prevents free radical oxidation by donating protons, which aids in the regeneration of acyl-glycerol molecules (*Reis et al.*, 2016), thus reducing the risk of hypertension by preventing damage to the endothelium responsible for maintaining balance between vasoconstriction and vasodilatation (*Grossman*, 2008).

Weight control

Overweight and obesity have been concomitant with a series of health issues ranging from diabetes, musculoskeletal disorders, cardiovascular diseases, and some cases of cancer. According to the WHO, 39% of the world's population 18 years of age and older in 2016 (1.9 billion people) were overweight, among which 650 million were obese. The obese or overweight population of adolescents and children in the same year (2016) within the age range of 5 and 19 years was 340 million (*WHO*, 2021).

The disturbing increase in cases of overweight and obesity could in large part be attributed to the consumption of high-calorie diets and physical inactivity, prompting the need for low-calorie diets. The inhibitory activity of roselle calyx extract against porcine pancreatic α -amylase (PPA) and ATP-citrate lyase, as observed by $Hansawasdi\ et\ al.$ (2000), indicates that the consumption of roselle calyx could prove to be an effective method of reducing glucose absorption in the body.

Studies indicate that obesity can be attenuated by chlorogenic acid (the predominant phenolic compound in roselle calyx) via mechanisms associated with the UCP-1 and PGC-1 α pathways, resulting in reduced blood lipid levels, reduced fat accumulation in the liver, and increased thermogenesis through fat metabolism (*Zhong et al.*, 2020).

Antioxidant activities of roselle calyx

Antioxidants are vital to humans, as they are known to prevent the oxidation of body metabolites by removing reactive oxygen species (*Brantley & Sternberg*, 2012). Reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as singlet oxygen (${}^{1}O_{2}$), superoxide radicals (O_{2} -), hydroxyl radicals (OH), hydrogen

peroxide (H_2O_2), nitric oxide (NO^2), and nitrogen dioxide (NO_2) in the body, could lead to the quick build-up of oxidative stress, a phenomenon resulting from an imbalance of production and accumulation of ROS and RNS in cells and tissues (*Pizzino et al.*, 2017).

Accumulation of free radicals in the body could result in health problems such as cancer, inflammatory and cardiovascular diseases, cataract and neurodegenerative diseases, and brain aging (*Lobo et al.*, 2010).

4. Conclusions

Consumption of roselle calyces is encouraged, as they provide a good percentage of the recommended daily mineral intake and have proven to be a cost-effective means of addressing lots of health-related issues. This review highlights the accomplishment of in-vivo studies on the anti-hypertensive effectiveness of roselle calyx extract. However, studies on the apoptosis of cancer cells by Hibiscus sabdariffa extract have been limited to in-vitro analysis; there is a need to scientifically establish the effectiveness of roselle extract on cancer patients. Few detailed studies exist on the potential application of organic acids present in roselle calyx and their possible role in addressing health-related issues.

Roselle calyx extract inhibitory effect against *S. aureus*, *L. monocytogenes*, *P. aeruginosa*, *E. coli*, *B. cereus*, *S. typhimurium*, *Candida tropicalis*, and *Candida krusei* in time- and dose-dependent manner highlights the need for more research to be conducted on the incorporation of roselle calyces as a cost-effective method for improving the shelf life of food products as compared to controls.

References

- [1] Abdel-Moemin, A. R., Effect of Roselle calyces extract on the chemical and sensory properties of functional cupcakes. *Food Science and Human Wellness*, 5. 4. (2016) 230–237. https://doi.org/10.1016/j.fshw.2016.07.003.
- [2] Abou-Arab, A. A., Abu-Salem, F. M., Abou-Arab, E., Physico-chemical properties of natural pigments (anthocyanin) extracted from Roselle calyces (*Hibiscus subdariffa*). *Journal of American Science*, 7. 7. (2011) 445–456.
- [3] Adanlawo, I. G., Ajibade, V. A., Nutritive value of the two varieties of Roselle (*Hibiscus sabdariffa*) calyces soaked with wood ash. *Pakistan Journal of Nutrition*, 5. 6. (2006) 555–557. https://doi.org/10.3923/pjn.2006.555.557.

