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Journal of Chemical and Pharmaceutical Research, 2016, 8(5):900-905

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In vitro antioxidant studies of fresh *Brassica oleracea* and the characterization of its bioactive compounds using Fourier transform infrared spectroscopy (FTIR)

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ABSTRACT

In the present study methanolic extract of the pulp of plant Brassica oleracea was investigated for the total phenolic content, total flavonoid content and antioxidant activity. Total phenolic content was evaluated by Folin-Ciocalteau method which revealed the presence of very good amount of phenolic content (14.56µg GAE mg-1) in methanol extract. Total flavonoid content was evaluated by aluminium chloride method which revealed the presence of high amount of flavonoid content (22.65µg QE mg-1) in methanol extract. The extract was also analyzed for antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and Ferric Reducing Antioxidant Potential (FRAP) assay. In vitro DPPH radical scavenging assay showed potent antioxidant power with IC50 values of 200µl and Ferric reducing antioxidant power for extract (54.16µM/ml) which are comparable to ascorbic acid. The bioactive compounds present in methanolic extracts were characterized by FTIR spectral analysis. The FTIR spectrum showed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, carboxylic acids and aromatics, aliphatic amines in methanolic extracts. The present study reveals that Brassica oleracea can be used as a potential source of natural antioxidant which may be used to treat various oxidative stress related diseases.

Key words: *Brassica oleracea*, Folin-Ciocalteau, 1,1-diphenyl-2-picrylhydrazyl(DPPH), Ferric Reducing Antioxidant Potential(FRAP), Fourier Transform Infrared Spectroscopy FTIR.

INTRODUCTION

Cruciferae family which is one of the largest families in the plant kingdom in medicinal plants. It includes 338 genera and 3350 species that are distributed worldwide [1]. Various studies indicate that consumption of large number of cruciferous vegetables (e.g., broccoli, cabbage, kale, and Brussels sprouts) are associated with a reduced incidence of cancer [2]. These vegetables contain various primary and secondary metabolites. Antioxidants can scavenge free radicals and protect the human body from oxidative stress, which is the main cause of some cancers and heart diseases [3]. Antioxidants are molecules that prevent the oxidation of other compounds. Brassica foods are very nutritive, providing nutrients and health-promoting phytochemicals such as vitamins, carotenoids, fiber, soluble sugars, minerals, glucosinolates and phenolic compounds [4,5]. Antioxidant enzymes, convert reactive oxygen species into nonreactive oxygen molecules [6]. The most important antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Fruits and vegetables are good sources of natural antioxidants such as vitamins, carotenoids, flavonoids and other phenolic compounds [7,8]. Human health benefits associated to *Brassica* consumption could be explained, in part, by their dietary antioxidants and consequently,

Brassica crops have been the focus of intense research based on the content of secondary metabolites [9,10]. The antioxidant potential of *Brassica* vegetables is high compared to other vegetable crops. The methanol extract of these vegetables show highest antioxidant activity against oxidative stress releated diseases.

In the present work, an attempt has been made to explore the phenolic content, flavonoid content, antioxidant activity of *Brassica oleracea* and characterization of the active compounds using FT-IR.

EXPERIMENTAL SECTION

Plant material

Fresh Red cabbage (*Brassica oleracea)* vegetables were purchased from a local market in Tamil Nadu-Chennai.

Chemicals and reagents

Methanol, 5% NaNO2, 10% Aluminium chloride, NaOH, Folin-Ciocalteau, Sodium carbonate (Na2CO3), FRAP reagent, Deionized water, Gallic acid, Quercetin, Potassium chloride, Sodium acetate, DPPH solution (2,2-diphenyl-1-picrylhydrazyl), Potassium ferricyanide, Phosphate buffer, 10% trichloroacetic acid, FeCl3, FeSO4.

Preparation of Extract

Fresh *Brassica oleracea* was cut in half. Then each vegetable was separately cut into small pieces and grounded with the usage of kitchen blender. Each of the slurries (200 g) was added to a glass beaker and homogenized with 200 ml of methanol. The mixture was incubated at 45°C within the water bath with gentle stirring for 30 min. The homogenate was filtered through Whatman No.1filter paper to obtain a clear supernatant. The supernatant become transferred to a clean flask and the residue became blended with another 100 ml of methanol to repeat the extraction. The resulting supernatant changed into mixed with the preceding one. The methanol inside the supernatant evaporated beneath vacuum at 45°C using a vacuum centrifuge evaporator and the extract decreased to 100 ml. The solution was sealed and stored at 4°C until use [11].

