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A Comparative Study of Anti-oxidative and Cardioprotective Efficacy of White and Pink Extract of *Brassica oleracea*

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A B S T R A C T

The Indian system of medicine such as Ayurveda, Siddha, Unani and Homeopathy rely on plant based crude materials and their formulations. The aim of this study was to investigate the comparative study to assess the phytochemical analysis, Antioxidant, cardioprotective effect and cytotoxic dose of methanolic extract of white and pink cabbage of *Brassica oleracea*. The results of methanolic extract of white and pink cabbage of *Brassica oleracea* showed the presence of carbohydrates, alkaloids, flavonoids, steroids, terpenoids, tannins, quinones and phenols and the absence of saponins and glycosides were respectively. The methanolic extract of pink cabbage of *Brassica oleracea* showed IC_{50} with minimum concentration and more effective in scavenging DPPH, ABTs radicals when compared to control ascorbic acid and white cabbage. In this study *in vitro* lipid peroxidation was carried out by Thiobarbituric acid reactive species (TBARS) assay method. The methanolic extract of pink cabbage of *Brassica oleracea* showed dose dependent prevention towards generation of lipid peroxides when compared to standard MDA and white cabbage. The concentration of methanolic extract of pink cabbage of *Brassica oleracea* yields the value of LC_{50} (50% mortality) as 15.6µg/ml which 88% mortality occurs for the methanolic extract when compared to white cabbage were respectively.

***Key words*:** Indian system of medicine, *Brassica oleracea,* MDA, TBARS

Cabbage (*Brassica oleracea* L. var. capitata) is one of the most essential vegetables grown worldwide. It belongs to the family *Cruciferae*, which contains broccoli, cauliflower and kale. The different cultivated types of cabbage show great variation in respect of size, shape and color of leaves as well as the texture of the head. Approximately 6.3 kg of *Brassica* vegetables are consumed per person yearly. Cabbage is consumed either raw or processed in diverse methods like boiled, fermented or salads. Due to its anti-inflammatory and antibacterial properties, cabbage has widespread use in traditional medicine, in alleviation of symptoms associated with gastrointestinal disorders such as gastritis, peptic and duodenal ulcers and irritable bowel syndrome as well as in treatment of minor cuts and wounds and mastitis [1]. Phytochemicals are non-nutritious chemicals that are derived from plants and provide defense against diseases in humans. They are oxidation preventive and sweep out free radicals, the byproducts of biochemical processes.

They provide safeguard against different neurological, cardiac and many other physiological ailments and protect important biomolecules from oxidative damage. *Brassica* plants are the rich source of phytochemical compounds of medicinal importance. A large no of *Brassica* plants has been studied for their bioactive phytochemical components and antioxidant potential. Antioxidants are the compounds which prevent the oxidation of the biomolecules by reducing the oxidizing agents and being self-oxidized. These compounds have the ability to scavenge the free radicals produced during the redox reactions occurring in the living and nonliving systems and prevent the free radical chain reactions [2].

Cardiovascular disease (CVD) is the leading cause of disability and death worldwide being estimated by 2030 about 23.6 million people affected. Hyperlipidemia is a risk factor of CVD involved in the production of reactive oxygen species (ROS) which cause lipid peroxidation resulting in loss of membrane integrity and cell death. As an end product of membrane lipid peroxidation, malondialdehyde (MDA) has shown to cross-link erythrocytes membrane causing impairment of its functions. MDA have been implicated in neurodegenerative diseases such as Alzheimer, Parkinson, depression and aging process [3].

Cytotoxicity tests for extracts are the first commonly used preclinical tests and thus cell culture and potential therapeutic agent screening have become commonplace. The present study entitle: "A Comparative Study of Anti-oxidative and Cardioprotective Efficacy of White and Pink extract of *Brassica oleracea*" following objectives, to assess the

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preliminary phytochemical analysis of methanolic extract of white and pink vegetables of *Brassica oleracea* and to determine the cardioprotective effect, cytotoxic dose of methanolic extract of white and pink vegetables of *Brassica oleracea* were carried out.

MATERIALS AND METHODS

Collection of cabbage: Fresh white and pink vegetables of *Brassica oleracea* were purchased from a local market in Tirupattur, dried and converted into a powder using an electric blender. The dried powders were used for further analysis.

Extraction of cabbage: 10 g of white cabbage of *Brassica oleracea* powder + 100ml of methanol and 10g of pink cabbage of *Brassica oleracea* powder + 100 ml of methanol powder was placed in a thimble and extracted for 8 cycles in a Soxhlet apparatus separately. After 8 cycles, extract was filtered by Whatman no.1 filter paper.

Filtrates were then concentrated in a rotatory evaporator. The concentrated extracts were further kept at room temperature to dry completely for 2-3 days. Once the extracts dried and kept in clean bottles till further use.

