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Free Radical Scavenging Activity of Methanolic and Ethyl Acetate Extracts of *Brassica oleracea* and its Antioxidant Role in Combating Degenerative Diseases

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Abstract: Focus on natural antioxidants in medicinal plants are gaining momentum in present days reflecting the immense valuability of Ayurveda, Naturopathy and Siddha medicine in treatment of ailments. The role played by free radicals which are highly reactive oxygen species has become increasingly relevant in etiology of degenerative diseases. The present study will focus on the antioxidant potential of methanolic and ethyl acetate extract of *Brassica oleracea* (var. *italica)* inflorescence which contribute to the scavenging of free radicals. *In vitro* free radical scavenging potential of aqueous extracts of *Brassica oleracea* inflorescence was assessed by 2,2-azino-bis (3-ethylbenzthiazoline -6-sulfonic acid, ABTS), 1,1-diphenyl-2-picryl hydrazyl (DPPH) and metal ion chelating assay (20, 40, 60, 80 and 100 µg/ml). Antioxidant potential was found more in the methanolic extracts of the florets in the present study.

Keywords: *Brassica oleracea,* Inflorescence, Free radical activity, ABTS, DPPH, Metal chelating assay

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Introduction

Herbals are considered to be promising source of medicine in the traditional healthcare system. The efficacy and safety of herbal medicine have turned the major pharmaceutical population towards medicinal plant research. There is a need for more effective, less toxic and cost effective antioxidants and antimicrobials from natural sources to treat various non- communicable and communicable diseases (Singh *et al*., 2002).

Cruciferous vegetables contain several hydrophilic and lipophilic antioxidant compounds and it is important to estimate the antioxidant

activity using different methods. Broccoli (*Brassica oleracea* var. *italica*) is a nutritionally important crop grown all over the world. Glucosinolates have been found to have anti-cancer properties. Neutraceutical and functional food ingredients have been popularly used in health products. The most important nutraceutical formulations contain vitamins and minerals in combination with antioxidant such as carotenoids, omega-3 fatty acids, glucosinolates and/or degradants in the finished products (Hedges and Lister, 2006; Soengas *et al*., 2011; Simlai *et al*., 2014). They may act together more effectively than singly because they function synergistically and are capable of quenching free radicals in both aqueous and lipid phases (Ohr, 2004). The role played by free radicals which are highly reactive oxygen species has become increasingly relevant. They initiate or propagate development of diseases such as cancer, liver and cardio-vascular disorders (Uttara *et al*., 2009). The oxidation induced by reactive oxygen species can result in cell membrane disintegration, membrane protein damage and DNA mutation. Antioxidants with free radical scavenging activity play an important role in protecting damage by reactive oxygen species (Darkwah *et al*., 2018).

Antioxidant compounds may also act as metal chelators and interfere with the pathways that regulate cell division and proliferation and detoxification; they also may regulate inflammatory and immune responses and may have antiulcerative properties (Trombino *et al*., 2004). They may inhibit or activate a large variety of mammalian enzyme systems, exhibiting biphasic dose responses in cells at low doses. Phytochemicals activate signaling pathways that result in the increased expression of genes encoding cytoprotective proteins, including antioxidant enzymes, protein growth factors, and mitochondrial proteins (Dragsted *et al.*, 2006). The aim of the present study was to evaluate and assess the free radical scavenging and antioxidant potential of the florets of cruciferous vegetable, *Brassica oleracea*.

Materials and Methods

Collection and Identification of Plant Material:

Inflorescence of *Brassica oleracea* were purchased from wholesale market, Chennai, Tamil Nadu during April 2021. Fresh plant specimens collected were authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, Tambaram, Chennai (Registration No. PARC/2021/4704).

Processing and Preservation of Plant Materials:

About one kg broccoli was weighed, the inflorescence was washed under running tap water followed by washing with distilled water to remove the surface debris. The heads were obtained by cutting the main stalk. The florets, together with about 1 cm of the stalk were cut off from the rest of the stalk and used for crude sample extraction. Finally, the above-prepared vegetable samples were chopped into small pieces using a cutter and later minced using a mixer grinder and finely macerated. After homogenization, it was extracted in 70% methanol and ethyl acetate solvent for 7 days in the dark at normal room temperature with intermittent shaking. After 7 days, the whole extracts were filtered using muslin cloth at first and then through Whatman No. 1 filter paper, and the filtrate was concentrated using rotary evaporator. The yield of crude extracts obtained was noted, stored in desiccators for maximum of 3 days. The crude extracts were later preserved in a deep freezer (−20°C) for further use (Jamuna *et al*., 2015).

