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Assessment of Antioxidant activity in *Brassica oleracea var. capitata rubra*

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Abstract:
Brassica oleracea var. capitata rubra extract with different solvents [methanol, ethanol. Water, acetone and chloroform] were analyzed for antioxidant activities. The antioxidant activities were evaluated based on the ability of the plant extracts to DPPH radical scavenging assay, FRAP assay, reductive activity, superoxide radical scavenging activity, Lipid peroxidation assay and Nitric oxide scavenging assay. Ascorbic acid is used as standard. It was found that methanol extract (70%) has potent antioxidant when compared to the other extracts. Ethanol and chloroform extract showed the second highest antioxidant activity (59%) followed by acetone extract (25%). Least activity was shown by water extract (22%) in DPPH assay. The results proved that the overall antioxidant activity were highest in methanol extract as compared to others The information of this study helpful to choose the solvent extracts for the further pharmacologic studies.
Key words: *Brassica oleracea var. capitata rubra, antioxidant activity, radical scavenging.*

Introduction

O_2 is an element indispensable for life. Oxidation processes are very important to living organisms. Most of the potentially harmful effects of O_2 are due to the formation of reactive oxygen species (ROS). The uncontrolled production of ROS and the unbalanced mechanism of antioxidant protection result in the onset of many diseases and accelerate ageing. ROS include superoxide anion radical, hydroxyl radical, H_2O_2, singlet O_2. There is a balance between the generation of ROS and inactivation of ROS by antioxidant system in organisms[1,2,3].

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reaction or suppressing formation of free radicals

by binding metal ions, reducing H_2O_2 and quenching superoxide and singlet O_2. Free radicals present in the human organisms cause oxidative damage to different molecules such as lipids, proteins and nucleic acids and thus are involved in the initiation phase of some neurodegenerative diseases, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation, gastric ulcer[4]. Many phytochemicals have antioxidant activity and reduce the risk of many diseases.

Materials and methods:

Vegetable collection:

Red cabbage *(Brassica oleracea var. capitata rubra)* was collected from local markets of Virudhunagar, Tamilnadu, India. The vegetable was washed thoroughly under running tap water to remove dirt and then shade dried at room temperature for a week. They were ground into fine particles after drying and kept in closed container.

Extraction and sample preparation:

Ten grams of ground sample of *Brassica oleracea var. capitata rubra*, was weighed and homogenized with 100 ml of ethanol, methanol, acetone, chloroform, water separately. The crude preparation was left for 72 hours in shaker at room temperature. The extract obtained by cold extraction was then concentrated by evaporating the solvent at room temperature.

Antioxidant Assay

Antioxidant activity in these extracts was evaluated by different methods. The methods are DPPH Photometric assay[4], superoxide radical scavenging activity[5], Nitric oxide scavenging assay[6],FRAP reducing assay[7,8], Reductive activity[9,10], Lipid peroxidation assay[11].

Results and Discussion

DPPH photometric assay was shown in Table 1. DPPH was reduced in the presence of extracts. Ascorbic acid was taken as positive control. Methanol extract (54%) showed highest amount of antioxidant activity followed by water (37%), acetone (34%), ethanol (25%) and chloroform (16%) extract.

From results of superoxide radical scavenging activity in Table 2, it was found that the ethanol extract (55%) showed potent free radical scavenging activity. Methanol (52%), water (47%), acetone (44%) and chloroform (22%) extract also showed good activity in the decreasing order of percentage inhibition. Results of nitric oxide scavenging assay are given in Table 3. From results, it was confirmed that methanol extract (70%) has potent antioxidant activity when compared to the other extracts. Ethanol and chloroform extract showed the second highest antioxidant activity (59%) followed by acetone extract (25%). Least activity was shown by water extract (22%).

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes including neurotransmission, vascular homeostasis, antimicrobial and antitumor activities. Excess concentration of nitric oxide is implicated in the cytotoxic effects observed in various disorders like AIDS, cancer, alzheimer's and arthritis. Oxygen reacts with excess nitric oxide to generate nitrite and peroxy nitrite anions, which act as free radicals. The FRAP assay is widely used in the evaluation of antioxidant components in dietary polyphenols. The percentage of FRAP inhibition was shown in Table 4. Among the five extracts subjected to the FRAP assay, water extract (48%) showed significantly greater antioxidant activities followed by methanol (39%), ethanol (32%), acetone (24%) and chloroform extract (11%).

When samples react with FRAP solution, a dark color will appear which corresponds to the ferrous tripyridyltriazine complex. The extracts which exhibit activity produced more ferrous tripyridyltriazine complex. Ferrous tripyridyltriazine complexes were product from the

reaction in which the samples had the ability to reduce Fe^{3+} to Fe^{2+}. The greater amount of Fe^{3+} reduced to Fe^{2+}, the higher the total antioxidant content observed[8].

Reducing power of various extracts of *Brassica oleracea* were found to be significant and are summarized inTable 5.Methanol extract (0.382 OD) showed significantly higher result followed by water (0.115 OD), chloroform (0.094 OD), ethanol (0.09 OD) and acetone (0.036 OD) extract. Reductive ability was investigated from the ability of the extract to perform $Fe^{3+}$to Fe^{2+} transformation. The reducing capacity of compound may serve as significant indicator of its potential antioxidant activity. Increased absorbance of the reaction mixture indicated increased reducing power.