The preliminary phytochemical analysis: The preliminary phytochemical analyses of white and pink cabbage of *Brassica oleracea* were carried out as per standard methods described by Harbone [4].

Detection of alkaloids

Mayer's test: The extract was treated with Mayer s reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Wagner s test: The extract was treated with Wagner s reagent. Formation of brown/reddish brown precipitate indicates the presence of alkaloids.

Detection of flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Sulphuric acid test: Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

Detection of steroids: Two ml of acetic anhydride was added to five ml of the extract and then added each with two ml of H_2SO_4. The color was changed from violet to blue or green indicates the presence of steroids.

Detection of terpenoids

Salkowski s test: Five ml of the extract mixed with two ml of chloroform and then added carefully the 3 ml of concentrated H_2SO_4 to form a layer. An appearance of reddish brown colour in the inner face indicates the presence of terpenoids.

Detection of phenols

Ferric chloride test: 10ml of the extract was treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Lead acetate test: 10 ml of the extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

Detection of saponins: About 0.5ml of the extracts was shaken with five ml of distilled water. Formation of frothing (appearance of creamy of small bubbles) shows the presence of saponins.

Detection of tannins: A small quantity of extract was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour was formed. It indicates the presence of tannins.

Detection of carbohydrates: 0.5ml extracts were dissolved individually in five ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

Detection of glycosides

Liebermann's test: Added 2.0 ml of acetic acid and 2 ml of chloroform with whole aqueous plant crude extract. The mixture was then cooled and we added H_2SO_4 concentrated. Green color showed the entity of aglycone, steroidal part of glycosides.

Salkowski's test: Added 2 ml H_2SO_4 concentrated to the whole aqueous plant crude extract. A reddish-brown color formed which indicated the presence of steroidal aglycone part of the glycoside.

Detection of quinones

Borntrager s test: About five ml of the extract was boiled with10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of Chloroform was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. Formation of pink colour indicates the presence of anthraquinones.

In vitro assessment of radical scavenging efficacy of methanolic extract of white and pink cabbage of Brassica oleracea
DPPH radical scavenging assay

This was assayed as described by Elizabeth and Rao [5]. The reaction mixture prepared containing 50ml of Methanol. DPPH (Diphenyl-2-picryl hydrazyl radical)- 1mM 3 ml of 1mM DPPH in methanol was added to 100µl of plant extract with concentrations ranging from 20µl, 40µl, 60µl,80µl and 100µl. DPPH solution with methanol was used as a positive control (ascorbic acid) and methanol alone acted as a blank. When DPPH reacts with antioxidant in the sample and the color changed from deep purple to light yellow. This was measured calorimetrically at 518 nm. The percentage for scavenging activity was calculated by the following formula:

$$\text{Scavenging activity (\%)} = \frac{\text{A518 (control) - A518 (sample)}}{\text{A518 (control)}} \times 100$$

ABTS radical scavenging assay

Free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay. ABTS·+ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. ABTS·+ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µl of

plant extract to 3.995 ml of diluted ABTS·+ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula:

$$\text{ABTS·+ scavenging effect (\%)} = \frac{(AB-AA)}{AB} \times 100 \text{ ----- (2)}$$

where, AB is absorbance of ABTS radical + methanol; AA is absorbance of ABTS radical + sample extract/standard. Trolox was used as standard substance.

Lipid peroxidation (TBARS)

Lipid peroxidation was estimated by the method of Ohkawa *et al*. [6]. The pink colored chromogen formed by the reaction of 2-thiobarbituric acid (TBA) with breakdown products of lipid peroxidation was read at 532 nm. The Reagents are 8.1% sodium dodecy sulphate (SDS), 20 % acetic acid, 0.5% TBA, N-Butanol and pyridine (15:1 v/v), Stock melondialdehyde solution and 1,1,3,3 - tetramethoxy propone (184 ug/ml). To 0.2 ml of sample, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid, solution (adjusted to pH 2 to 3.5 with NaOH) and 1.5 ml of 0.8% aqueous solution of TBA were added. The mixture was made up to 4.0 ml with distilled water and then heated in a water bath at 95°C for 60 minutes. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of a mixture of n-butanol and pyridine were added and shaken vigorously. After centrifugation at 4000 g for 10 minutes, the organic layer was removed and its absorbance at 532nm was measured.

