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In vitro antioxidant activity and total phenolic of *Brassica oleracea* var. *gemmifera* (Brussel sprouts)

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ABSTRACT

Aqueous and ethanolic extracts of brussel sprouts were investigated with different in vitro antioxidant activity test including diphenly picryl hydrazyl radical scavenging, lipid peroxidation, reducing power, superoxide anion radical scavenging, and metal chelation. Ethanolic extract showed strong inhibition of DPPH radical scavenging activity than aqueous with IC $_{50}$ of 1mg/ml and 1.2 mg/ml. Furthermore, both ethanolic and aqueous showed equipotent activity (IC $_{50}$ 0.4mg/ml) toward lipid peroxidation of microsomes isolated from rat liver homogenate induced by the $FeCl_2$- H_2O_2 model. Inhibition of superoxide anion and metal chelation of aqueous and ethanolic extracts showed IC $_{50}$ of 0.5 mg/ml, 0.6 mg/ml and 0.2 mg/ml, 0.3 mg/ml respectively. The extracts also showed a strong reducing activity compared to positive control, ethylenediaminetetraacetic acid (EDTA).

Keywords: Brussel sprouts, antioxidant activity, polyphenols.

INTRODUCTION

Free radical is an atom or group of atoms that have one or more unpaired electrons. They are formed as intermediates in a variety of normal aerobic life, but when generated in excess can damage macromolecules proteins, DNA, carbohydrates.

Oxidative stress, induced by free radicals, is known to cause several degenerative diseases such as cardiovascular, cataracts, Parkinson, diabetics [1]. Radicals derived from oxygen are known as Reactive oxygen species (ROS) sequential reduction of molecular oxygen leads to the formation of superoxide anion (.O2_), hydroxyl radical (.OH) and hydrogen peroxide (H_2O_2) which are highly reactive that targets peroxidation of unsaturated fatty acid present in membrane phospholipids leading to damage to membrane lipids, resulting in the accumulation of lipid peroxides, which further react with fatty acids and proteins.

Antioxidants are molecules that protect cells from the damage caused by the unstable free radical. They possess neutralizing effects by scavenging ROS, and chelating detoxifying enzymes, to prevent the generation of ROS [2]. At present there is a huge demand towards phytochemical derived from plants, since they have wide spectrum of health promoting properties which ameliorates free radical induced degenerative diseases [3]. Plant derived extracts comprises many components including phenolics, flavanoids, tannins, saponins, nitrogen compounds, lignin,

glycosides etc [4]. The antioxidant properties of several plants materials have been reported [5-6]. Therefore there is a thrust for new phytochemicals, antioxidant components that regulate the free radical damage.

Brassica oleracea var commonly known as brussel sprouts belongs to the family brassicaceae, cultivated commonly in Unites States, cannada, Europe etc, which is consumed as a common vegetable in daily life. Earlier reports have shown that it is rich in bioactive ingredients such as, sulforaphane, glucosinolate sinigrin, indole 3- carbinol that has anticancer properties, DNA repair mechanisms [7]. However, there is no exploration regarding the role of brussel sprout in free radical scavenging. Hence the present work investigates the crude extracts of aqueous and ethanolic of brussel sprout in various *in vitro* antioxidant assays systems, such as DPPH/superoxide scavenging, metal chelation, superoxide dismutase, reducing power and inhibition of lipid peroxidation to understand the amelioration of oxidative stress induced by free radical.

MATERIALS AND METHODS

Chemicals

Nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), Butylated hydroxyltoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid (TBA) and were purchased from M/sSigma Chemicals Co. (St.Louis, MO). Ferrozine, nicotinamide adenine dinucleotide-reduced (NADH), trichloroaceticacid (TCA), deoxyribose (DR), potassium ferricyanide, ethylenediamine tetra-acetic acid (EDTA) and ferric chloride were purchased from M/s Sisco Research Laboratories, Mumbai, India. All solvents used were of analytical grade.

