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Antioxidant and Anti Cholinesterase Potential of Red Cabbage (*Brassica oleracea var. capitata f. rubra*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author JA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MPK and CV managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study aims at *in-vitro* evaluation of *Red* cabbage extracts for their antioxidant and anticholinesterase activity.

Methodology: Red cabbage collected from the local market was dried and extracted with different solvents. Qualitative and quantitative phytochemical screening of these extracts was done. Then the Resulting extracts were evaluated for antioxidant activity using DPPH and H_2O_2 scavenging at various concentrations. Anticholinesterase activity was assessed qualitatively using TLC autobiography.

Conclusions: Maximum antioxidant activity was found with methanolic extract and Maximum anticholinesterase activity is found with Hexane extract.

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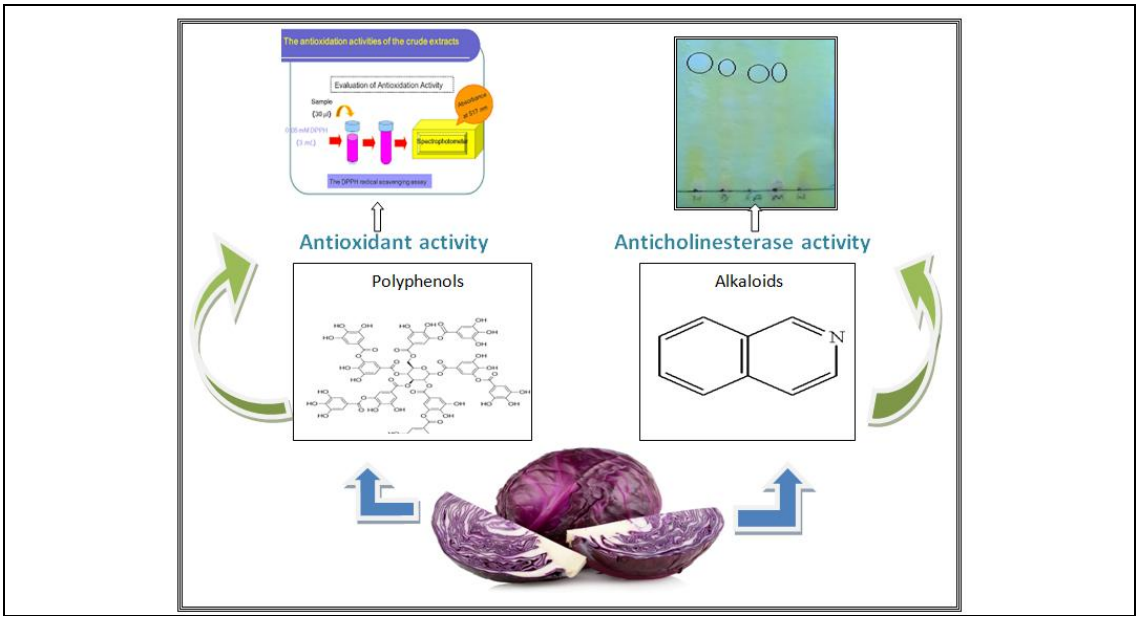

Keywords: Alzheimer's disease; acetylcholinestrase inhibitory effect; Brassica oleracea.

1. INTRODUCTION

The brain, in Alzheimer's disease, shows loss of cholinergic neurons in the basal forebrain, decreased acetylcholine (Ach) levels, and a decrease in the acetylcholine synthesizing enzyme choline acetyltransferase in the cerebral cortex. Animal models show that Ach plays a crucial role in information processing and memory. Although other neurotransmitter systems (noradrenalin, serotonin, somatostatin and other peptides) are also deficient in AD, the cognitive impairment correlates best with the loss of cholinergic input. Thus acteylcholinesterase (AChE) a biologically important enzyme that hydrolyzes acetylcholine (ACh), is considered to play role in the pathology of Alzhiemer's disease [1]. Conventional cholinesterase inhibitors like donepezil with selective facilitatory effect on intellectual performance, learning capability and memory have severe side effects like the miosis, salivation, hypothermia and tremors [2] have made their applicability limited [3]. One of the richest resources for new anticholinesterase drugs is natural products [4]. Many foods from plants have been found to exhibit anticholinesterase activity. These include ginger, a blend of black chokeberry, and lemon juice [5] as well as green tea [6].