Total phenolic content (TPC)

The concentration of phenolic content in Methanol extract was expressed as micrograms of Gallic acid equivalents,determined with Folin–Ciocalteu reagent (FCR), according to the method of Slinkard and Singleton [12]. 1 mL of the solution containing 1 mg of the tested extract in methanol was added to 46 mL of distilled water and 1 mL of FCR, and mixed thoroughly. After 3 minutes, 3 mL of sodium carbonate solution (2%) were added to the mixture and shaken intermittently for 2 hours at room temperature. The absorbance was read at 760 nm. The concentration of phenolic compounds was calculated according to the following equation that was obtained from the standard pyrocatechol graph:

Absorbance = 0.001 µg Gallic acid + 0.0095 (r^2 =0.988)

Total flavonoid content (TFC)

Measurement of flavonoid concentration of the extract was based on the method described by Park et al. in 1997 with a slight modification [13], and result was expressed as quercetin equivalents. An aliquot of 1 mL of the solution (contains 1 mg of extract in methanol) was added to test tubes containing 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1 M potassium acetate and 3.8 mL of ethanol. After 40 minutes at room temperature, the absorbance was determined at 415 nm. Quercetin was used as a standard. The concentration of flavonoid compounds was calculated according to following equation that was obtained from the standard quercetin graph.

Absorbance = 0.0046 µg Quercetin + 0.0149 (r^2 = 0.996)

Antioxidant activity by DPPH free radical scavenging assay:

The extract was taken at various concentrations (100, 200, 300, 400 and 500 µl), in small tubes and made upto 1ml using methanol. 1 ml of 0.01 mM DPPH dissolved in methanol was added to all the test concentrations and maintained within the dark for 30 min, at room temperature. The absorbance of the solution was read at 517 nm. The percentage inhibition and the IC_{50} values were calculated. The concentration of dry material per ml of solvent that inhibits the formation of DPPH radicals by 50% is defined as IC_{50} value[14].

$$\%\text{Inhibition} = \frac{(\text{Absorbance of the control} - \text{Absorbance of the sample}) \times 100}{\text{Absorbance of the control}}$$

Antioxidant activity by FRAP Assay:

1 ml of plant extract, 2.5 ml phosphate buffer (of 0.2 M, pH 7)and 1% potassium ferricyanide (2.5 ml) have been combined and incubated at 50◦C for 30 min. To the solution, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 6500 rpm for 10 min. Distilled water (2.5 ml) and 0.5 ml of 0.1% FeCl3were added to 2.5 ml of the supernatant. The absorbance of the solution was measured at 700 nm using UV–visible spectrophotometer. The reducing capability of the plant was evaluated in terms of percentage by relating to the standard, FeSO4 [15].

$$\%\text{Inhibition} = \frac{(\text{Absorbance of the control} - \text{Absorbance of the sample}) \times 100}{\text{Absorbance of the control}}$$

FTIR Spectroscopic analysis:

The extracts were examined under visible and UV light for proximate analysis. For FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper by using a high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. Then the FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm-1 and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

Statistical analysis

The experimental results are expressed as mean ± standard deviation of triplicate measurement and the results are processed using Graph pad prism 6.0 version.

RESULTS AND DISCUSSION

Total phenolic content (TPC)

Lipid oxidation is stabilized with polyphenolic compounds having antioxidant activity [16]. Polyphenolic compounds are also indispensable due to having inhibitory effects on carcinogenesis and mutagenesis in human. Therefore, the amount of phenolics in the studied extracts was measured by Folin–Ciocalteu method. The concentration of phenolics in the extract was expressed as micrograms of gallic acid equivalents per milligrams of the extract. Total phenolic content of methanol extract was found to be 14.56±0.76 According to Ahmed Elkhalifa Chemsa[17] *et al* shows Gallic acid equivalents of total phenolics of *B. oleracea* extracts. Content of phenolic compounds in the extracts varied from18.22 ± 4.44 µg GAE mg-1 in ethyl acetate extract 199.80 ± 0.55 µg GAE mg-1 in butanol extract. As displayed in Table 1, the total phenolic content (TPC) of the methanol extract was 14.56±0.76. Gaafar *et al*., 2014[18] reported that the red cabbage and white cabbage of methanolic extracts contained 29.13 to 11.36 mgGAE total phenolics/g DW.

