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SPECTROSCOPIC AND CHROMATOGRAPHIC CHARACTERIZATION OF PHYTOCHEMICALS IN *BRASSICA OLERACEA* VAR. CAPITATA F. RUBRA (PURPLE CABBAGE)

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ABSTRACT
The present study on the phytochemical characterization of Brassica oleracea var. capitata f. rubra (Purple Cabbage) using FT-IR and GC–MS analytical techniques revealed the presence of diverse chemical constituents with potential biological significance. The FT-IR analysis identified 25 characteristic absorption peaks within the range of 370.33–3757.33 cm^{-1}, indicating the presence of various functional groups, including hydroxyl, alkane, alkene, aromatic, amine, amide and ether-associated groups. These findings provided preliminary evidence of the complex phytochemical composition of the leaf powder. The GC–MS analysis of the ethanolic extract of Purple cabbage revealed several phytochemical constituents, including phenolic derivatives, organic acids, nitrogen-containing compounds and heterocyclic compounds. Some of the identified compounds were associated with potential antimicrobial, antioxidant, anti-inflammatory and other pharmacological activities based on their reported properties and PASS prediction results. The combined analytical findings highlight the chemical diversity and potential biological importance of Brassica oleracea var. capitata f. rubra.

KEYWORDS: Brassica oleracea var. capitata f. rubra (Purple Cabbage), FT-IR, GC-MS, Phytoconstituents.

INTRODUCTION
Purple Cabbage (Brassica oleracea L. var. capitata) is one of the most important vegetables grown worldwide. It belongs to the family Cruciferae, which includes broccoli, cauliflower, and kale. The different cultivated types of cabbage show great variation in respect of size, shape and colour of leaves as well as the texture of the head (Singh et al., 2006). Purple cabbage or (red cabbage) gets its name from the pigment anthocyanin, which turns the leaves purple or red. The colour of the soil varies depending on the PH level. Acidic soil produces reddish leaves, neutral soil produces purple leaves, and alkaline soil produces greenish yellow cabbage (Manasa et al., 2017). Red cabbage extract contains more than 30 anthocyanins, mainly cyanidin-3-diglucoside-5 glucoside in acylated and non-acylated forms. The acyl groups are mainly p-coumaric, sinapic, and ferulic acids. The primary carotenoids in Brassica species are lutein and β-carotene, but at least 16 carotenoids have been identified. Glucosinolates (GSLs) are sulfur-containing secondary metabolites found mainly in plants of the Brassicaceae family. Sinapic acid and its constituents are the main phenolic acids in various cultivars of both red and white cabbage. (Podsedek et al., 2006). Thomas et al., (2024) focused on the evaluation of proximate, elemental, and phytochemical composition, including the antioxidant and cytotoxic potentials of green and purple cabbage varieties. The findings revealed different percentages of moisture, crude fat, fibre, protein, and carbohydrates in both varieties. Calcium, magnesium, potassium, sodium, iron, copper, zinc, saponins, terpenoids, flavonoids, phenolics, tannins, anthraquinones, and steroids were also detected and quantified. Anthocyanins are natural plant pigments derived from the flavonoid family and their presence in food gives orange, red, purple, and blue colorations. (Oladzadabbasabadi et al., 2022). In the present research, we have studied the phytochemicals in Purple cabbage using GC- MS and the functional groups are characterized using FT-IR analysis. These studies gives the details of quantity of phytoconstituents and their biological property.

MATERIALS AND METHODS
Collection of Sample and Preparation of Brassica oleracea Ethanol Extract
The sample Purple cabbage (Brassica oleracea var. capitata f. rubra). was purchased from the commercial vegetable shops, Nagercoil, Kanyakumari District, Soon after collecting the Purple cabbage, it was washed and cleaned.

The cleaned Purple cabbage was separated, weighed and shade dried for 15 days and after fully dried it was powdered in to fine powder and was stored in the airtight container for further studies. Ethanol Extract of Brassica oleracea was prepared from 10 gm of the Plant powder using Soxhlet Hot Extraction method.