Polyphenolic compounds contained in vegetables, fruits, nuts, and spices exhibit remarkable antioxidant and anti-inflammatory activities which may exert an important role in reducing age-related oxidative stress and inflammation thus hampering the neurodegenerative processes [7,8]. The genus Brassica (family Brassicaceae, also known as Cruciferae) includes a high number of vegetables comprising amongst others broccoli, cauliflower, brussels sprouts, kohlrabi, cabbage, and mustard. The contribution of Brassica vegetables to health improvement has been associated with phenolic compounds, the major antioxidants of these plants [9,10].

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The vegetable *Brassica oleracea* L. var capitata rubra (red cabbage) obtained from local market. The plant can be identified authenticated by Department of Botany, RBVRRWCP, Hyderabad.

2.2 Collection and Preparation of Sample

The collected *Brassica oleracea* was sliced and dried under shade, ground into fine powder using a dry grinder. The grinded samples were sieved to get uniform particle size then kept in an airtight container and stored, until further analysis.

2.3 Extraction

75 gm of the grounded material was extracted in ethanol and later partitioned in with n-hexane,

dichloromethane (DCM), ethyl acetate (EtOAc), methanol and water. Filtered through Whatman No.1 filter paper. The filtrate was concentrated in a small volume using flash evaporator and further evaporated to dryness in a vacuum desiccators. The extracts obtained were in the form of thick paste due to the presence of resinous matter. The extracts were proceeded for phytochemical screening [11].

2.4 Phytochemical Screening

Phytochemical screening of extracts was done qualitatively by the following methods [12,13,14].

2.4.1 Qualitative screening

2.4.1.1 Alkaloids

Mayer's test: To a few ml of filtrate, a drop or two of Mayer's reagent was added to the side of the test tube. A white or creamy precipitate indicated the test as positive.

2.4.1.2 Glycosides

To few ml, aqueous extract of the sample, 5ml of Benedict's solution and few drops of dilute HCl was added and heated for minutes. The solution became red with precipitate which indicated the presence of glycosides.

2.4.1.3 Terpenoids

Libermann – Burchard's test: 2 ml of acetic anhydride solution was added to 1 ml extract chloroform, followed by 1 ml of concentrated sulphuric acid. A violet colour ring was formed indicating the presence of terpenoids.

2.4.1.4 Steroids

Libermann –Burchard's test: 2 ml of acetic anhydride solution was added to 1 ml extract in chloroform followed by 1 ml of concentrated sulphuric acid. A greenish colour developed turned to blue.

2.4.1.5 Saponins

In a test tube containing about 5 ml of an aqueous extract, a drop of sodium bicarbonate solution was added. The mixture was shaken vigorously and left for 3 minutes. Honeycomb-like froth was formed.

2.4.1.6 Tannins

To 1-2 ml of plant extract, a few drops of 5% $FeCL_3$ solution were added. A green colour indicated the presence of gallotannins which brown colour indicated tannins.

2.4.1.7 Phytosterol

Libermann–buchard's test: The extract (50 mg) was dissolved in 2 ml acetic acid anhydride. To this, one or two drops of concentrated sulphuric acid were added slowly along the side of the test tube. An array of colour changes showed the presence of phytosterols.

2.4.1.8 Flavonoids

Shonoda Test: In a test tube containing 0.5 ml of alcoholic extract, 5-10 drops of dilute HCl was added followed by small pieces of magnesium. In the presence of flavonoids, a reddish pink or brown colour produced.

2.4.2 Phenolic estimation

The total phenolic content (TPC) extracts of red cabbage were spectrophotometrically determined by FolinCiocalteu reagent assay using gallic acid for the preparation of calibration curve (20 – 120 mg/l). A suitable aliquot (1 ml) of each extract or standard solution was added to 25 ml volumetric flask, containing 9 ml of distilled water. One milliliter of Folin Ciocalteu`s phenol reagent was added to the mixture and shaken. After 5 min 10 ml of 7% Na_2CO_3 solution were added to the mixture. The solution was diluted to 25 ml with distilled water and mixed. After incubation for 90 min. at room temperature, the absorbance was determined at 750 nm with spectrophotometer (Unicum UV 300) against prepared reagent as blank. A total phenolic content in samples was expressed as mg gallic acid equivalents (GAE)/g dry weight. All samples were analyzed in triplicates [15].