Determination of in vitro Free Radical scavenging Activity of Brassica oleracea:

The methanolic and ethyl acetate solvent extracts of *Brassica oleracea* florets was assayed for free radical activity by the methods such as ABTS radical scavenging activity (Re *et al*., 1999), DPPH radical scavenging assay (Clarke *et al*., 2013) and metal chelation assay (Soler-Rivas *et al*., 2000). Respective standards were used for each assay.

Table 1: ABTS radical scavenging activity of methanolic and ethyl acetate extract of *Brassica oleracea*

Concentration (µl/ml)	Standard Ascorbic acid	Methanolic extract	Ethyl acetate extract
20	46.16±0.52	54.06±0.55	4.97±0.13
40	48.70±0.50	71.95±0.93	16.79±0.48
60	62.87±0.55	73.40±0.75	25.63±0.53
80	71.69±0.50	66.18±0.65	42.01±0.31
100	79.79±0.61	69.21±0.78	53.88±0.70

Values are Mean ± SD of three (n = 3) independent analysis of the extract; Values expressed as % radical scavenging activity

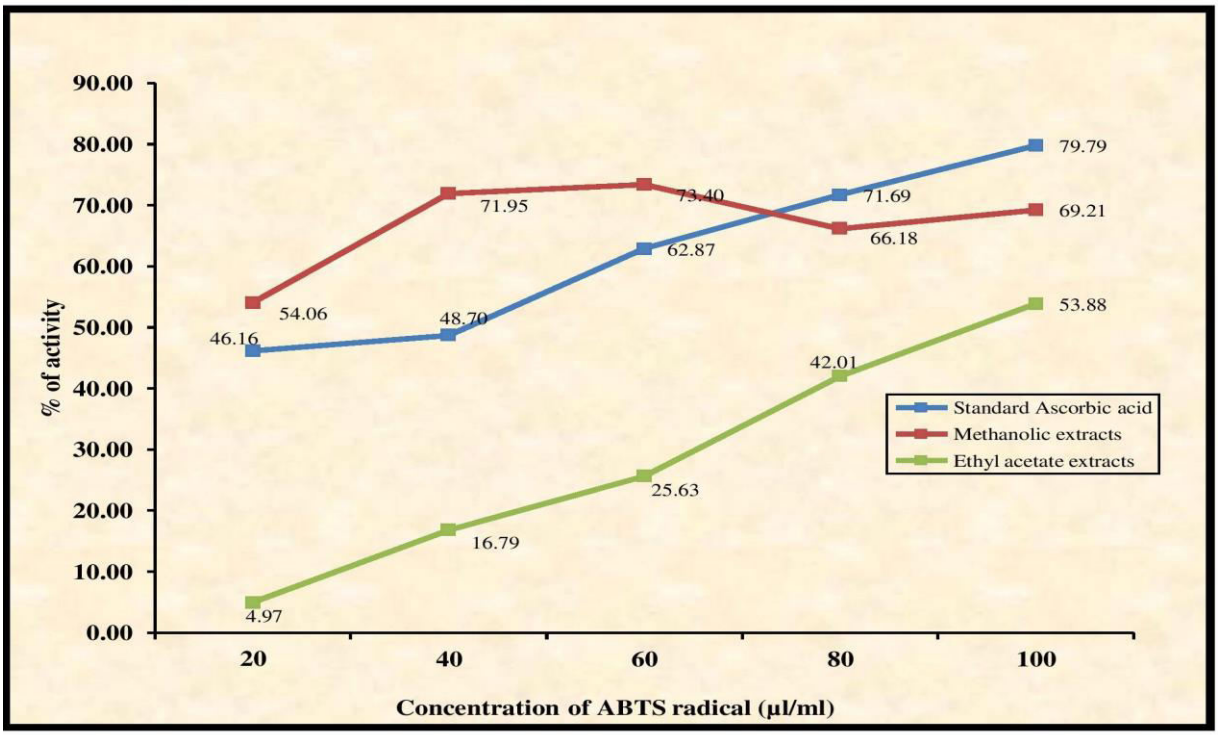

Fig. 1: ABTS radical scavenging activity of methanolic and ethyl acetate extract of *Brassica oleracea.*

Results

The antioxidant potential of the aqueous extracts of *Brassica oleracea* florets was assessed at various concentrations of 20 µl, 40 µl, 60 µl, 80 µl and 100 µl by *in vitro* studies.