Figure.1. Brassica oleracea var. capitata f. rubra (Purple Cabbage)



FT-IR Analysis

To know the functional groups, the FT-IR analysis was performed using Shimadzu 8400S Spectrophotometer system. Infrared spectra of the test plants were measured using the methods cited in Harborne (1999). The crude extract was measured in an automatic recording IR spectrophotometer (Shimadzu 7600) in the range of 500 to 4000 cm^{-1}. The peak values of the FT-IR were recorded.

Preparation of sample solutions for GC-MS analysis:

Brassica oleracea ethanol extract subjected to GC-MS analysis. The Purple cabbage ethanol extract was centrifuged at 3000 rpm for 10 min and the supernatant was used for further phytochemical analysis.

GC-MS analysis

To reveal the chemical constituents present in the selected Purple cabbage powder ethanol extract the GC-MS analysis was performed using the Clarus 500 GC-MS (Perkin Elmer). 2 µl of selected Purple cabbage powder ethanol extract were injected for GC-MS analysis (Merlin et al., 2009).

Identification of components

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Ver.2.0 - Year 2005 library (Sangeetha and Vijayalakshmi, 2011). Interpretation of GC-MS was conducted using the database of NIST having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components present in the test materials were ascertained. The compound biological activity prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA. The biological activity spectrum of PASS Prediction.