Total flavonoid content (TFC)

Flavonoids are the major components of the phenolic compounds. The total flavonoid content (TFC) extracts were estimated by using aluminum nitrate colorimetric assay. The concentration of flavonoids in the extract was expressed as micrograms of quercetin equivalents per milligrams of the extract (Table 1). Total flavonoid content of methanol extract was found to be 22.65±1.43 µg QE mg-1. According to Gaafar *et al*.,2014[18] reported that the red cabbage and white cabbage of methanolic extracts of total flavonoid contained 17.44 to 4.37 mgQE total flavonoid/gDW respectively.

Table 1: Extraction yield, total phenolic and flavonoid of *B.oleracea* extracts

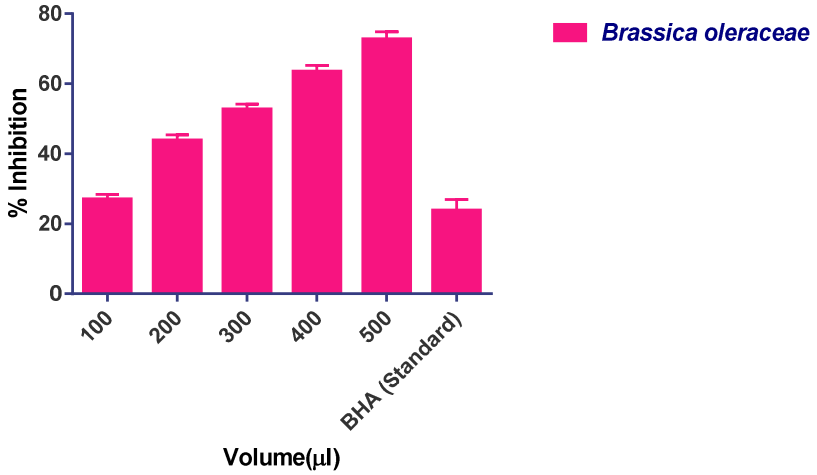
Samples	Yield(%)	Total Phenolic Content (µg GAE mg-1)	Total Flavonoid Content (µg QE mg-1)
Methanol Extract	17.68%	14.56±0.76	22.65±1.43

DPPH radical scavenging activity

The free radical scavenging ability of the methanolic extract of the vegetable extract was performed using DPPH. The IC50value of *Brassica oleraceae* was recorded as 200.453 µl. The red cabbage aqueous extract contained highest DPPH activity ranged from (62.51 to 89.04 µg/ml), from (31.58 to 59.42 µg/ml) in white cabbage. In this

study red cabbage has highest antioxidant activity than white cabbage. (Raghu KL *et al*.,2011)[19]. According to Turkmen et al[20] Antioxidant activity of fresh vegetables as determined by the DPPH radical scavenging method decreased in the order: broccoli>pepper>spinach>green beans>peas>squash>leek. Among all these test vegetables broccoli showed highest scavenging activity with a inhibition of 78.17% whereas leek had lowest activity with 12.20%.

Fig 1: Antioxidant activity of *Brassica oleracea* by DPPH Assay



Ferric reducing antioxidant potential (FRAP Assay)

FRAP assay is based on the ability of phenolics to reduce Fe3+ to Fe2+. In Table 2 Ferric antioxidant potential assay showed Methanol extract of B.oleracea was found to be 54.16±0.60 when compared to Ascorbic acid 34.13±0.15. According to Abha Shukla et al[21] leaves of *Casearia tomentosa* extract showed Hydroalcoholic 43.12 ± 0.60 when compared to Ascorbic acid 39.45 ± 0.14. In this study hydroalcoholic extract showed highest ferric antioxidant power when compared to ascorbic acid. Yazdizadeh et al.,2012[22] reported that the extracts from sweet bell pepper possess antioxidant and antiradical activity, which could vary in different varieties may be helpful in preventing or slowing the progress of various oxidative stress-related diseases.