2.5 Determination of Antioxidant Activity by DPPH Method [16]

The free radical scavenging activity was followed by the DPPH method. 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of methanolic extract at different concentration (0.05, 0.1, 0.3 and 0.5 mg/ml). Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic

acid at various concentrations (0.05, 0.1, 0.3 and 0.5 mg/ml) was used as standard. The experiment was repeated triplicate. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH Scavenged (%) = $[(A_o – A_1) / A_o] \times 100$

where A_o is the absorbance of the control reaction (containing all reagents except the sample extract), and A_1 is the absorbance of the sample extract. Ascorbic acid was used as positive controls.

2.6 Hydroxyl Radical Scavenging Assay [17]

Individual sample extracts (1 mL) at different concentrations (0.05, 0.1, 0.3 and 0.5 mg/ml) was added to the reagent containing 1 mL of 1.5 mM $FeSO_4$, 0.7 mL of 6 mM H_2O_2 and 0.3 mL 20 mM sodium salicylate. After incubation for 1 h at 37°C, the absorbance of the reaction mixture was read at 562 nm. The scavenging ability of hydroxyl radicals was calculated using the following equation:

Scavenging ability on hydroxyl radicals (%) = $[(A_o – A_1) / A_o] \times 100$

where A_o is the absorbance of the control reaction (containing all reagents except the sample extract), and A_1 is the absorbance of the sample extract. Ascorbic acid was used as positive controls.

2.7 TLC with Bioassay Detection for Acetylcholinestrase Inhibition

The TLC with bioassay detection for Acetylcholinestrase inhibition was modified from the study of Rhee et al. [18]. A 2.5 mm silica gel plate was used as a stationary phase. Two mobile phases, i.e. dichloromethane: Ethanol: water 4:4:0.5 (v/v/v) and chloroform: Methanol 9:1 (v/v) were used. 3 µl of extracts dissolved in methanol at concentration of 5 mg/ml was applied to the plate. After the plate had been developed, it was dried at room temperature and then sprayed with 30 mM acetylthiocholine iodide (ATCI) followed by 20 mM DTNB. The plate was dried at room temperature for 45 min, and then sprayed with 10.17 U/ml AChE. After 20 min, the plate was observed under visible light. A positive spot indicating AChE inhibitor was a colourless spot on the yellow background. The plates were immediately photographed, as the white spots gets disappear fast. To confirm the true inhibition, another TLC plate was developed in a similar manner without spotting the extract, called a false positive. Appearance of white spots at the similar place on the TLC plate with extract considered as false inhibition [19].

3. RESULTS

In the present study, the leaves of *Brassica oleracea was* extracted with various solvents like n-hexane, dichloromethane (DCM), Ethyl acetate (EtOAc), Methanol and Water. The extract was screened for phytochemical constituents (Table 1).

The preliminary phytochemical investigation of red cabbage extracts revealed the presence of various secondary metabolites such as alkaloids, glycosides, steroids, flavonoids, saponin, tannin, terpenoids and phytosterols in the different extracts.

The total phenolic content varied significantly between the different extracts of *Brassica oleracea* L. var capitata. Total phenolic content in different fractions was given in Table 2.

Table 1. Phytochemical screening of red cabbage powder extract

Name of the phytochemical constituent	n-hexane	Dichloromethane	Ethylacetate	Methanol	Water
Alkaloids	+	+	+	+	+
Glycosides	-	+	+	+	+
Terpenoids	+	-	+	+	+
Saponins	-	-	+	-	-
Tannins	-	-	+	+	+
Phytosterols	+	+	-	+	-
Flavinoids	+	+	+	+	-
Steroids	-	-	-	+	-

Table 2. IC $_{50}$ values (µg/ml) of *in vitro* antioxidant activity of *Brassica oleracea*

Extract	DPPH	H_2O_2	Total phenolic content in mg/g
Hexane	34.14±0.91	47.19±0.65	11.5±0.42
DCM	30.63±0.59	44.71±0.20	17.67±0.33
Ethyl acetate	28.02±0.85	39.9±0.84	19.43±0.29
Methanol	23.68±0.54	35.69±0.57	27.63±0.39
Aqueous	41.67±0.71	51.02±0.94	10.87±0.35
Ascorbic acid	10.37 ±0.54	27.78± 0.50	-

The highest concentration of total phenol content found in the methanol extract, followed by Ethyl acetate extract, DCM, Hexane and least in aqueous extract of *Brassica oleracea L. var capitata*. The IC $_{50}$ values for different extracts have been calculated and given in Table 2. From the result we concluded that there was a positive correlation between total phenolic content and antioxidant activity. This finding is similar to that reported by Katsube et al. [20]. Anticholinesterase determined by TLC autobiographic assay (Fig. 1). Anticholinesterase activity was maximum for n-hexane extract followed by DCM, Ethyl acetate, Methanol extracts and there is no anticholinesterase activity with aqueous extract which is in line with the results reported by Hassan and Bakar [21].