RESULT AND DISCUSSION

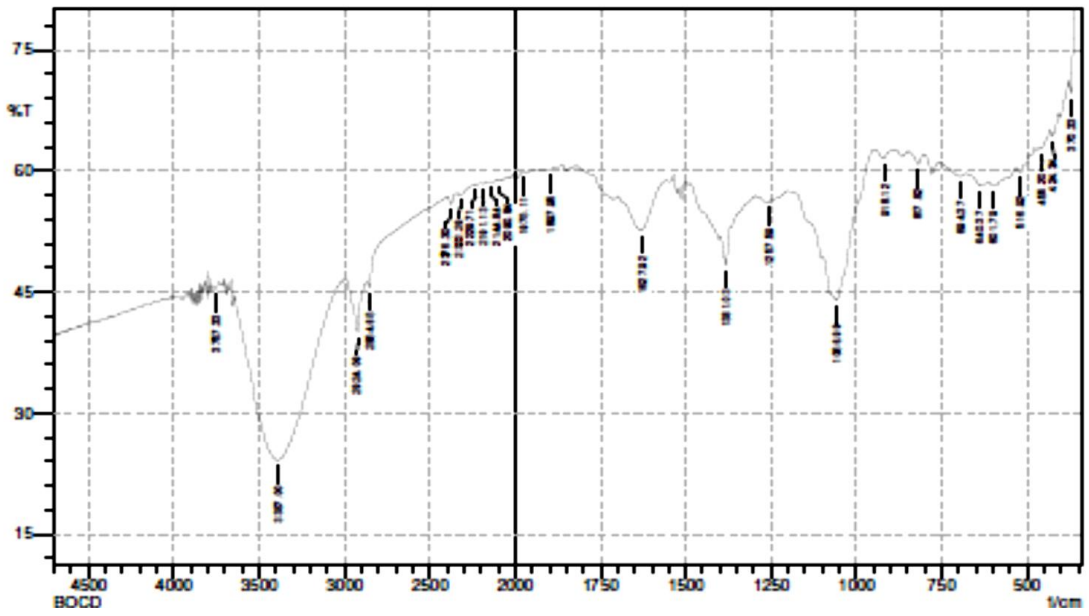
FT-IR ANALYSIS

The FT-IR peak values were used to identify the functional group of the active metabolites present in the Brassica oleracea var. capitata f. rubra powder. The crude powder of Brassica oleracea var. capitata f. rubra powder were passed in to the FT-IR and the functional groups of the components were identified based on its peak ratio and compared with the standard. In the Brassica oleracea var. capitata f. rubra powder, the presence of 25 different peak values ranging from 370.33-3757.33 were observed. The crude powder of Brassica oleracea var. capitata f. rubra showed different peak values are 370.33, 424.34,455.2, 516.92, 601.79, 640.37, 694.37, 817.82, 918.12, 1066.99, 1257.59, 1381.03, 1627.92, 1897.95, 1975.11, 2000.84, 2144.84, 2191.13, 2229.71, 2322.29, 2376.3, 2854.65, 2924.09, 3387 and 3757.33. This confirmed the existence of various functional groups such as iodides, bromides, primary alcohols, secondary alcohols, Alkane, germinal dimethyl, conjugated ethers, azides, aromatic homocyclic comp, alkenes, amido acids, amino acid hydrochlorides, changed amines, amino acids, aldehydes, alkenes, alkanes, alcohols , phenols free OH. The peak values presence confirmed the existence of various functional groups such as iodides (C-Istr), bromides (C-Brstr), Alkane germinal dimethyl esters(C-H def), Amino acid hydrochlorides, Aryl nitrites, alkanes(-CH2-) (C-Hstr), Amino acid hydrochlorides charged amine derivatives(NH3str), R-OH, amido acids C=O str, , alcohols, phenols free OH(O- Hstr). In this higher peak area was observed by Amido acids of showing peak value of 299.038% followed by Alkanes (-CH_2 -) with peak area of 128.757 and the least percentage of peak area was observed by Iodides of 2.694%. Among these functional group Bromide C-Bstr showed higher peak area of 5.882. The results of FT-IR analysis was displayed in Table. 1 and Figure. 2.

Table.1. FT-IR Prediction of types and groups of compounds in Brassica oleracea var. capitata f. rubra Powder

SI NO	PEAK	AREA	TYPES AND GROUPS	BOND
1.	370.33	2.694	Iodides	C- Istr
2.	424.34	8.092	Iodides	C- Istr
3.	455.2	4.557	Iodides	C- Istr
4.	516.92	11.52	Bromide	C-Brstr
5.	601.79	15.979	Bromide	C-Brstr
6.	640.37	12.51	Alkynes	C-H def
7.	694.37	17.136	Halides	C-Cl str
8.	817.82	8.125	Tri substituted alkenes	C-H str
9.	918.12	11.145	Di substituted alkenes	C-H def
10.	1066.99	68.888	Primary alcohols	C-OH str
11.	1257.59	22.841	Aromatic Esters	R-O-Ar str
12.	1381.03	47.543	Alkane, germinal dimethyl	CH_3def
13.	1627.92	33.841	Amino acids, amide I band-	C=C+C=N str
14.	1897.95	8.435	Amido acids	C=O str
15.	1975.11	8.579	Amino acid hydrochlorides	
16.	2000.84	8.857	Amino acid hydrochlorides	
17.	2144.84	10.704	Amino acids	NH_3^+str
18.	2191.13	8.984	Charged Amine derivatives	NH^+vib
19.	2229.71	5.413	Aryl nitrites	C=N str
20.	2322.29	18.465	Charged Amines($C=NH^+$)	NH^+str
21.	2376.3	9.57	Amido acids	
22.	2854.65	128.757	Alkanes (-CH_2 -)	C-H str
23.	2924.09	44.032	Alkanes (-CH_2 -)	C-H str
24.	3387	299.038	Amido acids	N-H str
25.	3757.33	13.11	Alcohols, Phenols, free OH	O-H str

Figure. 2. FT-IR Spectroscopic Graph representing the types and bonds of compounds in Brassica oleracea var. capitata f. rubra Powder