Extraction of aqueous and ethanolic extracts of brussel sprouts

Brussel sprouts acquired from market were washed two to three times with running water and after that dried at room temperature. Further, it was cut into little pieces and permitted to dry in hot air oven at temperature 50^0C for three days. Dried material was grinded to fine powder. Fine powdered material was extracted with Luke warm distill water, by keeping in temperature regulated orbital shaker overnight. Later it was filtered utilizing a muslin material and further, separated through Whatman No 1 filter paper, the resultant concentrate was lyophilized to dryness. Ethanolic extract was done utilizing a soxlet extraction at 50^0C for overnight, extract acquired was concentrated using flash evaporator. The concentrates were kept at 40^0c till utilization.

Inhibition of lipid peroxidation in rat liver microsomes

Liver extracted from adult male Albino rats was homogenized (15-25 g) in 0.02 mol/l tris buffer (Ph 7.4). Microsomes were isolate by the strategy portrayed by [8]. To 100 µl of liver microsomal suspension, 1 mmol/l each of $FeSo_4$ and ascorbic acid were included, with or without of both aqueous and ethanolic extracts in an aggregate volume of 1 ml in 0.1 mol/l phosphate buffer (ph 7.4) and incubated at 37 °c for1 h. After incubation, the reaction mixture was included with 2 ml each of 20% TCA and 1% TBA, accompanied by heating in a water bath for 10 min, cooled and centrifuged. Malondialdehyde (MDA), which is the byproduct, was measured at 535 nm. BHA was utilized as a positive control

DPPH Radical Scavenging Assay

The DPPH assay was measured as described by Guohua et al. [9] with some changes. Different concentrations of both aqueous and ethanolic extracts were blended with of 1 ml DPPH (0.1 mmol/l, in 95% ethanol (v/v)), and the reaction mixture incubated for 30 min at room temperature. The optical density was measured spectrophotometrically at 517 nm against a blank. BHA was utilized as a positive control. Diminish in the absorbance of DPPH demonstrates a higher radical scavenging activity.

Superoxide Anion Scavenging Activity

Superoxide anion was produced by the response of NADH and phenazine methosulphate (PMS) coupled with a reduction of Nitro Blue Tetrazolium chloride (NBT) [10]. The reaction mixture had NBT (100 lM), NADH (300 lm) with or without extracts in an aggregate volume of 1 ml Tris buffer (0.02 M, PH 8.3). The reaction was measured spectrophotometrically at 560 nm each 30 sec for 1 min by including PMS (30 lM) to the mixture.

Reducing Power

The reducing activity of the extracts was measured as per the technique described by Oyaizu [11]. 1 ml of reaction mixture holding extracts of both aqueous and ethanolic in phosphate buffer (0.2 mol/l, ph 6.6) was incubated with 3 ml of 1% potassium ferricyanide at 50 °c for 20 min. After incubating, the reaction was ceased by adding 1 ml of

10% TCA and the mixture was centrifuged at 3,000 rpm for 10 min. The supernatant was blended with distilled water (2.5 ml) and ferric chloride solution (0.1 g/ 100 ml), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance demonstrated increased reducing power.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

DPPH radical might be measured at absorbance maxima at 517 nm. Essentially, it is utilized to screen the antioxidant activity of different samples. It is a stable free radical which has the liking to acknowledge electrons to get stable atoms. Diminish in the absorbance shows the acceptance of electrons which is impelled by the antioxidants. The outcomes are demonstrated in Figure 1. This shows that both aqueous and ethanolic extracts of Brassica oleracea var. gemmifera are equipotent in scavenging the free DPPH radical with an IC_{50} of 1.2-1.0 mg/ml respectively. Extracts activity was low compared with BHA. Scavenging activity of aqueous and ethanolic, were concentration dependent. Percent restraints of aqueous and ethanolic extracts at their most astounding focus were found to be 80-88%. The malignancy anticipation development of the extracts is credited to their hydrogen giving limit [12], which both extracts has. Results suggest that both extracts have potential in scavenging the free radical, which could be attributable to its hydrogen giving capability.

