**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**
'A Bridge Between Laboratory and Reader'

www.ijbpas.com

**VOLATILE COMPOUND ANALYSIS USING GC-MS,
PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES
OF *BRASSICA OLERACEA* VAR. CAPITATA F. RUBRA**

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Received 23rd March, 2021; Revised 24th April 2021; Accepted 20th May 2021; Available online 1st Dec. 2021

<https://doi.org/10.31032/IJBPAS/2021/10.12.5784>

ABSTRACT

Eatable seedlings of vegetables and herbs have gotten expanding consideration and they are treated as nourishments or superfoods because of their important health-promoting properties. In specific, the red cabbage plays a vital role because of their significant amount of bioactive constituents present in it. This study deals about the identification of secondary metabolite, phytochemical, GC-MS, UV and FTIR analysis and antioxidant activity of *Brassica oleracea* var. capitata f. rubra (Red cabbage). From the ethanol and aqueous extract of red cabbage, phytochemical studies revealed the existence of alkaloids, tannins, flavonoids, saponins, aminoacids, sterols, proteins, carbohydrates, triterpenoids, anthraquinones and saponins. For major peaks in the red cabbage extracts, UV-visible spectrophotometer findings revealed a wavelength range of 260–900 nm. Primary, secondary, aromatic and aliphatic amines, carboxylic acids, amides and alkanes were identified using FT-IR analysis. The crude extracts contained significant bioactive compounds, according to the results of GC-MS research. These secondary metabolites are completely related to potential benefits of humans. Many useful constituents were discovered by GC-MS research, indicating that Red cabbage could be used to make nutraceuticals as potent antioxidants to treat a variety of human diseases and their complications.

Keywords: Red cabbage, Antioxidant, Bioactive Compounds, FTIR, Gas chromatography

INTRODUCTION

Aside from therapeutic medicines, medicinal plants and vegetables are a rich source of data on a wide range of chemical compounds that might be produced into exact selectivity medications. These are the source of possibly valuable chemical compounds that could be used as innovative which leads and clues in modern medication development [1]. Flavonoids, tannins, alkaloids and phenolic chemicals are the most important bioactive elements which are present in plants [2]. The relationship between phytoconstituents and plant bioactivity is important to understand for the synthesis of compounds with detailed activities to treat a variety of health problems and chronic diseases [3]. Because of the importance of such preliminary phytochemical screening of plants and vegetables, it is urgently needed in order to find and develop novel therapeutic molecules with improved efficacy. Such investigations have also been reported by a number of study groups around the world [4].

Phytochemical screening is a qualitative analytic method for identifying bioactive chemicals that are unknown in plant and vegetable extracts [5]. An early stage in a phytochemical study is phytochemical screening, which aims to provide an overview of the class of chemicals found in

the plants being researched. The colour testing reaction is examined using a colour reagent in the phytochemical screening method [6]. It is necessary to research the chemical components found in therapeutic plants. This knowledge will be useful in the production of complex active mechanisms of chemical compounds found in medicinal plants. In addition to identifying active compounds that are favourable to the body's health, phytochemical screening in medicinal plants can also be used to detect active molecules that produce toxicity. As a result, the phytochemical screening of medicinal plants is must before proceeding with further research [7].

The vegetable red cabbage (*Brassica oleracea* var. capitata f. rubra), which belongs to the Brassicaceae family, was chosen for this study (**Figure 1**). The purpose of this work is to screen the phytochemicals present in red cabbage extract and analyse the bioactive components present in it using GC-MS. The antioxidant activity of the extracts was determined using the DPPH method.

Figure 1: *Brassica oleracea* var. *capitata* f. *rubra*

MATERIALS AND METHODS

Plant Material

Whole vegetable of *Brassica oleracea* var. *capitata* f. *rubra* were collected in Thanjavur. The leaves were gathered and washed thoroughly with running tap water to remove any dirt, then dried in the shade for a few weeks and ground into a coarse powder then placed in a tightly sealed jar for extraction.

Plant extract preparation

Ethanol and Aqueous were used to extract known quantities of the lyophilized powder. The solvents were of analytical quality. In a rotary evaporator, the solvents were evaporated at 40°C–50°C under reduced pressure. Using a soxhelt apparatus, 0.5kg of the plant powder was extracted with different solvents in stages. After being suspended in water overnight, the leftover extract was filtered. After that, the filtrate was dried and stored at 40°C until it was needed.

Qualitative phytochemical screening

Secondary metabolites such as alkaloids, steroidal compounds, and phenol were

screened for the crude extract. Secondary metabolites such as alkaloids, tannins, flavonoids, saponins, aminoacids, sterols, proteins, carbohydrates, triterpenoids, anthraquinones and saponins were screened in the crude extract [8]. Individual solvent free extracts were subjected to phytochemical analysis. Since ethanolic extract contains a greater number of active ingredients, it was chosen for this analysis.