Lipid peroxidation is regarded as one of the basic mechanism of tissue damage caused by free radicals. Inhibition of lipid peroxide formation by various extract was determined using egg yolk as lipid source. The results obtained by this method were tabulated in Table 6. Water extract showed maximum percentage inhibition of 79%. It was followed by ethanol (74%) extract. Methanol extract showed (63%) activity. Least activity was shown by chloroform (60%) extract. Ascorbic acid was used as the positive control for all the above mentioned antioxidant experiments. The antioxidant activity of various extracts of *Brassica oleracea var capitata rubra* may be attributed to the presence of identified phytochemicals. Flavonoids, tannins, vitamin-C and plant phenolics are major group of compounds which act as primary antioxidants[12] and are known to react with hydroxyl radicals[13], superoxide anion radicals[14], lipid peroxyradicals[15], protect DNA from oxidative damage, inhibitory against tumor cell and possess anti-inflammatory and anti-microbial properties. The variations in phenolics and Flavonoids contents across population may be attributed to morphological as well as biochemical characters of the fruits and vegetables. This would, however, suggest source specific variation of antioxidant potential.

According to Amit K. Jaiswal *et.al*[16], 2011, methanolic extract was found to have the highest DPPH radical scavenging capacity with 0.71 ± 0.06 mg/ml of extract. As compared to methanol, ethanol and acetone extracts showed 39% and 47% lesser scavenging capacities, respectively. A similar trend was observed for white cabbage also, where methanolic extract showed the highest scavenging capacity for DPPH radical. The results from the present study were in agreement with this report which indicated that methanolic extracts have higher DPPH radical scavenging capacity. Ekrem koksal *et.al*[17], 2008 stated that the superoxide radical scavenging activity of *Brassica oleracea L* showed 51.5%, 43.9% inhibition for water and ethanol extract respectively. Antioxidant activity of *Pterospermum acerifolium* wild leaves by nitric oxide radical scavenging activity and reductive ability. Ethanol extracts showed maximum activity when compared to water and acetone extracts. The % inhibition was 82.74±0.02 µg/ml for nitric oxide scavenging activity and 1.3615±0.017µg/ml for reducing power[18]. In the strawberry, blackberry and raspberry leaves had good antioxidant activity and green tea was used as positive control. FRAP activity was maximum in blackberry (36.7±1.1 mmol/l) when compared to green tea (47.0±2.4 mmol/l)[19]. Peel and pulp of citrus fruits like kinnow, orange and shaddock were subjected to lipid peroxidation assay, superoxide scavenging assay[20]. In lipid peroxidation assay, maximum activity was observed in ethanol extract when compared with water and chloroform. The pulp fraction of orange (50%) and peel fraction of orange (10%) showed good antioxidant activity.

Ethanol extract (74%) of our source showed higher activity as compared to citrus fruits which were reported to have higher antioxidant activity. In superoxide scavenging assay also ethanol extract (55%) of our source showed higher activity compared to orange (50%).

Summary and conclusion

Antioxidant activities of *Brassica oleracea var capitata rubra* were estimated by six different assays. The results proved that the overall antioxidant activity were highest in methanol extract as compared to others. On the basis of the present results and available reports, it can be finally concluded that *Brassica oleracea var capitata rubra* can be used as an effective herbal medicine. This study also indicates that methanol is the most efficient solvent for extraction of polyphenolic compounds from vegetables. Their antioxidant activity may be responsible for their usefulness in the management and treatment of various diseases. Further research possible involves the isolation of individual components and formulation of a potent antimicrobial and antioxidant drug from *Brassica oleracea var capitata rubra.*

Table 1: DPPH photometric assay in % inhibition

Extracts [1 mg/ml]	**E**	**M**	**A**	**C**	**W**	**Std (0.1mg/ml)**
DPPH radical scavenging activity	25	54	34	16	37	79

Table 2 : Superoxide radical scavenging activity in % inhibition

Extracts [5 mg/ml]	**E**	**M**	**A**	**C**	**W**	**Std (0.5mg/ml)**
Superoxide radical scavenging activity	55	52	44	22	47	66

Table 3: Nitric oxide[NO] scavenging assay in % inhibition

Extracts [5 mg/ml]	**E**	**M**	**A**	**C**	**W**	**Std (0.5mg/ml)**
NO free radical scavenging assay	59	70	25	59	22	92

Table 4: FRAP reducing assay in % inhibition

Extracts [1mg/ml]	**E**	**M**	**A**	**C**	**W**	**Std (0.1mg/ml)**
FRAP reducing assay	32	39	24	11	48	28

Table 5 : Reductive ability in optical density

Extracts [1 mg/ml]	**E**	**M**	**A**	**C**	**W**	**Std(0.1mg/ml)**
Reductive ability	0.09	0.382	0.036	0.094	0.115	0.309

Table 6 : Lipid peroxidation assay in % inhibition

Extracts[1 mg/ml]	E	M	A	C	W	Std(0.1mg/ml)
Lipid peroxidation assay	74	64	63	60	79	23

E-Ethanol, M- Methanol, A-Acetone, C-Chloroform, W-Water extracts of *Brassica oleracea var capitata rubra*; Std- Ascorbic acid

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