- [4] Aliyu, B., Oyeniyi, Y. J., Mojiminiyi, F. B. O., Isezuo, S. A., Alada, A. R. A., The aqueous calyx extract of *Hibiscus sabdariffa* lowers blood pressure and heart rate via sympathetic nervous system dependent mechanisms. *Nigerian Journal of Physiological Sciences*, 29. 2. (2014) 131–136.
- [5] Alobo, A. P., Offonry, S. U., Characteristics of coloured wine produced from roselle (*Hibiscus sabdariffa*) calyx extract. *Journal of the Institute of Brewing*, 115. 2. (2009) 91–94. https://doi.org/10.1002/j.2050-0416.2009.tb00351.x.
- [6] Babalola, S. O., Babalola, A. O., Aworh, O. C., Compositional attributes of the calyces of Roselle (*Hibiscus sabdariffa* L.). *Journal of Food Technology in Africa*, 6. 4. (2001) 133–134. https://doi.org/10.4314/jfta.v6i4.19306.
- [7] Balarabe, M. A., Nutritional analysis of *Hibiscus sabdariffa* L. (Roselle) leaves and calyces. *Plant*, 7. 4. (2019) 62–65. https://doi.org/10.11648/j. plant.20190704.11.
- [8] Banwo, K., Sanni, A., Sarkar, D., Ale, O., Shetty, K., Phenolics-linked antioxidant and anti-hyperglycemic properties of edible Roselle (*Hibiscus sabdariffa* Linn.) calyces targeting type 2 diabetes nutraceutical benefits in vitro. Frontiers in Sustainable Food Systems, 6. (2022) https://doi.org/10.3389/ fsufs.2022.660831.
- [9] Barber, T. M., Kabisch, S., Pfeiffer, A. F. H., Weickert, M. O., The health benefits of dietary fibre. *Nutrients*, 12. 10. (2020) 3209. https://doi.org/10.3390/ nu12103209.
- Brantley, M. A., Sternberg, P., Mechanisms of oxidative stress in retinal injury.
 In: Ryan, S. J. et al. (eds.), *Retina*, 5th ed., vol. 1, ch. 22. (2012) 517–528.
 https://doi.org/10.1016/B978-1-4557-0737-9.00022-9.
- [11] Camaschella, C., Iron deficiency. *Blood*, 133. 1. (2019) 30–39. https://doi.org/10.1182/blood-2018-05-815944.
- [12] 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. *Toxicology and Applied Pharmacology*, 205. 3. (2005) 201–212. https://doi.org/10.1016/j.taap.2004.10.014.

- [13] Chao, C. Y., Yin, M. C., Antibacterial effects of roselle calyx extracts and protocatechuic acid in ground beef and apple juice. *Foodborne Pathogens and Disease*, 6. 2. (2009). https://doi.org/10.1089/fpd.2008.0187.
- [14] Cid-Ortega, S., Guerrero-Beltran, J. A., Roselle calyces particle size effect on the physicochemical and phytochemicals characteristics. *Journal of Food Research*, 3. 5. (2014) 83–95. https://doi.org/10.5539/jfr.v3n5p83.
- [15] Clark, S. F., Iron deficiency anemia. *Nutrition in Clinical Practice*, 23. 2. (2008) 128–141. https://doi.org/10.1177/0884533608314536.
- [16] Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., Hatab, S. R., Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Frontiers in Microbiology, 9. (2018) https://doi.org/10.3389/fmicb.2018.01639.
- [17] Grossman, E., Does increased oxidative stress cause hypertension? *Diabetes Care*, 31. 2. (2008) S185–S189. https://doi.org/10.2337/dc08-s246.
- [18] Hamrita, B., Emira, N., Papetti, A., Badraoui, R., Bouslama, L., Ben Tekfa, M.-I., Hamdi, A., Patel, M., Elasbali, A. M., Adnan, M., Ashraf, S. A., Snoussi, M., Phytochemical analysis, antioxidant, antimicrobial, and anti-swarming properties of *Hibiscus sabdariffa* L. calyx extracts: *In vitro* and *in silico* modelling approaches. *Evidence-Based Complementary and Alternative Medicine*, Special issue. (2022) https://doi.org/10.1155/2022/1252672.
- [19] Hansawasdi, C., Kawabata, J., Kasai, T., Alpha-amylase inhibitors from roselle (Hibiscus sabdariffa Linn.) tea. Bioscience, Biotechnology and Biochemistry, 64. 5. (2000) 1041–1043. https://doi.org/10.1271/bbb.64.1041.
- [20] Hernández-Nava, R. G., Anaya-Tacuba, J. D., Sánchez-Mundo, M. de la L., García-Barrientos, R., Flores-Castro, A., Suárez-Rodríguez, C. del P., Espinosa-Solis, V., Use of Roselle calyx wastes for the enrichment of biscuits: An approach to improve their functionality. *Processes*, 11. 1. (2023) 287. https://doi.org/10.3390/pr11010287.
- [21] Herrera-Arellano, A., Flores-Romero, S., Chávez-Soto, M. A., Tortoriello, J., Effectiveness and tolerability of a standardized extract from *Hibiscus sabdariffa* in patients with mild to moderate hypertension: A controlled and randomized clinical trial. *Phytomedicine*, 11. 5. (2004) 375–382. https://doi.org/10.1016/j.phymed.2004.04.001.

