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Research Article

Identification of ascorbic acid and phytochemical profiles of Scotch Bonnet pepper (*Capsicum chinense*) using High-Performance Liquid Chromatography-Ultraviolet Detector (HPLC-UV)

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Abstract
Researchers have discovered that red and green scotch peppers are extremely rich in phytochemicals and that these secondary metabolites are connected to several biological processes in the human body. This study aimed to determine the numerous phytochemicals present in scotch pepper seeds, flesh, and red and green varieties. The samples were pulverized after being air-dried. The phytochemicals and ascorbic acid contents were examined using a High-performance Liquid Chromatography-Ultraviolet detector (HPLC-UV). Twelve phytochemicals and ascorbic acid were identified in the flesh and seed of red scotch pepper, with lycopene having the highest content (83.10 mg/kg), the ascorbic acid content of 8.80 mg/kg; moreover, the seed of red scotch pepper revealed twelve phytochemicals and lycopene being the highest content (75.60 mg/kg) and ascorbic acid content of 6.80 mg/kg, furthermore, fourteen phytochemicals were identified in the flesh of red scotch bonnet pepper with lycopene the highest quantity (86.00 mg/kg) and ascorbic content of 10.60 mg/kg. Twelve phytochemicals were identified in the flesh and seed of green scotch bonnet pepper, lycopene (68.70 mg/kg) being the highest content, ascorbic acid content (6.50 mg/kg), twelve phytochemicals were revealed in the flesh of green pepper and lycopene had highest quantity (76.30 mg/kg), in the seed of green pepper, the lycopene content was 68.70 mg/kg. However, it has been shown that capsaicin, dihydrocapsaicin, and lutein concentrations varied, with red scotch pepper flesh having the highest concentration of capsaicin (7.50 mg/kg). The lycopene and ascorbic acid contents of flesh were the highest (86.00 mg/kg) and (10.60 mg/kg). The study's findings showed that red pepper flesh was better than other components that were looked at.

Keywords: Ascorbic acid, HPLC-UV Detector, Phytochemicals, Scotch Bonnet pepper

INTRODUCTION

The Capsicum genus is the most economically significant one in the *Solanaceae* family (Dong *et al*., 2014). Their fruits, often called peppers or chilli, are bell-shaped and come in various hues, including yellow, green, red, and orange (Dong *et al*., 2014). They have been extensively used as a herb and spice by human civilization, with the earliest reports of usage dating back to 7000 BC, as part of the diet of the Mexican Indians diets (Govindarajan and Sathyanarayana, 1991). With almost 50,000 different types of pepper, the Capsicum genus is one of earth's most widely consumed and grown plants. The chemical composition within the

genus exhibits a large deal of variation because of its extensive diversification. Since pepper has a chemical composition rich in capsaicinoids, carotenoids, flavonoids, and volatile chemicals, which is attributed to the fruit's ability to eliminate insipidity, produce smells, and combat oxidative diseases, its main applications are in the culinary and pharmaceutical industries (Antonio *et al*., 2018). Vegetables are essential to ensure access to food and nourishment (Natesh *et al*., 2017). Due to the vitamins, minerals, phytochemicals, and dietary fiber they contain are crucial for human health (Ulgeret *et al*., 2018). Gender, age, money, education, heritage, and one's state of health all impact how much one consumes vegetables (Giskes *et al*., 2002). Consuming green vegetables is a part of the African culinary tradition, and they are essential to an African family's tradition and food culture (Otitoju *et al*., 2014). Since the era of marine exploration, when various civilizations first began to consume and cultivate capsicum fruits, their production and use have spread across the globe (Dong *et al*., 2014; Garcés-Claver *et al*., 2006). Thanks to the hybridization process, these fruits could adapt to various environmental conditions and become domesticated (Dong *et al*., 2014; Ballard *et al*., 1970; Das *et al*., 2016). Due to their exceptional qualities, which include the fact that they were employed as food spices, colors, and remedies for headaches and stomach problems as early as 1600 BC, it is clear why their popularity grew so quickly (Govindarajan and Sathyanarayana, 1991; Govindarajan, 1985; Salgado-Roman *et al*., 2008). Numerous researchers have been drawn to peppers because of their use in food and medicine, and they are interested in learning more about each of the chemical features associated with peppers. The amount and quality of minerals and phytochemicals in pepper foliage (leaves) may impact the suitability of particular types or species for use in food and medicine (Amaechi *et al.,* 2021). Yet, many like eating the green and red scotch bonnet pepper's seed, flesh, or both without much knowledge of the health advantages of each portion of the ingested pepper. The purpose of this investigation was to identify and quantify the ascorbic acid and the phytochemicals that are present in each part of the seed, flesh and both Scotch Bonnet Pepper (*Capsicum chinense*)