Table 2: Ferric reducing antioxidant potential (FRAP Assay) of *B.oleracea* extract compared with ascorbic acid

S.No	Extract/Standard	Ferric reducing Antioxidant power (µM/ml)
1.	Methanol	54.16±0.60
2.	Ascorbic acid	34.13±0.15

FTIR Analysis

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of phenol, alkanes, aldehyde, secondary alcohol, amino acid, aromatic amines and halogen compound. The results of this study offer a platform of using *Brassica oleracea* pulp as herbal alternative for various diseases including diabetic, cardiovascular etc. And our results were similar to a study by Karpagasundari [23]. The bioactive principle of the Red Cabbage is useful for treating therapeutic infections.

Fig 2: FTIR spectrum of Brassica oleracea methanolic extract

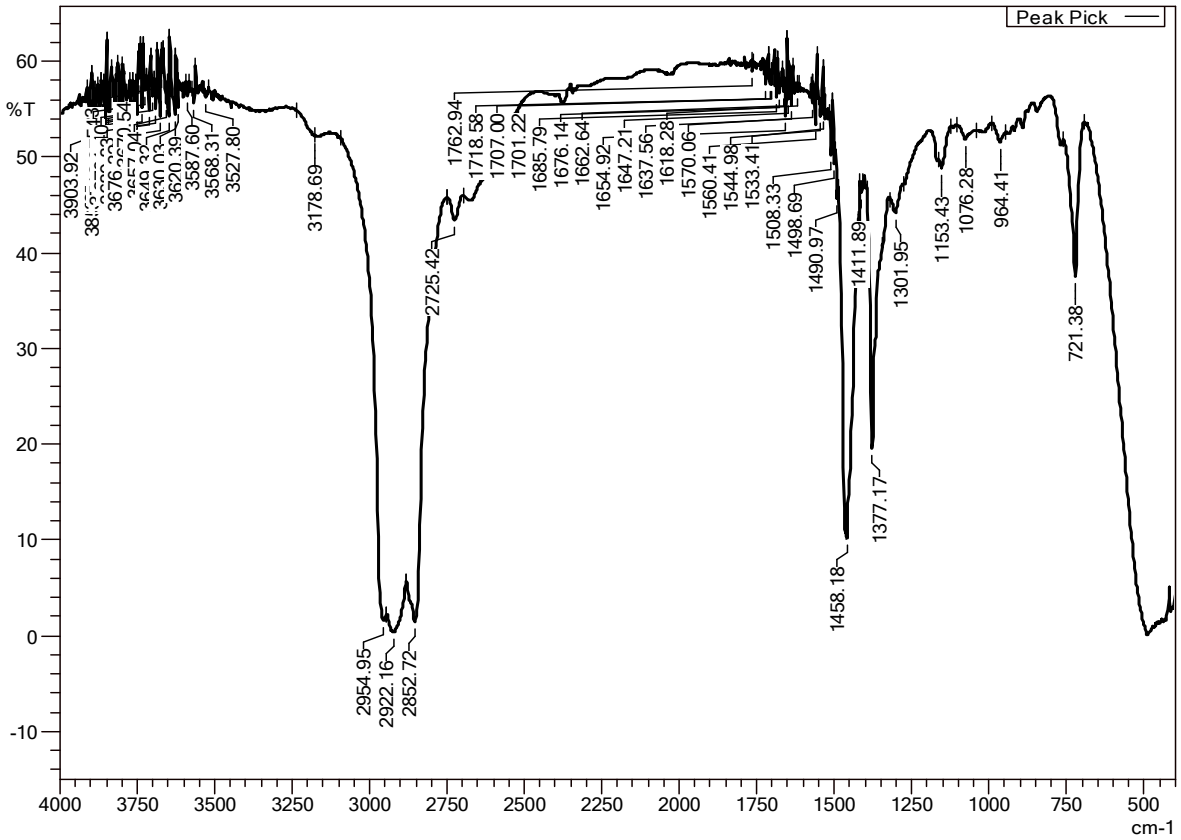


Table 3: FTIR peak values for methanol extract of *Brassica oleracea*

S.No	Peak values	Functional Groups
1.	3178.69	Alkynes
2.	2954.96	Alkanes
3.	2922.16	Alkanes
4.	2725.42	Aldehydes
5.	1458.18	Alkanes
6.	1411.89	Aromatics
7.	1153.43	Alkyl halides
8.	1076.28	Aliphatic amines
9.	964.41	Alkenes
10.	721.38	Alkanes

CONCLUSION

The present results revealed that the methanolic extract of *Brassica oleracea* showed good antioxidant on flavonoid content than phenolic content. Therefore vegetables of this family possess a high potential to manage against oxidative stress and thus act as strong anticancerous as well as antidegenerative foods.