QUANTITATIVE ANALYSIS

Determination of the total content of polyphenols

The Folin & Ciocalteu's reagent was used to calculate the total content of polyphenols in cabbage extracts, and the measurement was performed at 765 nm with the standard gallic acid. The results were given in milligrams of gallic acid equivalent (GAE) per gram of dry weight [9].

Determination of the total content of flavonoids

At 510 nm, flavonoids extracted with (5% NaNO₂, 10% AlCl₃. 6H₂O, and 1M NaOH) were measured, with catechin which is serving as a reference. The amount of flavonoids was measured in mg of catechin equivalents (CE) per gram of dry weight [9].

Determination of the total content of anthocyanins

A pH differential approach was used to determine total anthocyanins [10]. The

absorbance of cyanidin-3-glucoside was calculated at 510 nm and 700 nm in buffers with pH 1.0 and 4.5, respectively, using the equation $A = (A_{510} - A_{700})$ at pH 1 – $(A_{510} - A_{700})$ at pH 4.5, with a molar extinction coefficient of 29600. The results are given in milligrams of cyanidin-3-glucoside equivalent (CGE) per gram of dry weight

Components Identification

The National Institute of Standard and Technology (NIST) database, which contains over 62,000 patterns, was used for GCMS interpretation. The unknown component's spectrum was compared to the known component's spectrum contained in the NIST library. The components of the test materials were identified by their name, molecular weight, and structure.

UV and FTIR Spectroscopic analysis

The extracts were analysed under visible and UV light for proximate study. Using a high-pressure vacuum pump, the extracts were centrifuged at 3000 rpm for 10 minutes before being filtered onto Whatmann No. 1 filter paper for UV and FTIR spectrophotometer analysis. The sample is diluted to 1:10 with the same solvent. The extracts were scanned at wavelengths ranging from 260 to 900 nm with a Perkin Elmer Spectrophotometer, and the characteristic peaks were established. During the FTIR analysis, the Perkin Elmer Spectrophotometer was used

to detect the characteristic peaks in the range of 400-4000 cm^{-1} and their functional groups. The peak values of UV and FTIR were reported. Per analysis was duplicated twice for spectrum validation.

In vitro Antioxidant activity with 1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH):

DPPH is converted to 1,1-diphenyl-2-picryl hydrazine by antioxidants. The amount of discoloration suggests the antioxidant extract's capacity to scavenge free radicals. Antioxidant activity has been measured by the change in absorbance provided at 517nm.

RESULT AND DISCUSSION

Phytochemical compounds

This study investigated the phytochemical contents and antioxidant activities of crude extracts from selected *Brassica oleracea* var. capitata f. rubra. Phytochemical analysis revealed the presence of Alkaloids, Phenols, Glycosides, Flavonoids, Tannins, Proteins, Aminoacids, Carbohydrates, Saponins, and Triterpenoids (**Table 1**). These compounds are biologically active and may contribute to the antioxidant activities of the *Brassica oleracea* var. capitata f. rubra. On the aqueous and ethanolic extracts of red cabbage, phytochemical screening was carried out using colour forming and precipitating chemical reagents. Flavonoids, phenolic compounds, and anthocyanins were found to glycolate with mono or di-molecules of

saccharides in red cabbage extract (RCE). Anthocyanins such as cyanidin 3-galactoside and cyanidin 4-galactoside are the main constituents of red cabbage extract. Anthocyanins such as cyanidin 3-glucoside, acetylated anthocyanins with Coumaric acid, cyanidin 3-galactoside, cyanidin 3-5 diglycoside, Sinapic acid, isothiocyanatesglucosinolate, Ferulic acid, vitamins A, B, C, and -carotenes are the main constituents of red cabbage extract [11]. RCE contains anthocyanins, a type of phenolic natural pigment that was discovered to have antimicrobial properties, anti-inflammatory properties [12]. Therefore it can be used pharmacologically to develop new compounds for health benefit. The presence of flavonoids and terpenoids indicates these plant extracts potentially have antioxidant activities. Flavonoids are known to exert antioxidant activity through a scavenging or chelating process.

Evaluation of Total Phenolics, Flavonoids and Anthocyanine

The table shows the quantitative contents of total phenolics, total flavonoids, and total anthocyanine in red cabbage extracts. Total phenolics were found to be higher in the ethanolic extract (3.96 ± 0.98 mg GAE/g d.w) than in the aqueous extract (3.78 ± 0.75 mg GAE/g d.w). When the results were measured and recorded according to fresh and dry matter of the

substance analyzed, the same trend was observed, and their findings are consistent with those of Ismail *et al.*, [13], who found that the total phenolic content of the cabbage extract was 4.1 mg/g. Other researchers found 0.4 mg total phenolics/g, 0.36 mg total phenolics/g and 1.10-1.53 mg total phenolics/g in fresh white cabbage [14, 15]. Flavonoids and anthocyanins are potent antioxidants, neutralising free radicals and interrupting the oxidative damage chain reaction. According to Fahey *et al.* [16]. Anthocyanins have antioxidant effects *in vitro* (CaCO₂).