ABTS radical scavenging activity:

The reaction capability of ABTS radical scavenging activity was determined by the percentage inhibition induced by antioxidants at 20–100 µl concentration of methanolic and ethyl acetate extract of *Brassica oleracea* florets. At lowest concentration of 20 µl methanolic extracts showed 54.06 % inhibition while ethyl acetate extracts showed 4.97 % inhibition, at highest concentration of 100 µl 69.21 % and 53.88 % inhibition was recorded, respectively (Table 1, Fig. 1). Methanolic extract of *Brassica oleracea* showed remarkable results up to 60 µl concentration while a decline was noticed in 80 µl and 100 µl when

Table 2: DPPH radical scavenging activity of methanolic and ethyl acetate extract of *Brassica oleracea.*

Concentration (µl/ml)	Standard Quercetin	Methanolic extract	Ethyl acetate extract
20	23.58±0.51	23.11±0.38	37.18±0.64
40	42.05±0.88	31.21±0.64	40.73±0.64
60	48.27±0.49	36.01±0.77	41.57±0.38
80	72.76±0.57	42.73±0.75	44.58±0.49
100	92.15±0.81	48.24±0.91	56.07±0.23

Values are Mean ± SD of three (n = 3) independent analysis of the extract; Values expressed as % radical scavenging activity

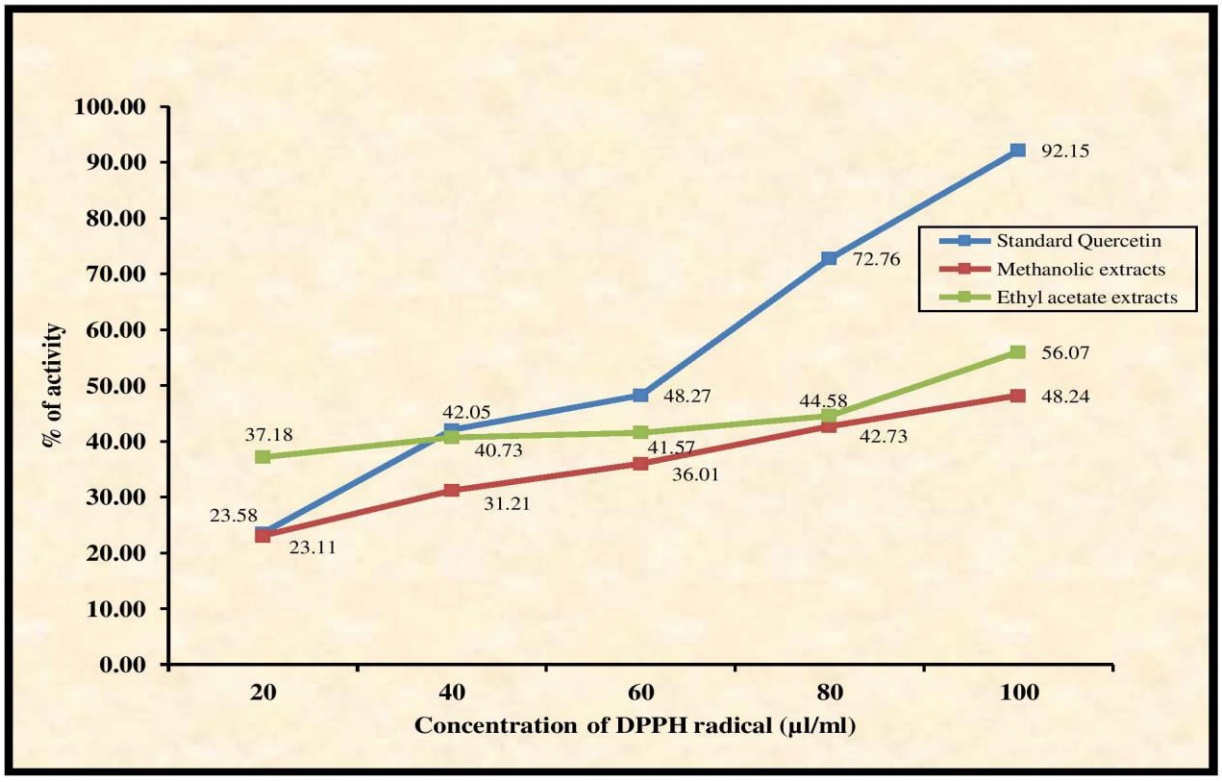

Fig. 2: DPPH radical scavenging activity of methanolic and ethyl acetate extract of *Brassica oleracea.*

compared to ethyl acetate extracts and standard ascorbic acid.