MATERIALS AND METHODS

Sample collection:

One hundred pieces of red and green scotch bonnet peppers *Capsicum chinense*) each was bought in October 2021 at the main market in Ado-Ekiti with herbarium number (UILH/002/1269/2022). The seeds, flesh, and the whole were dried in the air for five days. To obtain powdered Scotch bonnet pepper samples, each color variant was ground using an electric grinder and stored in airtight plastic containers until needed for analysis. The study duration was two months and the study was conducted in the Chemistry Laboratory, Afe Babalola University Ado-Ekiti, Nigeria.

Phytochemical and ascorbic acid determination

Sample for analysis:

The dried powdered flesh, seed, and the flesh and seed of the red and green Scotch bonnet peppers (*Capsicum chinense*) were used for the analysis. HPLC - UV Detector was used for the phytochemical analysis

Extraction of test sample

Ten grams of the samples were measured into the amber bottle. To it was added 20 ml of Acetonitrile, and both were shaken vigorously for 30 minutes. After shaking, the aqueous end was run off and the organic (solvent) was collected into a 25 ml standard flask, made up to the mark, and ready for the analysis.

Analytical method

The standard form of analyte profile was first injected into the HPLC, generating a chromatogram with a given peak area and profile. These were used to create a window in the HPLC in preparation for the test sample analysis. The aliquot of the extracted test sample was injected into the HPLC to obtain a curve pending peak area and profile in a chromatogram. Then the peak area of the sample is compared with that of the standard, relative to the concentration of the standard to obtain the concentration of the sample, the following chromatographic conditions were employed; Agilent Technologies HPLC 1200, Column: uBondapak C18, Carrier: Acetonitrile/Water 70:30 and 5µL injected @ 2ml / min flow rate (Akira *et al*., 2020).

RESULTS AND DISCUSSION

The study's findings have demonstrated the distinction and diversity of the numerous phytochemicals present. Tables 1-3 show that the ascorbic acid content of red scotch bonnet pepper flesh was greatest (10.6 mg/kg), followed by that of the seed (6.80 mg/kg), and the combined content of the flesh and seed (8.80 mg/kg). As indicated in Tables 1-3, the lycopene content of the flesh of the red scotch bonnet pepper (86.00 mg/kg) was the greatest, followed by that of the flesh and seed (83.10 mg/kg), the seed (75.60 mg/kg) had the least. Tables 1 and 2 show that lutein in the flesh (33.00 mg/kg) was the highest and seed (27.00 mg/kg) was the lowest. The red scotch bonnet pepper's flesh had the highest capsaicin level (7.50 mg/kg), followed by the flesh and seed (6.30 mg/kg) and the seed (3.20 mg/kg). The dihydrocapsaicin content of flesh was the greatest (3.40 mg/kg) based on the findings of Tables 1

Plate 1. *Showing red and green Capsicum chinense*

-3. When compared to seed (13.90 mg/kg) and flesh (30.50 mg/kg), the concentration of delphinidin in the flesh and seed was highest (31.00 mg/kg).

The ascorbic acid concentration (6.50 mg/kg) was the same as per the results of Table 4-6. In addition, the lycopene content of the flesh (76.30 mg/kg) was higher than that of the seed and flesh and seed (68.70 mg/kg), respectively. Compared to the lutein contents of the seed, flesh, and seed (20.80 mg/kg) of the green scotch bonnet pepper, the lutein content of the flesh was the highest (26.00 mg/kg). Capsaicin content in the flesh was high (3.40 mg/kg), and delphinidin content was highest (11.70 mg/kg). The findings showed that the red scotch bonnet pepper's flesh had more capsaicin (7.50 mg/kg) than the green scotch bonnet pepper's (3.40