The flavonoid concentration in ethanolic extract is 1.80.21mg CE/g dry weight, but there is no flavonoid production in aqueous extract. Flavonoids are a wide group of naturally occurring phenolic compounds found in high concentrations in plants. Humans are unable to synthesize this class of naturally occurring polyphenolic compounds. Because of the polarity variations, the solute's solubility in the solvent varies. The most widely used polar solvents for extraction are water, methanol, and ethanol. Non-polar solvents include chloroform, ethyl alcohol, and others. This may explain the differences in flavonoid content between genotypes. Flavonoids have long been known to have antioxidant properties, and their impact on human nutrition and health is important.

The maximum anthocyanin content of 38 ± 5.68 mgCGE/gd.w was observed in ethanolic extract and the minimum of 27 ± 4.32 mgCGE/gd.w was observed in aqueous extract of red cabbage (**Table 2**). Anthocyanins are widely regarded as nature's most significant and diverse group of water-soluble pigments [10]. They are responsible for the colours blue, black, red, and orange in a variety of fruits and vegetables. Nasunin, delphinidin-3-(p-coumaroylrutinoside) - 5- glucoside, is a representative anthocyanin in eggplant peel, according to Noda *et al.* [17]. Since anthocyanin concentration rises as the fruit ripens, cultivation practices have a significant effect on anthocyanin content. The results clearly show that phenol is an essential component of this plant, and that its existence may be responsible for some of the pharmacological effects.

GCMS analysis

The GCMS research revealed thirty-one compounds were present in red cabbage. The active principles are mentioned, along with their molecular weight, retention time, molecular formula and concentration (%). Hexadecadienoic acid, 9,12-15 Octadecadienoic acid, Stearic acid, Octadecanoic acid and 9 Octadecadienoic acid were the most common compounds which is present in the plant extract. Modern practitioners use the entire plant for various illnesses due

to the presence of various bioactive compounds (**Figure 2**).

Spectrophotometric analysis

Based on the proper baseline and sharpness of the peaks, the UV-VIS profile of plant extract was taken wavelength at 200 to 800nm. The UV-visible spectra were used to distinguish compounds with π -bonds, σ -bonds, and lone pair of electrons, as well as aromatic rings and chromophores. Peaks were found at 211.15, 213.01, 354.40, and 470.64, with absorption values of 2.681, 2.682, 0.213, and 0.154, respectively. Peaks at 234-676 nm indicate the presence of alkaloid and phenolic compounds in *Brassica oleracea* var. capitata f. rubra (**Figure 3**). A comparison of the spectra of seeds and flowers reveals that the extract contains alkaloids, flavonoids, and glycosides compounds that are identical to those found in the seeds and flowers reported by Sofowora [18].

Functional groups identification

Based on the peak values in the IR radiation region, the FTIR spectrum was once used to classify the functional groups of the active elements existing in the extract. The functional groups of the components were divided based on the peaks ratio when the extract was passed through the FTIR. The existence of phenol, alkanes, alcohol, aromatic and aliphatic amines was confirmed by FTIR analysis (**Figure 4**).

Antioxidant activity

All of the tests were repeated three times in triplicate. The DPPH radical scavenging operation was used to measure the percentage inhibition. The IC₅₀ value was determined using the obtained DPPH radical Scavenging effect (%), which represents the concentration of the

scavenging compound that caused 50% neutralization. The absorbance's of *Brassica oleracea* var. capitata f. rubra plants in various solvent extracts and percentage scavenging capability, as well as the IC₅₀ value of various extracts and a normal, are presented (Table 3).

Table 1: Phytochemical compounds in *Brassica oleracea* var. capitata f. rubra

Phytochemical Compounds	Results
Flavonoids	+ve
Aminoacids	+ve
Saponins	+ve
Alkaloids	+ve
Tannins	+ve
Triterpenoids	+ve
Phenols	+ve
Carbohydrates	+ve
Glycosides	+ve
Proteins	+ve

Table 2: Quantitative analysis of *Brassica oleracea* var. capitata f. rubra

Evaluated parameters	Ethanollic Extracts of <i>Brassica oleracea</i> var. capitata f. rubra	Aqueous extracts of <i>Brassica oleracea</i> var. capitata f. rubra
Total Phenolics (mgGAE/gd.w)	3.96 ± 0.98	3.78 ± 0.75
Total Flavonoids (mgCE/gd.w)	1.8 ± 0.21	-
TotalAnthocyanine (µgCGE/gd.w)	38 ± 5.68	27 ± 4.32