In Vitro assay for cytotoxicity (MTT assay) [7]

Cell line and culture

MCF 7 cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in DMEM supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37°C. Cells (1×10^5/well) were plated in 24-well plates and incubated in 37°C with 5% CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

$$\text{\% Cell viability} = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}} \times 100$$

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

RESULTS AND DISCUSSION

Phytochemical analysis

The preliminary phytochemical screening of methanolic extract of white and pink cabbage of *Brassica oleracea* showed the presence of carbohydrates, alkaloids, flavonoids, steroids, terpenoids, tannins, Quinones and phenols and the absence of saponins and glycosides were respectively. The table I shows the Phytochemicals analysis of methanolic extract of white and pink cabbage of *Brassica oleracea.*

Table 1 Phytochemicals analysis of methanolic extract of white and pink cabbage of *Brassica oleracea*

Phytochemical constituents	Methanolic extract of *Brassica oleracea*	
	Pink cabbage	White cabbage
Carbohydrates	+	+
Alkaloids	+	+
Flavonoids	+	+
Steroids	+	+
Terpenoids	+	+
Tannins	+	+
Quinones	+	+
Saponins	-	-
Glycosides	-	-
Phenols	+	+

+: Presence, -: Absence (not detected)

The phytochemicals investigation of red cabbage and green cabbage powder showed that the many phytochemical compounds are present in powder extract. Some phytochemical such as alkaloid, flavonoids, saponin, tannin, and phytosterols are present in distilled water extract since in petroleum ether, chloroform, distilled water extract and methanol extract some phytochemicals were absent [8].

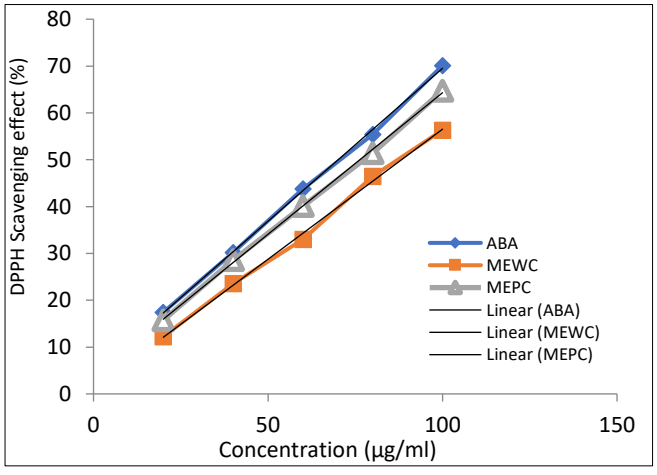

Fig 1 DPPH scavenging activity of MEPC of *Brassica oleracea*

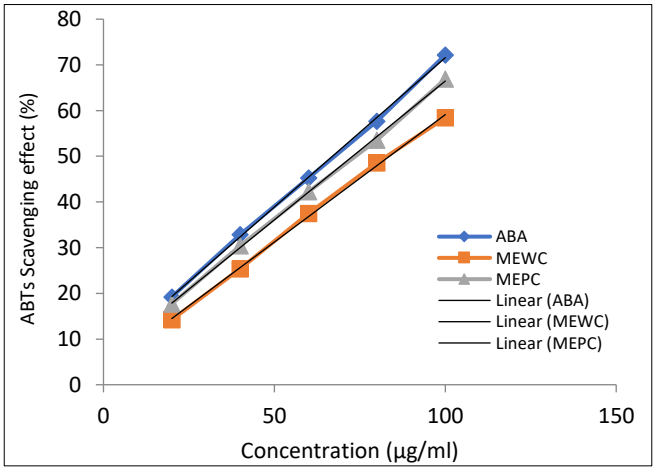

Fig 2 ABTs scavenging activity of MEPC of *Brassica oleracea*

DPPH scavenging activity

The methanolic extract of pink cabbage of *Brassica oleracea* showed IC_{50} with minimum concentration and more effective in scavenging DPPH radicals when compared to control ascorbic acid and white cabbage. The DPPH radical IC_{50} values of pink cabbage of *Brassica oleracea* were found to be 43.59µg, control ascorbic acid 43.51µg and 48.20µg white cabbage were respectively. The (Fig 1) shows the Radical DPPH Scavenging activity of methanolic extract of pink cabbage of *Brassica oleracea.*

ABTs scavenging activity

The methanolic extract of pink cabbage of *Brassica oleracea* showed IC_{50} with minimum concentration and more effective in scavenging ABTs radicals when compared to control ascorbic acid and white cabbage. The ABTs radical IC_{50} values of pink cabbage of *Brassica oleracea* were found to be 40.58µg, control ascorbic acid 40.49µg and 44.02µg white cabbage were respectively [9]. The (Fig 2) shows the Radical ABTs Scavenging activity of methanolic extract of pink cabbage of *Brassica oleracea.*

Lipid peroxidation (TBARS)