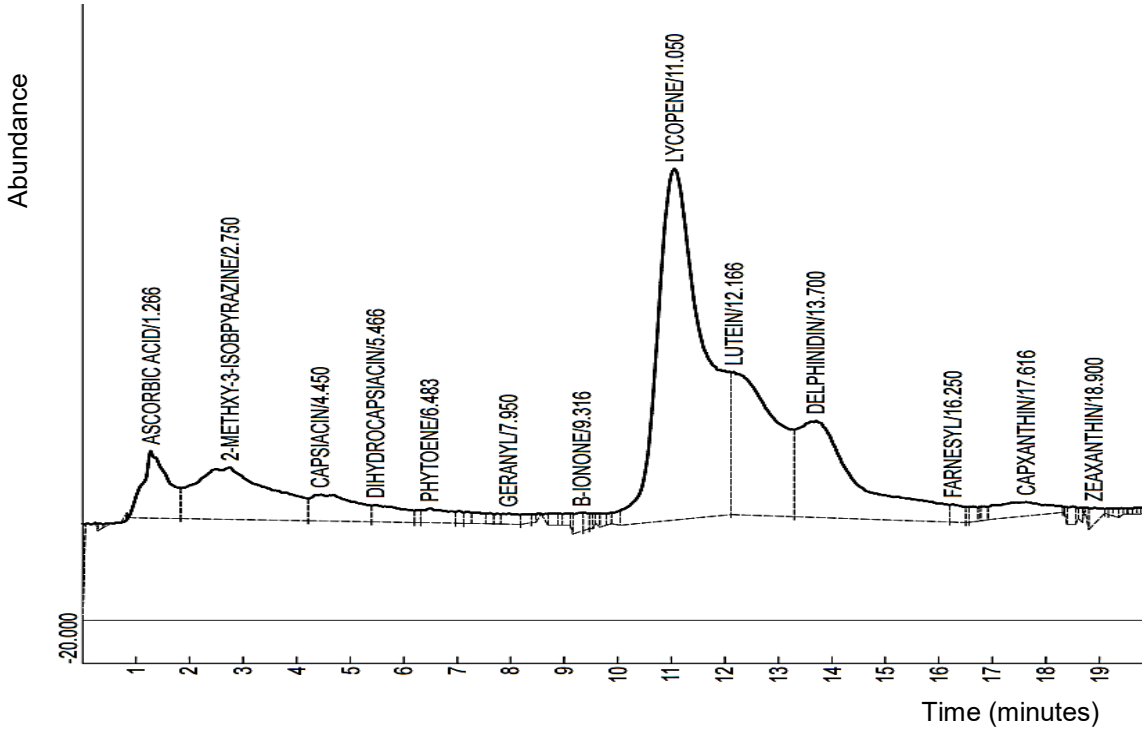

Fig. 1. *Chromatogram of flesh and seed of red scotch bonnet pepper*

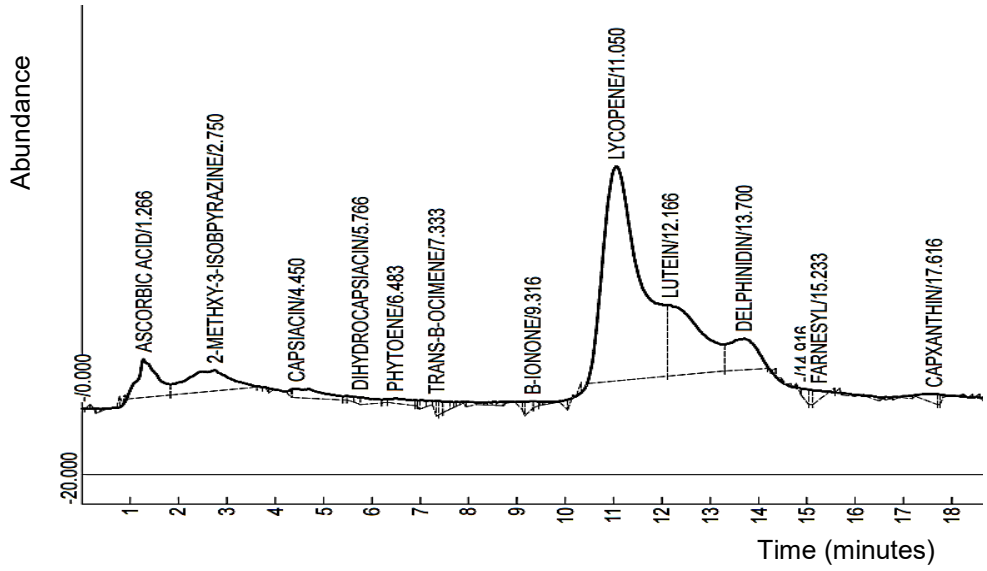

Fig. 2. *Chromatogram of the seed of red scotch bonnet pepper*

Table 1. Identified ascorbic acid and phytochemical contents of flesh and seed of red scotch