DPPH radical scavenging activity:

At 20–100 µl the superoxide radical scavenging activity of both extracts of *Brassica oleracea* florets varied from 23.11 % - 48.24 % in methanolic extracts and 37.18 – 56.07 % in ethyl acetate extracts when compared with standard quercetin. From 20 µl to 100 µl superoxide anion radical scavenging activity was higher in the ethyl acetate extracts of *Brassica oleracea* florets (Table 2; Fig. 2).

Ion chelation assay:

Ion chelation activity of both solvent extracts of *Brassica oleracea* florets was minimal at 20 µl concentration of the extract, whereas the maximum activity was observed at 100 µl concentration of the extract (Table 3; Fig. 3). The percentage antioxidant potential was higher in the methanolic extracts of *Brassica oleracea* florets

Table 3: Ion chelation assay of methanolic and ethyl acetate extracts of *Brassica oleracea*

Concentration (µg/ml)	Standard Na_2 EDTA	Methanolic extracts	Ethyl acetate extract
20	41.99±0.22	44.71±0.83	27.81±0.59
40	47.08±0.78	54.85±0.54	40.86±0.33
60	56.49±0.19	58.85±0.55	45.24±0.99
80	67.72±0.92	66.71±0.50	47.02±0.27
100	91.26±0.40	70.80±0.47	65.87±0.77

Values are Mean ± SD of three (n = 3) independent analysis of the extract; Values expressed as % radical scavenging activity

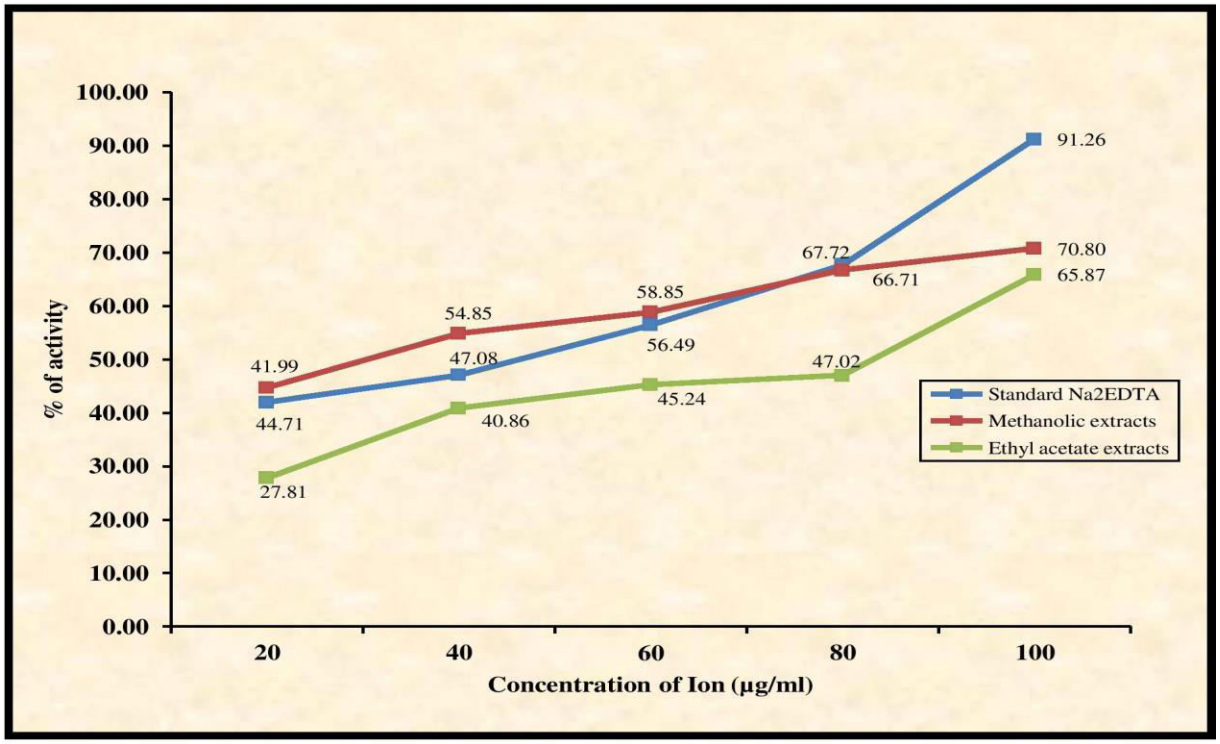

Fig. 3: Ion chelation assay of *Brassica oleracea* solvent extracts.

