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PHYTOCHEMICAL AND GC-MS ANALYSIS OF BRASSICA OLERACEA VAR. CAPITATA F. RUBRA

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ABSTRACT

The present study was aimed to carry out the detailed phytochemical analysis of Brassica oleracea var. capitata f. rubra. Qualitative phytochemical screening of the aqueous extract of the Brassica oleracea var. capitata f. rubra revealed the presence of many components such as anthocyanins, carbohydrates, flavanoids, reducing sugars,etc., GC-MS analysis was also carried out to detect the phytoconstituents present in the ethanolic extract of Brassica oleracea var. capitata f. rubra.

KEYWORDS: Brassica Oleracea var. capitata f. Rubra, Phytochemical screening, GC-MS.

INTRODUCTION

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different part of country.[1] The medicinal use of plants is probably as old as mankind plants have continued to be a valuable source of natural products for maintaining human health, as studies natural therapies have intensified. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. More than 150,000 plants species have been studied and several of them contain therapeutic substances and the use plant compounds for pharmaceutical purposes has gradually increased. About 1500 plants are systematically used in indigenous system of medicine, like ayurveda, unani and siddha. It is ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing.[2]

Red cabbage (*Brassica oleracea* L.) is an excellent source of food colorant. Red cabbage is rich in a number of bioactive substances, including anthocyanins. The anthocyanin profile of red cabbage consisting of twenty derivatives of cyanidin glucosides was described by means

of HPLC-DAD-MS/MS. The base structure of anthocyanins identified was cyanidin-3-diglucoside-5-glucoside. Their glucoside residues were nonacylated, monoacylated and diacylated. Sinapic, ferulic, caffeic and *p*-coumaric acids were recognized as main phenolic acids in this structure. The predominant anthocyanin in red cabbage was nonacylated form of cyanidin-3-diglucoside-5-glucoside, followed by cyanidin-3-(sinapoyl) (sinapoyl)-diglucoside-5-glucoside and cyanidin-3-(*p*-coumaroyl)-diglucoside-5-glucoside.

MATERIALS AND METHODS

EXTRACTION

Red cabbage was brought from the departmental store and ethanolic extract was prepared by putting the small quantity of leaves into the jar containing absolute ethanol. Smashed with Morton and Pistal, filtered. This filtrate is used for phytochemical and GC-MS analysis.

PHYTOCHEMICAL ANALYSIS

Estimation of Carbohydrates

Carbohydrates are the important components of storage and structural materials in the red cabbage. They exist as free sugars and polysaccharide. They are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural which forms a green coloured product with phenol and has an absorption maximum at 490 nm. The total carbohydrate content of the red cabbage, Brassica oleracea var. capitata f. rubra was estimated by phenol-sulphuric acid method. (Dubois, et al, 1956).

Estimation of Proteins

The amino acids present in proteins react with Folin-Ciocalteau agent, which contains phosphomolybdic acid and tungstate, to produce a blue coloured complex, which absorbs maximally at 620 nm. (Lowry *et al.*, 1951)

Estimation of Lipids

The lipid content of the Brassica oleracea var. capitata f. rubra was estimated by using chloroform methanol mixture as described by Folch *et al*, 1957.

Estimation of total Phenol

The type of phenolic content is influenced by seasons, species and place. It is assumed that the antioxidant properties of phenolics are related to the number of phenol rings that makes

them more effective hydrogen donors and quenchers. The phenolic hydroxyl groups present in the phenolic compounds of the red cabbage reacts with Folin reagent to produce a coloured product which absorbs maximally at 750 nm. The total phenol content of Brassica oleracea var. capitata f. rubra was estimated by Folin Ciocalteus method (Tagae *et al*, 1984).

Estimation of Alkaloides

Dragendroff's test

Little amount of the sample was treated with the Dragendroff's reagent; the appearance of reddish brown precipitate indicated the presence of alkaloids.

Mayer's test

Sample (2-3ml) was treated with few drops of Mayer's reagent. Appearance of white precipitate indicated the presence of alkaloids.

Wagner's test

Sample (2-3ml) was mixed with few drops of Wagner's reagent. Appearance of reddish brown precipitate indicated the presence of alkaloids.

Estimation of Glycosides

Free content of the sugar extract was determined. The sample was hydrolysed with mineral acid (dilute hydrochloric or dilute sulphuric acid). Again the total sugar content of the hydrolysed extract was determined. Increase in the sugar content indicated the presence of glycoside in the extract.

Estimation of flavonoides

Alkaline test: To 3 ml of the extract few magnesium ribbons are dipped and concentrated Hydrochloric acid was added over them and observed for the formation of magenta (brick red) colour indicating the presence of flavonoides.