Peak no.	Retention time	Area	Height	Compound	Quality (mg/g)	Quantity (mg/10g)	Quantity (mg/kg)
1	1.266	1025.2880	31.073	Ascorbic Acid	0.88	0.088	8.80
				PHYTOCHEMICALS			
2	2.750	2421.1895	24.052	2-Methxy-3-Isobpyrazine	2.08	0.208	20.80
3	4.450	729.8910	12.220	Capsaicin	0.63	0.063	6.30
4	5.466	322.6330	6.659	Dihydrocapsiacin	0.28	0.028	2.80
5	6.483	227.4410	6.659	Phytoene	0.20	0.020	2.00
6	7.950	102.7540	4.902	Geranyl	0.09	0.009	0.90
7	9.316	96.133	8.452	Beta -Ionone	0.08	0.008	0.80
8	11.050	9672.4920	162.614	Lycopene	8.31	0.83	83.10
9	12.166	3754.2900	66.108	Lutein	3.22	0.32	32.00
10	13.700	3577.9430	44.666	Delphinidin	3.07	0.31	31.00
11	16.250	140.1990	8.269	Farnesyl	0.12	0.01	1.00
12	17.616	461.1530	6.467	Capsanthin	0.40	0.04	4.00
13	18.900	108.0220	7.565	Zeaxanthin	0.09	0.01	1.00

Table 2. Identified ascorbic acid and phytochemical contents in the seed of red scotch bonnet

Peak no.	Retention time	Area	Height	Compound	Quality (mg/g)	Quantity (mg/10g)	Quantity (mg/kg)
1	1.266	790.8745	27.015	Ascorbic Acid	0.68	0.068	6.80
				PHYTOCHEMICALS			
2	2.750	1447.9695	18.357	2-Methxy-3-Isobpyrazine	1.24	0.124	12.40
3	4.450	371.4475	7.629	Capsaicin	0.32	0.032	3.20
4	5.466	120.5050	2.363	Dihydrocapsiacin	0.10	0.01	1.00
5	6.483	199.7400	5.168	Phytoene	0.17	0.017	1.70
6	7.333	96.8820	10.326	Trans-Beta-Ocimene	0.08	0.008	0.80
7	9.316	68.9710	5.042	B-Ionone	0.06	0.006	0.60
8	11.050	8798.7480	155.598	Lycopene	7.56	0.756	75.60
9	12.166	3140.8145	59.134	Lutein	2.70	0.27	27.00
10	13.700	1615.1030	32.974	Delphinidin	1.39	0.139	13.90
11	15.233	290.9525	8.860	Farnesyl	0.25	0.025	2.50
12	17.616	259.6055	3.637	Capsanthin	0.22	0.022	2.20

Table 3. Identified ascorbic acid and phytochemical contents in the flesh of red scotch bonnet pepper

Peak no.	Retention time	Area	Height	Compound	Quality (mg/g)	Quantity (mg/10mg)	Quantity (mg/kg)
1	1.266	1194.7065	33.746	Ascorbic Acid	1.06	0.106	10.60
				PHYTOCHEMICALS			
2	2.750	2721.8470	26.244	2-Methxy-3-Isobpyrazine	2.42	0.242	24.20
3	4.450	838.0950	13.860	Capsaicin	0.75	0.075	7.50
4	5.466	380.3530	9.212	Dihydrocapsiacin	0.34	0.034	3.40
5	6.483	263.6720	7.639	Phytoene	0.23	0.023	2.30
6	7.333	90.5950	5.949	Trans-Beta-Ocimene	0.08	0.008	0.80
7	7.950	108.6790	5.407	Geranyl	0.10	0.010	1.00
8	9.316	96.1330	8.452	Beta-Ionone	0.09	0.009	0.90
9	11.050	9647.9400	162.423	Lycopene	8.60	0.860	86.00
10	12.166	3718.1155	65.702	Lutein	3.31	0.331	33.10
11	13.700	3421.1690	43.966	Delphinidin	3.05	0.305	30.50
12	15.233	142.3390	7.080	Farnesyl	0.13	0.013	1.30
13	17.616	703.2400	9.008	Capsanthin	0.63	0.063	6.30
14	18.900	157.3420	9.391	Zeaxanthin	0.14	0.014	1.40