when compared with the ethyl acetate extracts. Na_2 EDTA was used as standard.

Discussion

Cruciferous vegetables belongs to the Brassicaceae family. *Brassica* vegetables have medicinal, pharmaceutical as antioxidant and anticancer properties that are beneficial to the general human health, due to their content of diverse and widely useful natural compounds such as secondary metabolite compounds include phenols, flavonoids, terpenes, vitamins and plant pigments which include carotenoids, anthocyanins, lycopene and tocopherol (Sousa *et al*., 2009; Ahmed *et al* ., 2012; Kamal *et al*., 2015). One of the most significant health concerns of cruciferous vegetables is the presence of biologically active compounds, glucosinolates. Due to the presence of

these compounds, *Brassica* plants show biological activities against various diseases and have been found to be effective in treating various diseases in human. The edible parts of these plants show anti-microbial, anti-bacterial, anti-diabetic, anti-malarial, anti-aging, anti-ulcer, anti-hyper-glycemic, anti-hyperlipidemic, anti-proliferative, neuroprotective, antidiabetic, anti-genotoxic and antioxidant activities (Fidrianny *et al*., 2014; Sharma *et al* ., 2015).

Methanolic extracts show higher ABTS radical scavenging activity and ferrous ion chelating ability, Ethyl acetate were considerably less effective as radical scavenger compared to methanolic extracts. Methanol may be the best solvent for such purpose. This result is in accordance with the findings of other researchers (Kapusta-Duch *et al*., 2012). Oxidative stress causes many damages to the human body, which leads to many diseases and can be prevented by consuming foods rich in natural antioxidants such as vegetables and fruits (Hwang and Lim, 2015; Santos-Sanchez *et a*l., 2019). Antioxidants can intervene with the oxidation process by interacting with free radicals, chelating catalytic metals, and also by acting as oxygen scavengers (Nawaz *et al* ., 2018)

DPPH is widely used to evaluate the free radical scavenging capacity. The antioxidant potential of methanolic, ethyl acetate extracts and quercetin as reference solution was evaluated for its ability to quench the synthetic DPPH radical in the present study. The ethyl acetate extracts of *Brassica oleracea* florets exhibited an increased percentage of concentration- dependent radical scavenging activity.

The antioxidant potential increased with an increase in the concentration of the ethyl acetate extracts. Dose-dependent DPPH scavenging activity was recorded. The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity (Fitriansyah *et al.,* 2018). Earlier studies showed that hydro-alcoholic extract of *Desmodium gangeticum* have the proton-donating ability and can serve as free radical inhibitors or scavengers, acting possibly as primary antioxidant (Usha and Suriyavathana, 2012).

The results of the recent research clearly indicated the importance of fruit and vegetables as the richest potential source of these substances and emphasize the need to increase the proportion of these products in the diet. A prominent role in this process is played by the popular cruciferous vegetables, which contain several bioactive compounds and which not only act as antioxidants, but also have other health promoting properties (Podsedek, 2007; Sturm and Wagner, 2017). The data, therefore suggested that the extracts of Cruciferae are a potential source of natural antioxidants. This is due to the presence of phytoconstituents, viz. alkaloids, sterols, phenolic compounds (flavonoids), glycosides and glucosinolates which have been known for their antioxidant property (Sosnowska *et al*., 2006).

Conclusion

Plants are considered to be promising source of medicine in the traditional healthcare system. The present study indicated the antioxidant potential of methanolic and ethyl acetate extracts *Brassica oleracea* florets paves way to test whether it serves as inhibitors, scavengers or act as primary antioxidants in prevention of free radical formation. From the results, it can be concluded that the Cruciferae vegetables have potent antioxidant activity contributing to the use for health benefits in addition to their nutritive role as a vegetable.

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