Estimation of Tannins

A fraction of the extract was dissolved in water and then it was subjected to water bath 37°C for 1 h and treated with ferric chloride solution and observed for the formation of dark green colour.

Lead acetate test

The sample was treated with 10% lead acetate solution; appearance of white precipitate indicated the presence of tannins. When the extract was treated with aqueous bromine solution, appearance of white precipitate indicated the presence of tannins.

Ferric chloride test

To 1 ml of extract, 2 ml of 5% ferric chloride was added. Formation of greenish black colour indicated the presence of tannins. A fraction of the extract was dissolved in water and then it was subjected to water bath 37°C for 1hr and treated with ferric chloride solution and observed and for the formation of dark green colour.

Test for Saponin

Foam test: To 1 ml of the extracts 5 ml distilled water was added and shaken vigorously. Formation of foam indicated presence of saponins.

Test for terpenoides

Chloroform test: To 5 ml of the extract few drops of chloroform and concentrated H2SO4 was added carefully along the sides of the test tube. Formation of brown color at interface was a positive indicator.

GC-MS ANALYSIS

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min, to 150ºC, then 8ºC/min to 250ºC, ending with a 20min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan *et al*., 2013).

Identification of components

Interpretation on GCMS was conducted using the database of National Institute Standard and Technology (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 of the test materials were ascertained (Dr. Dukes, 2013).

Identification of bioactive compounds in ethanolic extract of Brassica oleracea var. capitata f. rubra by GC MS analysis

Twenty compounds were identified in ethanolic extract of Brassica oleracea var. capitata f. rubra by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds are 1,5-Pentanediol, Phenol, 2,4-Bis(1,1-Dimethylethyl), 1-Heptadecanamine, N,N-Dimethy, l Hexadecanoic Acid Ethyl Ester, and 1-Tetradecanol were found in this ethanolic extract of Brassica oleracea var. capitata f. rubra. The biological activity of the selected compounds were listed in table 2. The presence of various bioactive compounds justifies the use of the ethanolic extract of Brassica oleracea var. capitata f. rubra for various ailments by traditional practitioners. However isolation of individual phytochemical constituents and subjecting its biological activity will definitely give fruitful results.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis of aqueous extracts of Brassica oleracea var. capitata f. rubra.

The results of qualitative phytochemical analysis of aqueous extract of Brassica oleracea var. capitata f. rubra given in Table 1. Results indicate the presence of many phyto-components in the extract.

Phytochemical constituents of Brassica oleracea species were reported by various authors.[10] Anthocyanins, Tannins, phenolics, alkaloids and flavonoids have been suggested to be involved in anti-inflammatory and anti-cancer activities.[11] GC-MS of the ethanolic extract of the Brassica oleracea is presented in Table 2. The fragmentation patterns of the mass spectra were compared with those of the known compounds stored in the National Institute of Standards and Technology (NIST) research library. In the GC-MS analysis, many active

components were detected. The identification of phytochemical compounds was based on peak area, molecular weight and molecular formula. The results of the GC-MS profile can be used as pharmacognostical tool for the identification of the compounds with different chemical structures. The presence of various bioactive compounds confirms the application of Brassica oleracea for various ailments by traditional practitioners.

Table 1: Phytochemical Screening of Brassica oleracea.

S.No	Name of the Constituent	Water Extract	Ethanol Extract	Methanol Extract
1	Anthocyanins	++	++	++
2	Alkaloids	-	-	-
3	Phenols	+++	+++	+++
4	Flavonoids	+	+++	+
5	Anthroquinones	-	-	-
6	Tannins	+++	+++	+++
7	Saponins	-	-	-
8	Coumarins	+	+++	+
9	Carbohydrates	+++	++	++
10	Proteins	+++	+++	+++
11	Quinines	+++	++	++
12	Glycosides	-	-	-
13	Terpenoids	-	-	-