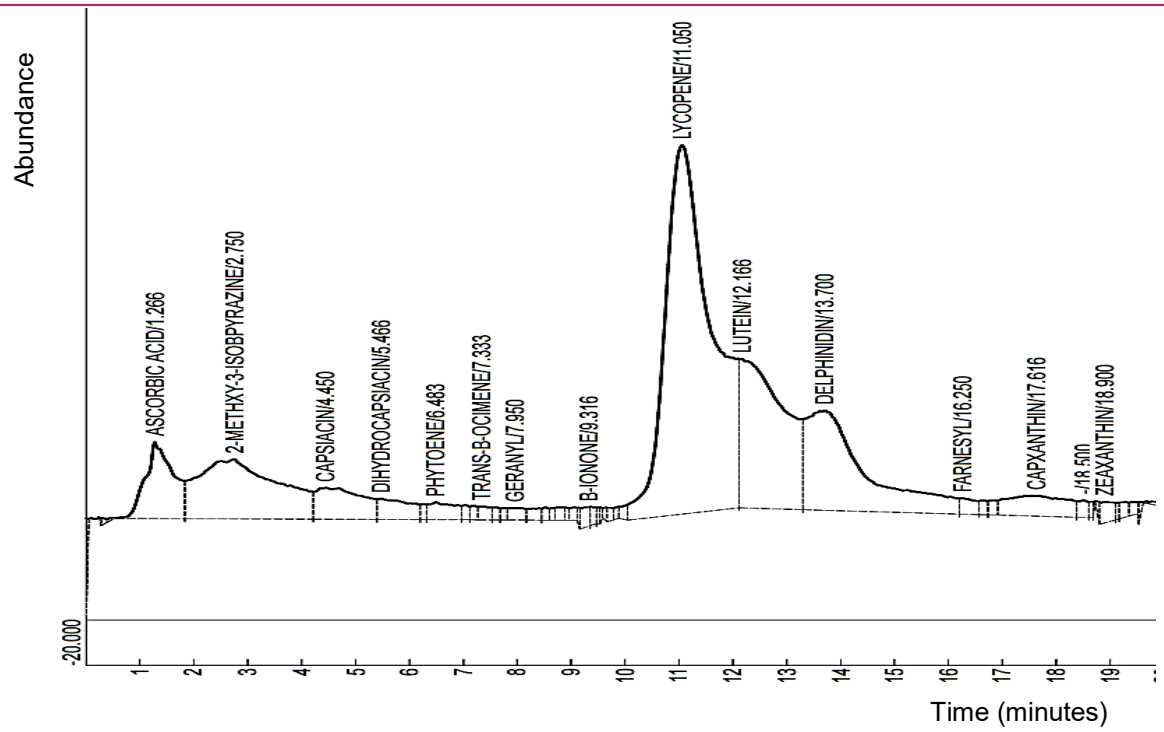

Fig. 3. *Chromatogram of the flesh of red scotch bonnet pepper*

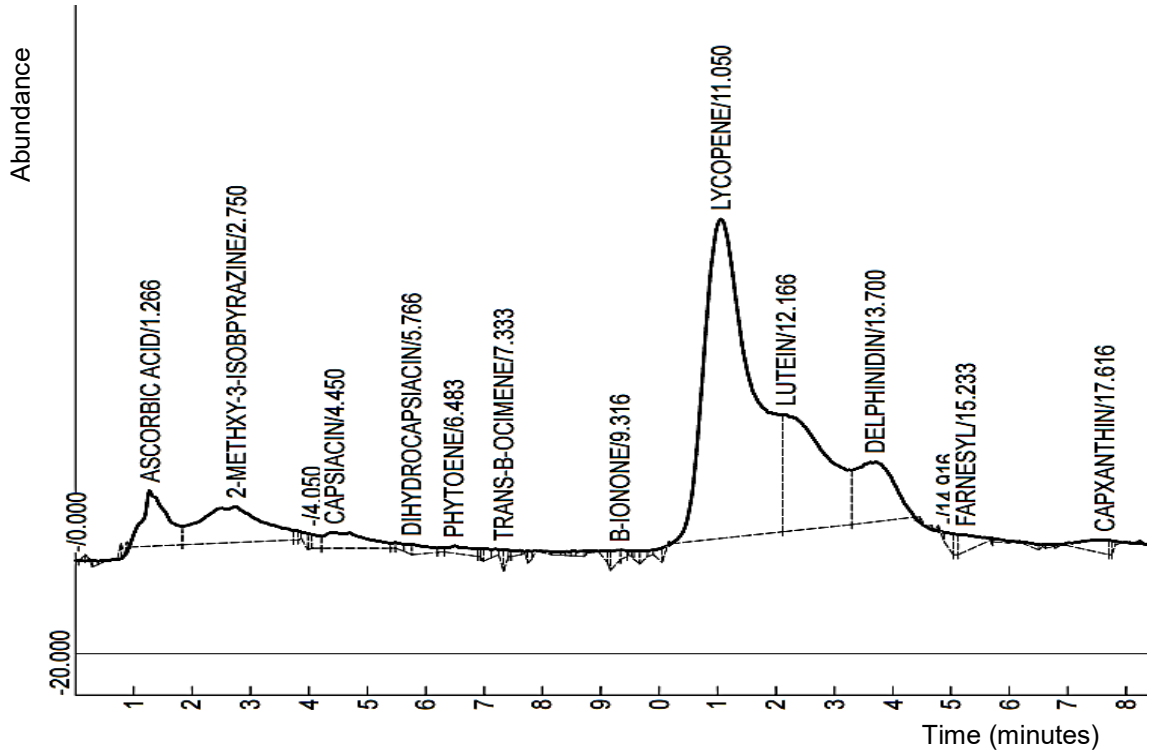

Fig. 4. *Chromatogram of flesh and seed of green scotch bonnet pepper*

mg/kg). The red scotch bonnet pepper's flesh also had more lutein (33.00 mg/kg) than its green counterpart (26.00 mg/kg). Red Scotch Bonnet peppers had more lycopene in their flesh, seeds, and combined portions than green peppers did. Red Scotch Bonnet peppers had more delphinidin in the flesh, seeds, and combined than green ones. The researchers have well discussed the health benefits of some of the identified phytochemicals.

Fattori et al. (2016) reported the usefulness of Capsaicin, the hot-producing chemical in chili peppers, to include an analgesic in topical creams, nasal sprays, and dermal patches to buttress these health benefits. The ratio of capsaicin to Dihydrocapsiacin identified has