GC-MS ANALYSIS

The results of GC-MS analysis led to the identification of 15 different phytocompounds in the ethanolic extracts of Brassica oleracea var. capitata f. rubra with a wide range of RT ranged from 3.824 – 18.917 min. Among those 16 compounds, four of the compounds were denoted with prominent peak area and retention time with a wide range of RT ranged from 4.861 to 19.654 RT showing peak area of 3.84 to 18.36% and their structures and nature were identified based on the mass spectrometry and FT-IR peak values. The identified compound bioactivities

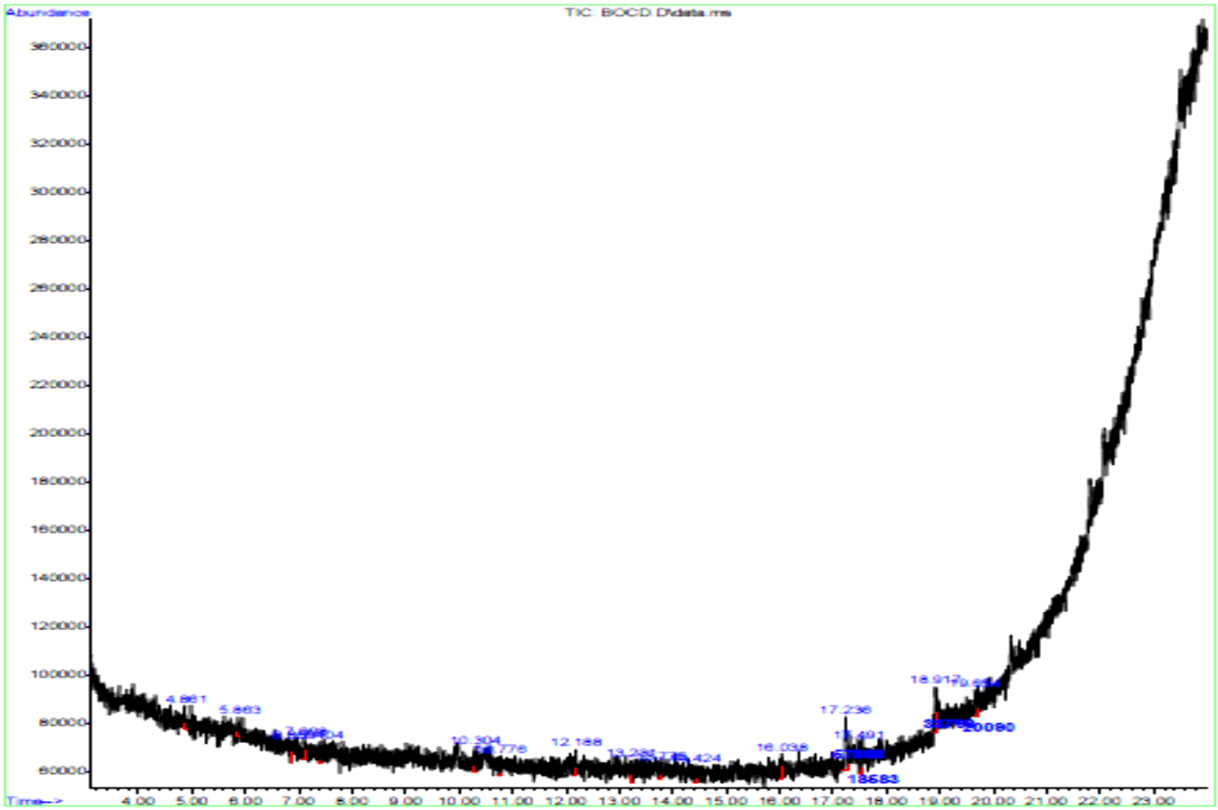
were predicted based on online PASS Prediction. The results of ethanolic extract of Brassica oleracea var. capitata f. rubra GC-MS analysis revealed the presence of few prominent compounds viz., Chloro-methyl-methoxy-amine, Trisilane, Phenol, 2,4-dibromo-, Phenol, 2,4-dibromo-, 5-(4-Chloro-2-nitro-phenylsulfanyl, Phenol, 2,6-dibromo-, Hydrazine carboxamide, .beta.-D-Glucopyranose, 2,4(1H,3H)-Quinazolinedione, Propanoic acid, Hydrazine carbothioamide, 2,3,6,7-Tetrahydro-5H-thiazolo[3,2, -a]-S-triazine-5-one-7-thione, 2,6-dibromo-, Acetamide, 2-Acetamido-d-mannitol, 9-Bromononanoic acid, Heptanoic acid, n-Decanoic acid, Pyrimidine, Ethanone, Phenanthridinium and Furo [2',3':4,5] thiazolo [3,2-g]purine-8-methanol. Among the identified compounds some of the compounds showed higher peak area viz., 9-Bromononanoic acid, Heptanoic acid, and n-Decanoic acid showed retention time of 17.491 and peak area of 18.36% revealing the biological property of Antimicrobial activity, Anti-inflammatory and Insecticidal / Repellent properties followed by Ethanone, isosemicarbazone revealed 18.917 retention time and peak area of 10.71% representing Antibacterial and antifungal activity**,** Anticancer and antiproliferative properties. Followed by the least peak area was represented by Trisilane, 5-Hexenoic acid, Furo [2',3', 4, 5]thiazolo[3,2-g] purine-8-methanol showing the retention time of 5.863 RT and peak area of 3.84%. Thus the compounds identified in the GC-MS analysis reported higher antimicrobial, anticancer, hepatoprotective, anti-inflammatory and Antioxidant Activity. Based on the reports of the GCMS analysis antimicrobial property of the ethanolic extract of Brassica oleracea var. capitata f. rubra was carried out. The results were displayed Table.2. and Figure. 3.