Superoxide radical scavenging

Inhibitory impacts of aqueous and ethanolic extracts of Brassica oleracea var. gemmifera on superoxide radicals are indicated in Figure 2. Scavenging of superoxide radicals with IC_{50} of 0.5 and 0.6 mg/ml was observed in aqueous and ethanolic extracts. Aqueous extract was extraordinarily a more intense forager of superoxide anion than the ethanolic extract. Superoxide radicals are created throughout the typical physiological process, principally in mitochondria. It is well-realized that superoxide anion is a weak oxidant and further undergoes oxidation to give hydroxyl radical and singlet oxygen, which are unsafe, expediting oxidative stress [13-14]. In this way, superoxide radical scavenging by antioxidants has physiological implications

Lipid peroxidation

Inhibition of lipid peroxidation was viewed in both aqueous and ethanolic extracts of Brassica oleracea var. gemmifera with an IC_{50} of 0.40 mg/ml respectively (Figure 3). Oxidation of polyunsaturated fattening acids in the cell layer produces Malondialdehyde (MDA), which is the record of lipid peroxidation and marker of tissue damage. Cell damage can happen in any interior organ by free radicals, prompting different issue, viz., atherosclerosis; hepato and nephro damage [15-16]. Our results demonstrated inhibition of lipid peroxidation with concentration dependent showing that both aqueous and ethanolic extracts have certain antioxidant particles which have the capacity to repair the damage caused by the free radicals. The mechanism in repressing the lipid peroxidation by the extracts could be by counteracting the chain start of polyunsaturated fatty acid chain by giving the hydrogen atom to the damaged lipid bilayer.

Reducing power

The reducing activity of the extracts and BHA qualities are indicated in Figure 4. The extracts demonstrated increase in activity with increase in the concentration of extracts. BHA was more intense, with a concentration of 1 mg indicating most elevated reducing power contrasted with the extracts. Reducing power of the extracts could be acknowledged a marker towards its potential antioxidant. Add upto potential of the antioxidant activity has been ascribed to different components viz., anticipation of chain start, binding of transition metal ion, and restraint of hydrogen abstraction, radical scavenging and avoiding lipid bilayer damage [17-18]. Duh [19] has indicated that reducing power are connected with the vicinity of reductones. Reductones play a major role in antioxidant by giving hydrogen atom and counteracting the free radical chain damage. It also responds with antecedents of peroxide and counteracts the peroxide formation [20]. Results suggest that both the extracts of Brassica oleracea var. gemmifera have potential to free radical damage by donating hydrogen atom thereby preventing oxidative stress.

Metal ion chelation

The ferrous particle chelating impact was indicated by both aqueous and ethanolic concentrates of Brassica oleracea var. gemmifera with IC_{50} values of 0.2 and 0.3 mg/ml, respectively (Figure 5). Extracts were compared with a positive control. It was observed that both the extracts were equipotent towards EDTA, which has IC_{50} of 0.4 mg/ml. Fenton reaction which happens in the vicinity of transition metal (Fe2+) produces different free radicals that are embroiled in some ailments [21]. Our outcome concurs with the consequence reported by Duh et al. [19] proposing

that both the extracts have critical part towards metal chelation, subsequently lessening the centralization of move metal, which expedites lipid peroxidation.

Phenol content

Phenolic substance in the aqueous extract of Brassica oleracea var. gemmifera was higher than that of the ethanolic extract (20.20±5.4 and 12.96±3.7 mg guaicol equal for every gram, separately). Phenols assume a major part in radical scavenging on account of their hydroxyl groups [22]. Downright phenolic substance present in the extract is directly related with antioxidant activity [23]. It is accounted that polyphenolic compound secure people from mutagenesis and carcinogenesis [24]. In our study, there is a relationship between antioxidant action and phenol content. The various antioxidant activities of aqueous and ethanolic extracts of Brassica oleracea var. gemmifera exhibited in this study obviously shows the potential application of nutraceutical.