Table 4. Identified ascorbic acid and phytochemical contents in the flesh and seed of green scotch bonnet pepper

Peak no.	Retention time	Area	Height	Compound	Quality (%)	Quantity (mg/10 g)	Quantity (mg/kg)
1	1.266	730.9245	26.183	Ascorbic Acid	0.65	0.065	6.50
				Phytochemicals			
2	2.750	923.3350	13.998	2-Methxy-3-Isobpyrazine	0.83	0.083	8.30
3	4.450	282.7915	6.044	Capsaicin	0.25	0.025	2.50
4	5.766	88.3580	4.897	Dihydrocapsiacin	0.08	0.008	0.80
5	6.483	108.0905	3.458	Phytoene	0.10	0.010	1.00
6	7.333	96.8820	10.326	trans-beta-ocimene	0.09	0.009	0.90
7	9.316	68.9710	5.042	Beta-Ionone	0.06	0.006	0.60
8	11.050	7708.5100	145.468	Lycopene	6.87	0.687	68.70
9	12.166	2332.6570	48.160	Lutein	2.08	0.208	20.80
10	13.700	864.6220	21.224	Delphinidin	0.77	0.077	7.70
11	15.233	94.3790	6.728	Farnesyl	0.08	0.008	0.80
12	17.616	111.3025	6.124	Capsanthin	0.10	0.010	1.00