+++ = Highly present ++ = Moderately Present + = Present = Absent

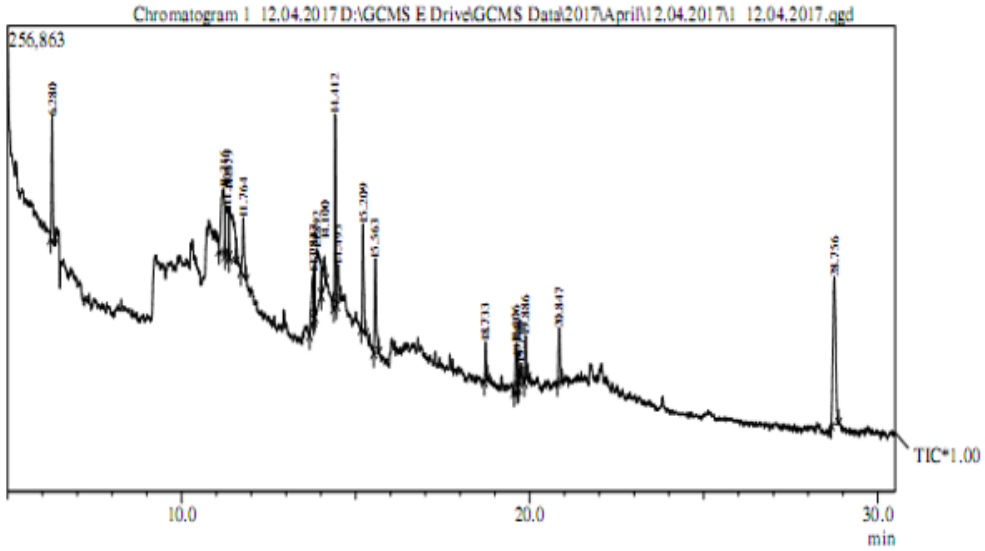

Fig. 1: Chromatogram of Brassica oleracea var. capitata f. rubra.

Table 2: GCMS Analysis of ethanolic extract of Brassica oleracea.

Peak#	R.Time	Area%	Height%	Molecular Formal	Name of the compounds
1	6.280	5.62	9.21	$C_2H_6S_3$	Trisulfide,Dimethyl
2	11.216	8.42	4.73	$C_7H_{14}O_2$	1-Butanol, 3-Methyl-, Acetate
3	11.308	5.60	3.71	$C_{10}H_{16}N_2O_5$	L-Proline, 1-L-.Gamma.-Glutamyl-
4	11.379	8.75	3.80	$C_8H_{13}NO_2$	Butan-2-One, 4-[Pyrrolidin-2-One-5-Yl]-
5	11.764	3.55	4.23	$C_9H_{10}O_2$	2-Methoxy-4-Vinylphenol
6	13.783	4.63	3.64	$C_8H_{16}O_3$	Carbonic Acid, Hexyl Methyl Ester
7	13.817	1.92	3.93	$C_8H_{10}F_4N_2O_2$	1-[4-(2,2-Difluoroacetyl)Piperazin-1-Yl]-2,2- D
8	13.892	9.67	4.44	$C_8H_{18}O_2$	1,5-Pentanediol
9	14.100	2.69	2.12	C_7H_{16}	Pentane, 2,4-Dimethyl-
10	14.412	8.03	14.17	$C_{14}H_{22}O$	Phenol, 2,4-Bis(1,1-Dimethylethyl)-
11	14.495	2.56	2.87	$C_7H_{12}O_5$	Alpha.-D-Galactopyranoside
12	15.209	5.59	7.89	$C_8H_8N_2O_3$	1,2-Dimethyl-3-Nitro-4-Nitroso-Benzene
13	5.563	5.40	6.94	$C_{20}H_{26}O_4$	1,2-Benzoldicarbonsaeure, Di-(He)
14	18.733	1.51	2.85	$C_{15}H_{29}ClO_2$	Chloroacetic Acid, 4-Tridecyl Ester
15	19.606	2.97	3.35	$C_{12}H_{22}O_{11}$	Beta.-D-Glucopyranose, 4-O-.Beta.-D-Galactop
16	19.667	1.19	2.28	$C_{19}H_{41}N$	1-Heptadecanamine, N,N-Dimethyl-
17	19.750	1.84	1.64	$C_{18}H_{16}N_{2O}S$	(2-Phenyl-1,3-dioxolan-2-YL)Methyl
18	19.886	2.21	3.33	$C_{16}H_{32}O_2$	Hexadecanoic Acid, Ethyl Ester
19	20.847	3.32	4.03	$C_{14}H_{30}O$	1-Tetradecanol
20	28.756	14.54	10.86	$C_{24}H_{38}O_4$	Diisooctyl Phthalate
		100.00	100.00		

Table 3: Biological activity of selected compounds in Brassica oleracea var. capitata f. rubra.

S.No.	R.Time	Name of the compounds	Biological activity**
1.	13.892	1,5-Pentanediol	Antimicrobial
2.	14.412	Phenol, 2,4-Bis(1,1-Dimethylethyl)	Antioxidant, Anticarcinogenic, Antiinfammatory
3.	19.667	1-Heptadecanamine, N,N-Dimethyl-	Anti-inflammatory activity and Antibacterial activity
4.	19.886	Hexadecanoic Acid, Ethyl Ester	Nematicide, Pesticide, Lubricant, Antiandrogenic, Hypocholesterolemic Flavor, Hemolytic 5-Alpha reductase inhibitor, Antimicrobial,
5.	20.847	1-Tetradecanol	Antifungal activity

**Dr.Duke's Phytochemical and Ethno botanical Databases, Phytochemical and Ethnobotanical Databases. www.ars-gov/cgi-bin/duke/. 2013.

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