Table.2. GC-MS Analysis of Phytochemicals and their Biological property in Ethanaolic Extract of Brassica oleracea var. capitata f rubra using PASS Prediction

Peak no	Name of the compound	RT (min)	Peak area %	Molecular formula	Molecular weight	Biological Property
1.	Chloro-methyl-methoxy-amine 8-Chlorocapric acid N-Methoxy-1-ribofuranosyl-4 olecarboxylic amide	4.861	4.32	$C_4H_9C_lNO$ $C_{10}H_{20}O_2$	122.57 g/mol. 206.7 g/mol	Antimicrobial Activity,
2.	Trisilane 5-Hexenoic acid	5.638	3.84	Si_3H_8 $C_6H_{10}O_2$	92.33 g/mol 114.14 g/mol	Antidiabetic Property
3.	Phenol, 2,4-dibromo-	6.869	4.99	$C_6H_4Br_2O$	251.9 g/mol	Antimicrobial Activity Low to Moderate Toxicity
4.	Phenol, 2,4-dibromo- 5-(4-Chloro-2-nitro-phenylsulfanyl)-[1,3,4]thiadiazol-2-ylamine	7.098	4.90	$C_8H_7N_3S_2$	209.31 g/mol	Antimicrobial activity Antiinflammatory, Anticancer Property
5.	Hydrazine carbothioamide	10.304	5.01	CH5N3S	91.15 g/mol	Antimicrobial activity Antitubercular, and anticancer potential
6.	.beta.-D-Glucopyranose, 1,6-anhydr o- 2,4(1H,3H)-Quinazolinedione	10.776	5.46	$C_6H_{12}O_6$	180.16 g/mol	Energy role, structural integrity, regulate blood sugar level
7.	Propanoic acid 3-hydroxy-Hydrazine carbothioamide	12.188	4.93	$C_3H_6O_2$ CH_6N_3OS	74.08 g/mol 108.16 g/mol	Antimicrobial activity Anticancer Enzyme Inhibition
8.	Phenol, 2,6-dibromo-	13.231	4.42	$C_6H_4Br_2O$	251.9 g/mol	Antimicrobial activity
9.	2,3,6,7-Tetrahydro-5H-thiazolo[3,2 -a]-S-triazine-5-one-7-thione	13.775	4.62	C_5H_5NS	111.17 g/mol	Mucolytic agent Antioxidant Hepatoprotective Potential
10.	Hydrazine carbothioamide ntylidene-	14.424	5.34	CH_5N_3S	91.15 g/mol	Antimicrobial potential Anticancer, antitubular, enzyme inhibition