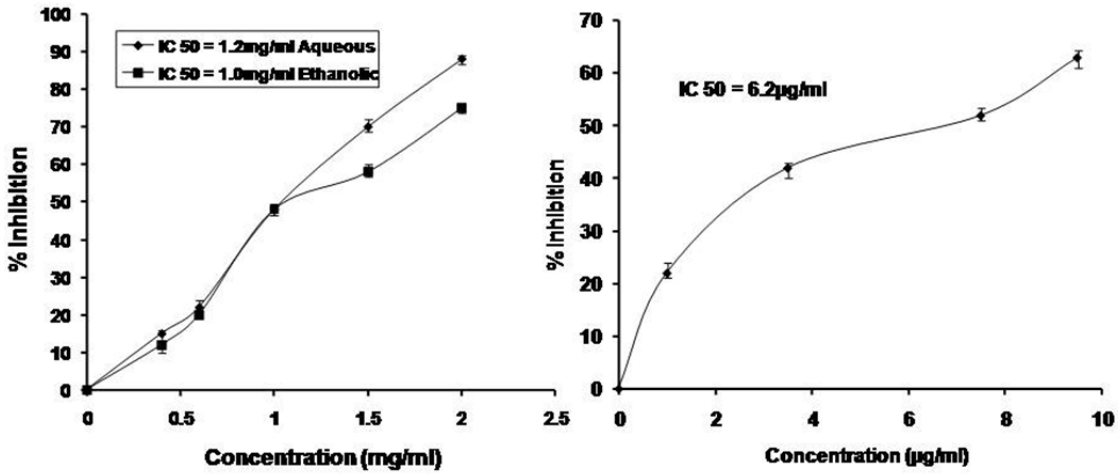

Figure 1: DPPH Radical Scavenging by the Extracts of brussel sprouts

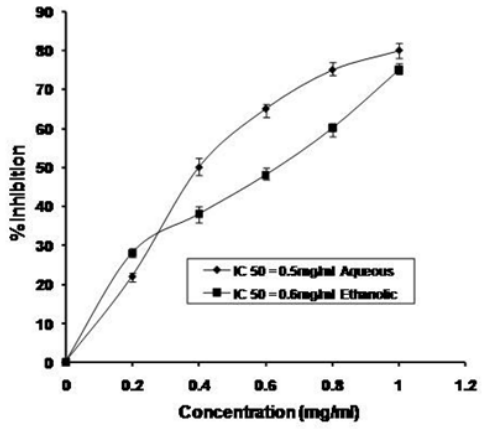

Figure 2: Superoxide Radical Scavenging by the Extracts of brussel sprouts (Aqueous and Ethanolic)