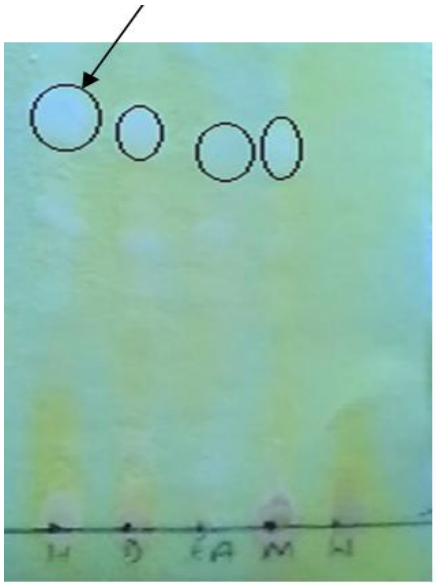

Fig. 1. Cholinesterase inhibitor activity of *Brassica*

Large colourless spot with Hexane Extract

4. DISCUSSION

The natural products have emerged as promising hope in the treatment and prevention of Alzheimer's disease. Polyphenols and other food phenolics is the subject of increasing scientific interest because of their possible beneficial effects on human health. Previous studies have repeatedly shown an inverse association between the risk of chronic human diseases and the consumption of polyphenolic-rich diet [22]. Oxidative stress and damage to brain macromolecules is an important process in neurodegenerative diseases. Because polyphenols are highly antioxidative in nature, their consumption may provide protection in neurological diseases [23]. Alzheimer's is firmly associated with impairment in cholinergic transmission. A number of cholinesterase inhibitors have been considered as candidates for the symptomatic treatment of Alzheimer's disease as the most useful relieving strategy [24]. The alkaloids are the major compounds isolated from plant different plant species and shows inhibitory activity for the acetylcholinesterase. Majority of Acetylcholine esterase inhibitors are alkaloidal in nature, including indole, isoquinoline, quinolizidine, piperidine and steroidal alkaloids. In the present study, phytochemical screening revealed the presence of alkaloids in all the extracts except aqueous extract and all the extracts exhibited anticholinesterase activity in TLC autobiography assay. Alkaloids from natural sources are acetylcholine and butyryl cholinesterase inhibitors in which their activity decreases with increasing polarity and also proved their promising role in the treatment of Alzheimer's disease [25,26]. Significantly high Anti Acetylcholine esterase activity was observed for Hexane extract in the present study. Glucobrassicin is the important alkaloid present in red cabbage [27]. This alkaloid-rich fraction has shown strong positive reaction than other fractions when fractions were assessed by general reagents for alkaloids detection. Glucobrassicin may be the compound responsible for the detected activity. The relationship of this effect between the alkaloid-rich fraction and Glucobrassicin could also be established not only by the appearance of a

stronger white spot amongst all others developed, but especially having the same Rf value, as that of Glucobrassicin reference [28].

5. CONCLUSION

The methanol extracts of Brassica oleracea presented high antioxidant activities in the DPPH and H2O2 assays and n-hexane extracts exhibited maximum acetylcholinesterase (AChE) inhibition activity. DPPH free radical scavenging activity and H2O2 scavenging activity are related to the presence of bioactive compounds such as phenolic compounds in the extract. A positive correlation was found between antioxidant activity and total phenolic content and these findings are similar to previous findings [29].

Houghton et al. [30] reviewed that terpenoids are acetyl cholinesterase inhibitors, which were identified to be present in Red cabbage. In view of the significant properties found in this study, including possible beneficial effects on the brain, we recommend that their consumption be encouraged and further studies need to be carried out to isolate and identify the bioactive components, especially from hexane fraction of Brassica oleracea crude extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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