11.	Acetamide N-(4-hydroxycyclohexyl) trans- Acetamide	16.038	6.05	C_2H_5NO $C_7H_{14}O2$	59.07 g/mol 130.18 g/mol	chemical intermediate fragnances and flavours
12.	9-Bromononanoic acid Heptanoic acid n-Decanoic acid	17.236	18.36	$C_9H_{17}BrO_2$ $C_7H_{14}O_2$	237.1 g/mol 75.08 g/mol	Antimicrobial / anticancer potential
13.	Pyrimidine 5-bromo-2,4-bis(methyl thio)-	17.491	5.90	$C_4H_4N_2$	80.09 g/mol	Nucleoside and nucleotide synthesis, antiviral and anticancer property
14.	Ethanone 1-(5-acenaphthenyl)- iosemicarbazone	18.917	10.71	C_2H_4O $C_{14}H_{15}N_3S$	44.05 g/mol 257.4 g/mol	fat metabolism Antimicrobial, anticancer, antiviral property
15.	Furo[2',3':4,5]thiazolo[3,2-g]puri ne-8-methanol henanthridinium	19.654	6.38	$C_{11}H_8N_4OS$ $C_{13}H_{10}N^+$	244.3 g/mol 180.2 g/mol	Antimicrobial, anticancer, antiviral property, metal binding

Figure. 3. Graphical Representation of phytocompounds present in Ethanolic Extract of Brassica oleracea var. capitata f. rubra



Adnan Wahhab Habeeb Al-Mudhafr (2020) studied the Gas Chromatography/ Mass Spectrometer (GC/MS) method to detect the active ingredients in the water extract of the red cabbage and showed several peaks. It was found that most active groups of esters, phenols, Alkanes and carboxylic acids, including (1- octadecene, acid, 5-trime thylsilyl, oxy-methyl ester, 3-Carboxamide, Oxime, 2-trifluoromethylephenyl, Heptacosane, Triacontane and Hexacosane), all of which are antibacterial, different concentrations of water extract of red cabbage. Afzal Hossain et a1. (2026) carried out GC–MS analysis and identified a diverse range of bioactive compounds in red cabbage extract, including phenolics, flavonoids, and fatty acid derivatives, where 2-deoxy-D-erythro-pentose (18.3901 ± 0.8754 %), and 3-acetoxy-3-hydroxypropionic acid, methyl ester (17.9127 ± 1.4014 %) are the most prominent. In the present research, 15 different phytocompounds with different peak values with prominent peak area were identified and the biological property of the compound were identified using PASS Prediction software. Mahadeva Rao et al. (2019) studied that ethanolic crude extract of Brassica oleracea var acephala showed that

eleven compounds were identified through GC-MS and FTIR analysis. Cem Baltacıoglu et al., (2024) carried out the chemo metric evaluation to predict the antioxidant capacity, anthocyanin content, and individual anthocyanins using FT-IR spectra. In the present research, FT-IR spectroscopic analysis recognized 25 different peak values ranging from 370.33-3757.33 were observed revealing functional and side groups of the crude powder of Purple cabbage.

CONCLUSION

The study provides preliminary scientific evidence supporting the potential of red cabbage leaves as a source of biologically interesting phytochemical constituents. The observed chemical profiles provide a basis for further investigation of their antimicrobial and antioxidant properties through experimental validation. Further studies involving compound isolation, structural confirmation, quantitative estimation and biological evaluation are recommended to establish the therapeutic and functional applications of this Purple cabbage.