Values are expressed as Mean±SD for triplicates

Peak#	R.Time	I.Time	F.Time	Area%	Mark	Name
1	6.029	5.983	6.083	0.62		3-Thiazolidinecarboxamide, 2-imino-
2	6.136	6.083	6.200	0.68	V	2,5-ANHYDRO-1,6-DIDEOXYHEXO-3,4-D
3	6.317	6.200	6.375	0.93	V	S-Methyl methanethiosulphonate
4	6.452	6.408	6.542	2.21		2,4(1H,3H)-PYRIMIDINEDIONE, 5-METHY
5	7.396	7.350	7.458	1.12		ETHANAMINE, N-ETHYL-N-NITROSO-
6	7.566	7.508	7.675	11.15		4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6
7	8.622	8.575	8.733	1.95		2,3-DIHYDRO-BENZOFURAN
8	8.800	8.733	8.967	1.33	V	5-Hydroxymethylfurfural \$\$ 2-Furancarboxal
9	9.044	8.967	9.142	2.12		6-UNDECANOL \$\$ 4,4,8,8-TETRADEUTE
10	9.603	9.533	9.833	5.84		2,4(3H,5H)-FURANDIONE, 3-PROPYL- \$
11	10.158	10.117	10.217	0.86		2-Methoxy-4-vinylphenol \$\$ Phenol, 4-etheny
12	11.142	11.050	11.300	4.01		1,2,3-BENZENETRIOL \$\$ PYROGALLOL
13	11.333	11.300	11.450	1.56	V	2-Butene-1,4-diol, bis(trimethylsilyl) ether
14	13.884	13.825	13.958	1.46		3',5'-Dimethoxyacetophenone \$\$ 3,5-Dimetho
15	17.937	17.883	18.017	1.03		1H-Indole-3-acetonitrile \$\$ Indole-3-acetonitr
16	19.516	19.458	19.675	12.29		n-Hexadecanoic acid \$\$ Hexadecanoic acid \$\$
17	19.905	19.858	19.958	1.91		HEXADECANOIC ACID, ETHYL ESTER \$
18	21.550	21.458	21.567	9.63		9-Octadecenoic acid, (E)- \$\$ trans- Δ^9 (sup
19	21.595	21.567	21.717	13.62	V	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- \$\$ Lin
20	21.758	21.717	21.775	2.67	V	Octadecanoic acid \$\$ Stearic acid \$\$ n-Octade
21	21.800	21.775	21.833	2.60	V	Methyl 9-cis,11-trans-octadecadienoate
22	21.886	21.833	21.950	4.10	V	Ethyl 9,12,15-octadecatrienoate \$\$ Ethyl 9.alp
23	22.075	21.950	22.125	4.70	V	TETRADECANOIC ACID, ETHYL ESTER
24	22.155	22.125	22.358	5.46	V	Tetrapentacontane
25	23.869	23.742	24.017	6.17		Dotriacontane \$\$ n-Dotriacontane \$\$ Bicetyl S
				100.00		

Figure 2: *Brassica oleracea* var. capitata f. rubra extract GCMS analysis Figure 3: *Brassica oleracea* var. capitata f. rubra extract UV-Vis Spectral analysis

Figure 4: FTIR analysis of *Brassica oleracea* var. capitata f. rubra

Table 3: DPPH radical scavenging activity of *Brassica oleracea* var. capitata f. rubra

Concentrations ($\mu\text{G/ML}$)	Butylated hydroxytoluene (Standard BHT)	Ethanollic extracts of <i>Brassica oleracea</i> var. capitata f. rubra	Aqueous extracts of <i>Brassica oleracea</i> var. capitata f. rubra
750	96.20%	68.90%	65.19%
500	98.31%	49.27%	54.66%
250	89.45%	28.44%	40.54%
100	46.21%	17.10%	25.26%
50	36.20%	7.43%	19.07%
10	19.02%	2.89%	3.85%
IC ₅₀ $\mu\text{g/ml}$	101 $\mu\text{g/ml}$	205 $\mu\text{g/ml}$	300 $\mu\text{g/ml}$

CONCLUSION

The present study justifies the claimed uses of the vegetable *Brassica oleracea* var. capitata f. rubra in the traditional system. Future studies to determine other pharmacologically active compound by preparing the extracts with other solvents should be carried out. The Bioactive compounds present in *Brassica oleracea* var. capitata f. rubra suggests that the vegetable has therapeutic value. The quantitative analysis of these bioactive

compounds needs further studies. Detailed studies are required to evaluate the potential effectiveness of the crude extracts of this vegetable as the antimicrobial agents.

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