The *in vitro* lipid peroxidations were carried out by Thiobarbituric acid reactive species (TBARS) assay method. The methanolic extract of pink cabbage of *Brassica oleracea* showed dose dependent prevention towards generation of lipid peroxides when compared to standard MDA and white cabbage [10-11]. The (Fig 3) shows the Lipid peroxidation activity of methanolic extract of pink cabbage of *Brassica oleracea.*

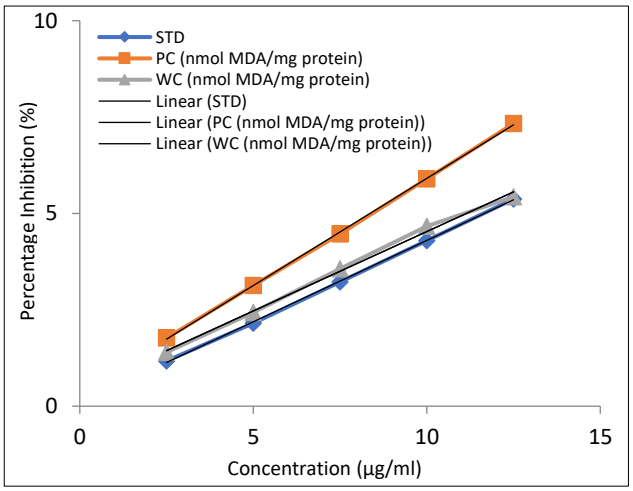

Fig 3 Lipid peroxidation activity of MEPC of *Brassica oleracea*

Cell viability (MTT) assay

The concentration of methanolic extract of pink cabbage of *Brassica oleracea* yields the value of LC_{50} (50% mortality) as 15.6µg/ml which 88% mortality occurs for the methanolic extract when compared to white cabbage were respectively [12]. The (Fig 4) shows the cell viability (MTT) activity of methanolic extract of pink cabbage of *Brassica oleracea.*

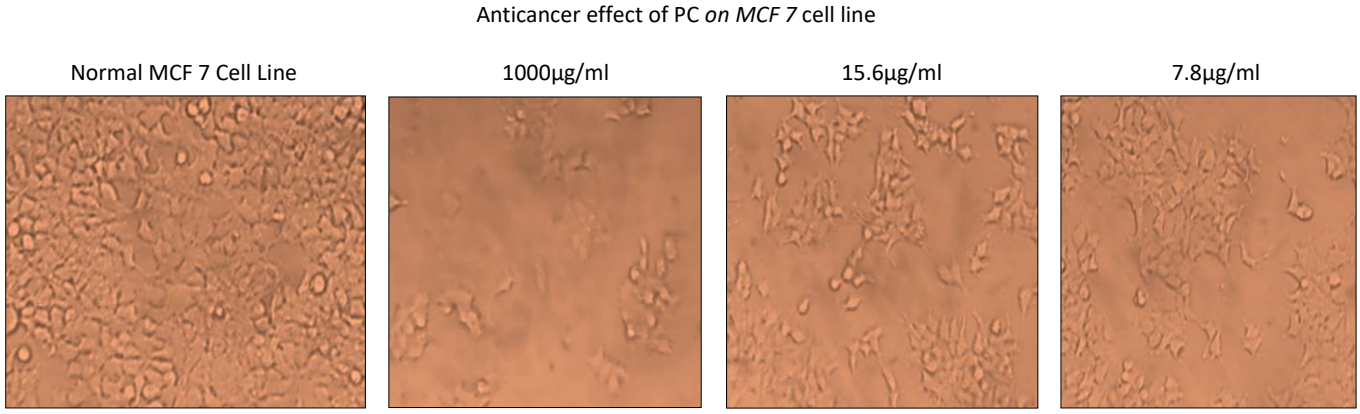

Fig 4 Cell viability (MTT) activity of MEPC of *Brassica oleracea*

CONCLUSION

It can be concluded that the results of methanolic extract of white and pink cabbage of *Brassica oleracea* showed the presence of bioactive compounds like alkaloids, flavonoids, phenols, tannins, saponins, carbohydrates, amino acids, steroids, terpenoids and protein were respectively. The methanolic extract of pink cabbage of *Brassica oleracea* showed IC_{50} with minimum concentration and more effective in scavenging DPPH, ABTs radicals when compared to control ascorbic acid and white cabbage. The methanolic extract of pink cabbage of *Brassica oleracea* showed dose dependent prevention towards generation of lipid peroxides when compared to standard MDA and white cabbage. The concentration of methanolic extract of pink cabbage of *Brassica oleracea* yields the value of LC_{50} (50% mortality) as 15.6µg/ml which 88% mortality occurs for the methanolic extract when compared to white cabbage were respectively. These findings reveal that pink cabbage of *Brassica oleracea* extract protects against oxidative stress and suggest that it can be used as an alternative therapeutic strategy to prevent the oxidative stress in the heart.

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