REFERENCES

16. Adnan Wahhab Habeeb Al-Mudhafr, Detection of Active Compounds by GC/MS In Extract of Red Cabbage (Brassica Oleracea) and their Effect in the Preservation of Raw Milk, Plant Archives, Vol. 20, Supplement 1, 2020 pp. 4-8.
17. Afzal Hossain, Md. Tariqul Islam, Mst. Sarmina Yeasmin, Farida Siddika, G.M. Masud Rana, Jaytirmoy Barmon, Bijoy Chandra Ghos, Tahmina Akter Chowdhury, Md. Farhad Sarker, Md. Mahfuzur Rahman, Md. Ashraful Islam, Nusrat Jahan Mouri, Rasheda Akter, Evaluating bioactive contents from ethanolic extract of red cabbage (Brassica oleracea var. capitata): In vitro and in silico approaches, Applied Food Research Volume 6 Issue 1 Pagination 101783 ISSN 2772-5022.
18. Baltacıoglu C, Keskin O, Baltacıoglu H, Agçam E. Encapsulation and drying methods in the production of powdered red cabbage (Brassica oleracea L.): Chemometrics and Fourier transform infrared spectroscopy. Food Sci Technol Int. 2025 Oct; 31(7):613-624. doi: 10.1177/10820132241238261. E.pub 2024 Mar 15. PMID: 38488461.
19. Harborne JB. Phytochemical methods, Chapman and Hall, Ltd. London. 1999. 60-66.
20. Mahadeva Rao U. S., C. Shanmuga Sundaram, J. Sivakumar. Isolation and Characterization of Phytochemical Constituents and its Antibacterial Activity of Brassica oleracea var acephala. Research J. Pharm. and Tech 2019; 12(1): 297-302. doi: 10.5958/0974-360X.2019.00055.6
21. Mallikharjuna PB, Rajanna LL, Seetharam YN, Sharanbasappa GK. Phytochemical studies of Strychonos potatorum L. f-A medicinal plant, E. J. Cehem. 2007; 4: 510-518.
22. Manasa, S., Mukunda, L., Sadarunnisa, S. And Rajasekharam, T. 2017. Studies on effect of spacing on yield and yield attributing parameters of red cabbage (Brassica oleracea var. capitata f. rubra). International J. Current Microbiology and Applied Sciences. 6(12): 3143-3147.
23. Merlin NJ, Parthasarathy V, Manavalan R, Kumaravel S, Chemical Investigation of Aerial Parts of Gmelina asiatica Linn by GC-MS. Pharmacognosy Res. 1(3): 152-156, (2009).
24. Oladzadabbasabadi, N., Mohammadi Nafchi, A., Ghasemlou, M., Ariffin, F., Singh, Z., & Al-Hassan, A. A. (2022). Natural anthocyanins: Sources, extraction, characterization, and suitability for smart packaging. Food Packaging and Shelf Life, 33, 100872. https://doi.org/10.1016/j.fpsl.2022.100872
25. Podsedek A., Sosnowska D., Redzynia M., Anders B. Antioxidant Capacity and Content of Brassica oleracea Dietary Antioxidants. Int. J. Food Sci. Technol. 2006;41: 49–58. doi: 10.1111/j.1365-2621.2006.01260.x
26. Sangeetha J, Vijayalakshmi K. Determination of Bioactive Components of Ethyl Acetate Fraction of Punica granatum Rind Extract. International Journal of Pharmaceutical Sciences and Drug Research. 2011; **3(2):** 116-122
27. Thomas Abu, Blessing Obianuju Ezea , Saka Alabi Yusuf , Olorunsola Olasunkanmi Bamidele, Latifat Olabimpe Sidiq, Aminat Omope Yusuf, Omonike Oluyemisi Ogbole, Phytochemicals, proximate and elemental analysis, antioxidant and cytotoxic potentials of purple (Capitata F. rubra) and green (Capitata Linn.) Brassica oleraceae (cabbage), J. Pharmacy & Bio resources 21(2), 74-86 (2024).