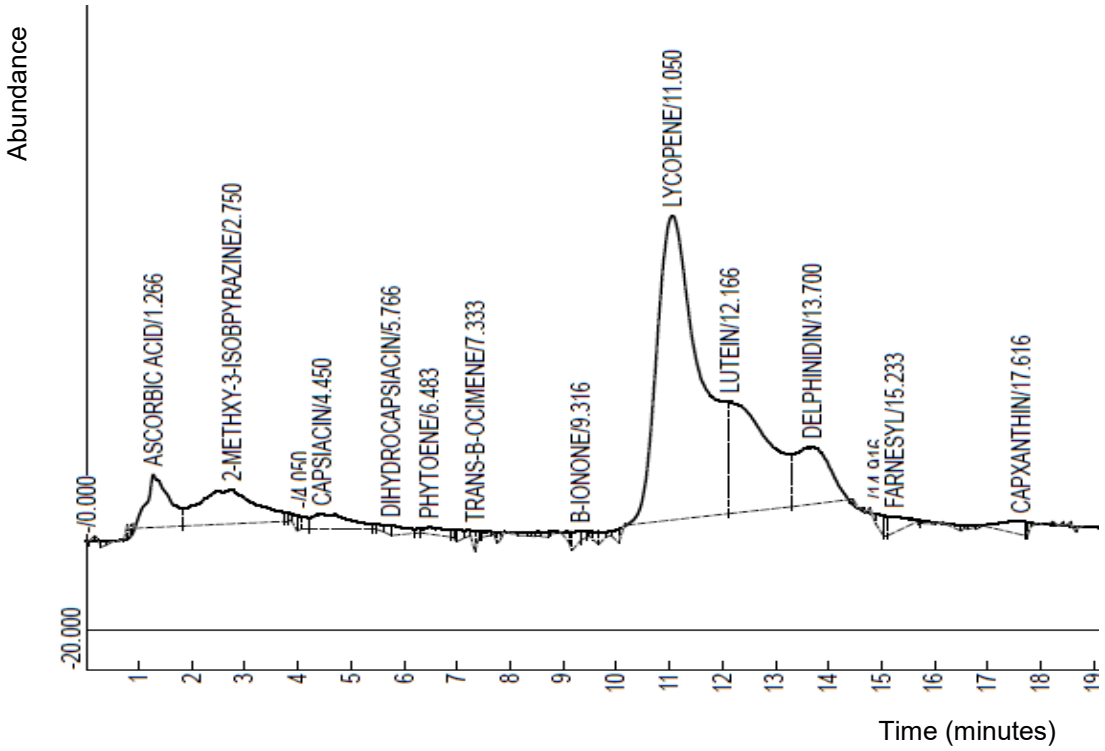

Fig. 5. *Chromatogram of the flesh of green scotch bonnet pepper*

been reported to be 1:1 or 2:1 (Giuffrida *et al.,* 2013; Sricharoen *et al*. 2017; Sweat *et al*., 2016; Liljana *et al*., 2013). Studies have reported the associated health benefit of delphinidin in humans. Patel *et al*. (2013) reported that delphinidin's pharmacological properties include antioxidant, antimutagenic, anti-inflammatory, and antiangiogenic. Delphinidin is said to be an example of Anthocyanins, which are classified as flavonoids. In Asia, notably in China, medicinal plants high in flavonoids are utilized to treat or prevent disease. Numerous investigations have been conducted to understand the molecular mechanisms behind flavonoids' medicinal effects (Liu *et al*., 2011).

Anthocyanin is the primary pigment that gives fruits, vegetables, flowers, and grains their many hues of red, purple, blue, and other colors. They are crucial in preventing various illnesses, including diabetes, cancer, mutagenesis, carcinogenesis, and cardiovascular conditions. Anthocyanins exhibited anti-inflammatory, antiproliferative, antioxidant, and anti-angiogenic activity (Miguel, 2011; Ichiyanagi *et al*., 2004; Vesna and Marica, 2009; Shipp and Abdel-Aal, 2010; Hou *et al*., 2004). Because of their vivid hues, anthocyanins have been utilized in the food sector as natural colorants (Azevedo *et al*., 2007; Ichiyanagi *et al*., 2008). Humans are thought to consume between a few milligrams to hundreds of milligrams of anthocyanins each day, depending on their diet (Miguel, 2011; Ichiyanagi *et al*., 2004; Vesna and Marica, 2009; Shipp and Abdel-Aal, 2010; Hou *et al*., 2004). There are many health benefits associated with the intake of lycopene and many studies have been conducted to uncover its relevance as anti-

Table 5. Identified ascorbic acid and phytochemical contents in the flesh of green scotch bonnet pepper

Peak no.	Retention time	Area	Height	Compound	Quality (%)	Quantity (mg/10g)	Quantity (mg/kg)
1	1.266	776.4370	26.815	Ascorbic Acid	0.69	0.069	6.90
			Phytochemicals				
2	2.750	1308.2580	17.308	2-Methxy-3-Isobpyrazine	1.16	0.116	11.60
3	4.450	382.0620	7.470	Capsaicin	0.34	0.034	3.40
4	5.766	88.3580	4.897	Dihydrocapsiacin	0.08	0.008	0.80
5	6.483	108.0905	3.458	Phytoene	0.10	0.010	1.00
6	7.333	96.8820	10.326	trans-beta-ocimene	0.09	0.009	0.90
7	9.316	68.9710	5.042	Beta-Ionone	0.06	0.006	0.60
8	11.050	8560.6505	153.598	Lycopene	7.63	0.763	76.30
9	12.166	2909.8870	56.290	Lutein	2.60	0.260	26.00
10	13.700	1312.6905	28.830	Delphinidin	1.17	0.117	11.70
11	15.233	179.0700	8.128	Farnesyl	0.16	0.016	1.60
12	17.616	186.3640	6.631	Capsanthin	0.17	0.017	1.70