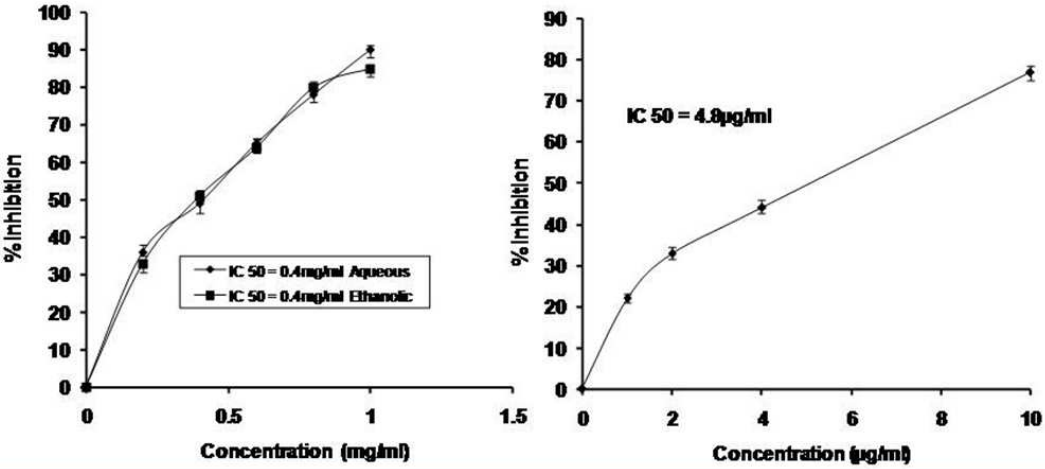

Figure 3: Inhibition of Lipid Peroxidation by the Extracts of brussel sprouts

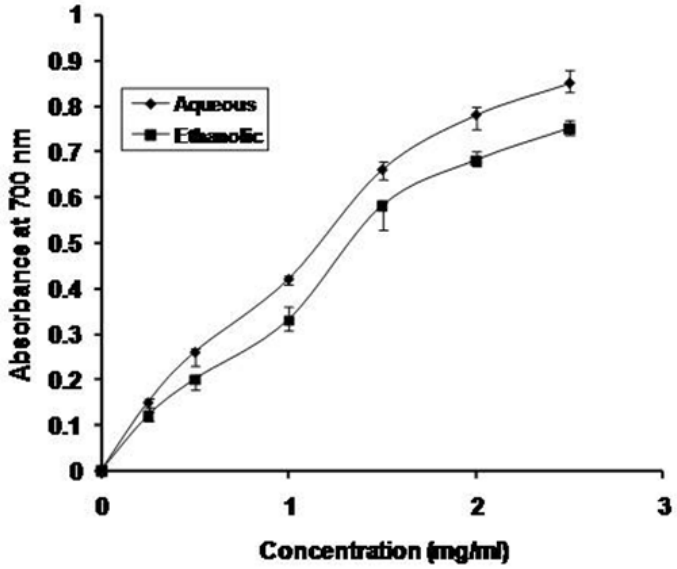

Figure 4: Reducing Power by the Extracts of brussel sprouts

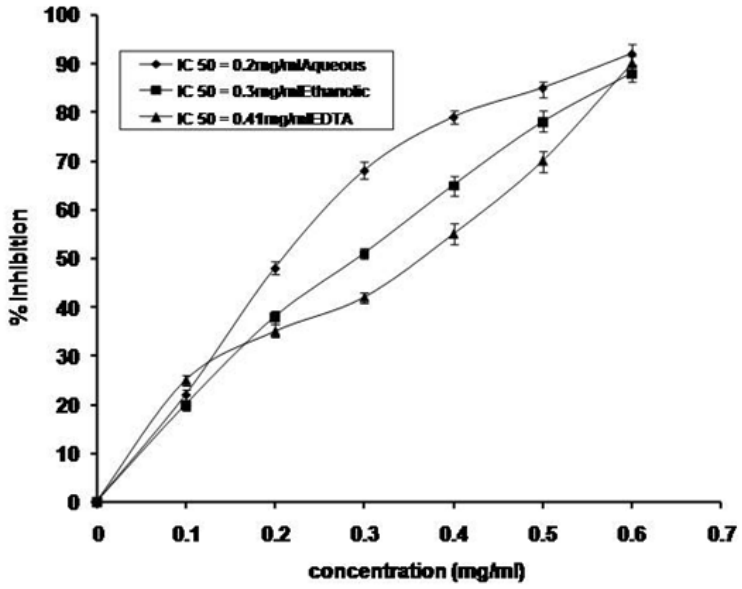

Figure 5: Metal Ion Chelating Activity of Aqueous and Ethanolic Extracts and EDTA

CONCLUSION

The results acquired in the present study infer that the aqueous and ethanolic extracts of Brassica oleracea var. gemmifera might hold various antioxidant biomolecules which can scavenge different ROS/free radicals under in vitro conditions. The expansive extend of action of the extracts recommends that Brassica oleracea var. gemmifera is a wellspring of antioxidants which could be acknowledged as nutraceutical with health promoting properties and prevention of degenerative maladies. Despite the fact that we have not isolated and described the antioxidant molecules answerable for the activity, we conjecture that it could be with the phenolic and nonphenolic molecules exhibit in the extracts. Accordingly, further work will be done to confine and distinguish the successful antioxidant molecules.

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