- [22] Holm, S. W., Cunningham, L. L., Bensadoun, E., Madsen, M. J., Hypertension: Classification, pathophysiology, and management during outpatient sedation and local anesthesia. *Journal of Oral and Maxillofacial Surgery*, 64. 1. (2006) 111–121. https://doi.org/10.1016/j.joms.2005.09.023.
- [23] Lobo, V., Patil, A., Phatak, A., Chandra, N., Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4. 8. (2010) 118–126. https://doi.org/10.4103/0973-7847.70902.
- [24] Morales-Luna, E., Pérez-Ramírez, I. F., Salgado, L. M., Castaño-Tostado, E., Gómez-Aldapa, C. A., Reynoso-Camacho, R., The main beneficial effect of roselle (*Hibiscus sabdariffa*) on obesity is not only related to its anthocyanin content. *Journal of the Science of Food and Agriculture*, 99. 2. (2019) 596–605. https://doi.org/10.1002/jsfa.9220.
- [25] Ojeda, D., Jiménez-Ferrer, E., Zamilpa, A., Herrera-Arellano, A., Tortoriello, J., Alvarez, L., Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin- and cyanidin-3-O-sambubiosides from *Hibiscus sabdariffa. Journal of Ethnopharmacology*, 127. 1. (2010) 7–10. https://doi.org/10.1016/j.jep.2009.09.059.
- [26] Olthof, M. R., Hollman, P. C. H., Katan, M. B., Chlorogenic acid and caffeic acid are absorbed in humans. *Journal of Nutrition*, 131. 1. (2001) 66–71. https://doi.org/10.1093/jn/131.1.66.
- [27] Pham, P., Pham, P. A., Pham, S., Pham, P. T., Pham, P. M., Pham, P. T., Hypo-magnesemia: A clinical perspective. *International Journal of Nephrology and Renovascular Disease*, 7. (2014) 219–230. https://doi.org/10.2147/IJNRD. S42054.
- [28] Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., Bitto, A., Oxidative stress: Harms and benefits for human health. In: Oxidative Medicine and Cellular Longevity, vol. 2017, Special issue. (2017) https://doi.org/10.1155/2017/8416763.
- [29] Puro, K., Aochen, C., Ghatak, S., Das, S., Sanjukta, R., Mahapatra, K. P., Jha, A. K., Shakuntala, I., Sen, A., Studies on the therapeutic properties of Roselle (*Hibiscus sabdariffa*) calyx: A popular ingredient in the cuisine of North East India. *International Journal of Food Science and Nutrition*, 2. 5. (2017) 1–6.

- [30] Reddy, M., Mushrif, S. K., Manjula, G. S., Krishna, H.C., Shankarappa, T. H., Standardization of anthocyanin extraction from Roselle (*Hibiscus sabdariffa* L.) calyces for edible colour. *The Pharma Innovation Journal*, 11. 3. (2022) 1337–1342.
- [31] Reis, J. F., Monteiro, V. V. S., Souza Gomes, R., Carmo, M. M., Costa, G. V., Ribera, P. C., Monteiro, M. C., Action mechanism and cardiovascular effect of anthocyanins: A systematic review of animal and human studies. *Journal* of *Translational Medicine*, 14. 1. (2016) 315. https://doi.org/10.1186/s12967-016-1076-5.
- [32] Riaz, G., Naik, S. N., Garg, M., Chopra, R., Phytochemical composition of an underutilized plant sorrel/Roselle (*Hibiscus Sabdariffa* L.) cultivated in India. *Letters in Applied NanoBioScience*, 10. 2. (2021) 2138–2147. https://doi.org/10.33263/LIANBS102.21382147.
- [33] Shruthi, V. H., Ramachandra, C. T., Roselle (Hibiscus sabdariffa L.) calyces: A potential source of natural color and its health benefits. In: Sankar Chandra, D. et al. (eds.), Food Bioactives, 1st ed. Apple Academic Press, New York. (2019) 22. https://doi.org/10.1201/9780429242793-8.
- [34] Sirag, N., Elhadi, M. M., Algaili, A. M., Hassan, H. M., Ohaj, M., Determination of total phenolic content and antioxidant activity of Roselle (*Hibiscus sabdariffa* L.) Calyx ethanolic extract. *Standard Research Journal of Pharmacy and Pharmacology*, 1. 2. (2014) 34–39.
- [35] World Health Organization (WHO), *Antibiotic resistance*. (2020). https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance?
- [36] World Health Organization (WHO), *Obesity and overweight*. (2021). https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight?
- [37] Wu, H. Y., Yang, K. M., Chiang, P. Y., Roselle anthocyanins: Antioxidant properties and stability to heat and pH. *Molecules*, 23. 6. (2018) 1357. https://doi.org/10.3390/molecules23061357.
- [38] Zhong, Y., Ding, Y., Li, L., Ge, M., Ban, G., Yang, H., Dai, J., Zhang, L., Effects and mechanism of chlorogenic acid on weight loss. *Current Pharmaceutical Biotechnology*, 21. 11. (2020) 1099–1106. https://doi.org/10.2174/1389201021666200318124922.