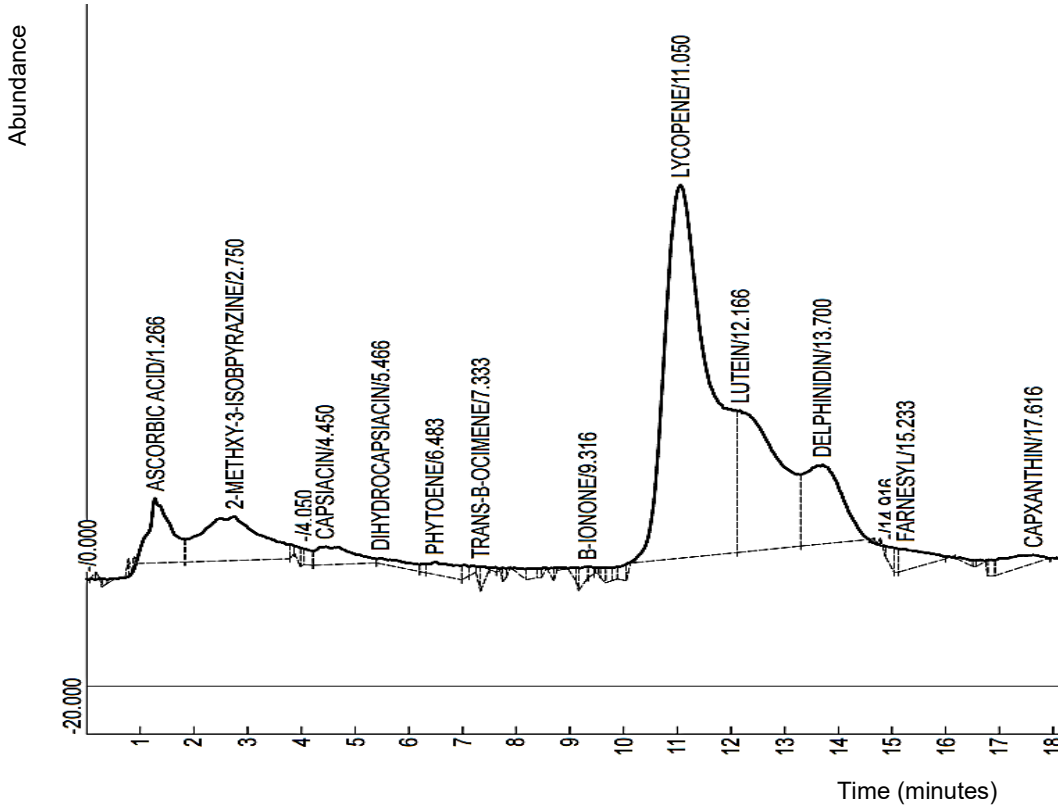

Fig. 6. *Chromatogram of the seed of green scotch bonnet pepper*

oxidant potential, anticancer, antioxidant, cardioprotective, neuroprotective, improves sleeping behaviour, anti-inflammatory, antiplatelet aggregative, and antihypertensive action (Khan *et al*., 2021).

Khan *et al*., 2021 reported that Lycopene is a vital cardioprotective because it may control some crucial processes, including apoptosis and inflammation. Furthermore, dietary lycopene is said to confer cardiovascular benefits as reported by Sesso *et al*. (2003), e.g., consuming at least servings/week of lycopene-based products significantly decreased cardiovascular risk within seven years in postmenopausal women, free from prior cardiovascular disorders and cancer (Sesso *et al*., 2003). Kennedy *et al*. (2021) had reported that the brilliant orange-red pigment called capsanthin, which gives paprika fruits (*Capsicum annuum*) their unusual red color, is a member of the group of carotenoids called xanthophylls. In addition, the high radical scavenging and singlet oxygen quenching properties of capsanthin were due to its distinctive chemical structure, which included a keto group and a lengthy chain of 11 conjugated dienes. Due to its strong antioxidant effect, Cap-

santhin has been shown to have chemopreventive, antitumor, skin photoprotective, anti-inflammatory, and anti-diabetic properties. Some of the more significant properties of capsanthin are its anti-obesity, anti-adipogenic, and antihyperlipidemic properties (Kennedy *et al*., 2021).

Conclusion

The present study concluded that the red Scotch bonnet pepper's flesh was superior to the seed, flesh, and seed of red and green Scotch bonnet peppers because it was particularly rich in ascorbic acid and phytochemicals. The lycopene (86.00 mg/kg) and ascorbic acid (10.60 mg/kg) contents of flesh were the highest. The physiological benefits of this lycopene have been well enumerated, and people who are deficient in this compound can be recommended to take the flesh of the red pepper. Significantly, lycopene from the flesh of red pepper can also be isolated for usage.

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Conflict of interest

The authors declare that they have no conflict of interest.

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