Acknowledgements
Authors are thankful to Department of Biotechnology, Jeppiaar Engineering College, Chennai for providing all the required facilities that helped us for the successful completion of the project work.

REFERENCES

[1] Shalabia Shahat Emam; Heba Ibrahim Abd El-Moaty, *Journal of Applied Sciences Research.,* **2009,** 5(12), 2315-2322.
[2] A Theresa Shapiro; W Jed Fahey; L Kristina Wade; K Katherine Stephenson; Paul Talalay, *Cancer Epidemiology, Biomarkers & Prevention.,* **2001**, 10, 501-508.
[3] A.J Verlangieri; J.C Kapeghian; S. el-Dean; M. Bush, *Medical Hypotheses.*, **1985**, 16(1), 7-15.
[4] A Podsedek; *Food sci Technol*., **2007,** 40, 1-11.
[5] M Jahangir; HK Kim; YH Choi; R Verpoorte, Compr Rev *Food Sci Food Saf*., **2009,** 8, 31-43.
[6] J Lee; N Koo; DB Min, *Compr Rev Food Sci Food Saf.,* **2004**, 3, 21-33.
[7] R.C Minussi; M Rossi; L Bologna; L Cordi; D Rotilio; G.M Pastore, *Food Chemistry.*, **2003**, 82, 409-416.
[8] D Zhang; Y Hamauzu, *Food Chemistry.*, **2004**, 88, 503-509.
[9] M Traka; R Mithen, *Phytochemistry*., **2009**, 8, 269-282.
[10] R Verkerk; M Schreiner; A Krumbein; E Ciska; B Holst; I Rowland; M Dekker, *Food Research.*, **2009**, 53, S219.
[11] T Sun; Z Xu; CT Wu; M Janes;W Prinyawiwatkul; HK No, *J Food Sci*.,**2007**,72, S98-102.
[12] K Slinkard, VL Singleton, *Am. J. Enol. Vitic.*, **1977**, 28(1), 49-55.
[13] YK Park, MH Koo, M Ikegaki, J Contado, *Braz. Arch. Biol. Technol*., **1997**, 40(1), 97-106.
[14] NK Udayaprakash; S Bhuvaneswari, N Sripriya; L Prameela; R Bhagya; B Radhika; A Balamurugan; S Arokiyaraj, *Int. J. Pharm. Pharm. Sci*.,**2014**, 6, 128–132.
[15] Kalita; B.K Tapan; TK Pal; R Kalita, *J. Drug Delivery Ther*.,**2013**, 3, 33–37.
[16] F Shahidi, P Janitha, P Wanasundara, *Crit. Rev. Food Sci. Nutr.*, **1992**, 32(1), 67-103.
[17] Ahmed Elkhalifa Chemsa, Sara Derdouri; Zohra Labbi; Smail Acila;
Djilani Ghemam Amara; Atef Chouikh; Khaled Kherraz; Ahmed Allali; Amar Zellagui, *Journal of Chemical and Pharmaceutical Research*, **2016**, 8(4): 1317-1321.
[18] Alaa A Gaafar, Hanan F. Aly, Zeinab A. Salama, *World Journal of Pharmaceutical Research*, **2014**,Vol 3, Issue 4,171-186.
[19] KL Raghu; CK Ramesh; TR Srinivasa; KS Jamuna, *Asian Journal of Experimental Biological Science*, **2011**, 2, 58-62.
[20] Nihal Turkmen; Ferda Sari; Y.Sedat Velioglu, *Food Chemistry*, **2005**, 93, 713-718.
[21] Abha Shukla; Ritu Tyagi; Swati Vats; Rishi Kumar Shukla, Journal of Chemical and Pharmaceutical Research, **2016**, 8(1):136-141.
[22] Narmin Yazdizadeh Shotorbani; Rashid Jamei; Reza Heidari, *Avicenna Journal of Phytomedicine* , **2012**,Vol **3**, 25-34.
[23] C. Karpagasundari; S. Kulothungan, *Journal of Pharmacognosy and Phytochemistry*, **2014**, 3(4): 196-201.