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BRASSICA OLERACEA: A POTENT ANTIOXIDANT THERAPEUTIC IN HEALTH AND DISEASES

Komal Talreja and Archana Moon*

University Department of Biochemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur - 440033, Maharashtra, India

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Correspondence to Author:
Dr. Archana Moon

Associate Professor
Département of Biochemistry, L. I. T. Premises, Mahatma Gandhi Marg, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur -440033, Maharashtra, India

E-mail:moon.archana@gmail.com

ABSTRACT: Oxidative stress contributes towards initiation and progression of hepatic damage in a variety of liver disorders (e.g. Hepatitis C, Hepatitis B, non alcoholic fatty liver disease amongst others). Hence, there is a great demand for the development of agents with potent antioxidant effect. There are various plant derived products which act as antioxidants. The aim of the present investigation is to evaluate the efficacy of *Brassica oleracea* extract as a hepatoprotective antioxidant. Brassica genus encompassing broccoli, cabbage, cauliflower and radish, have received much attention, due to their glucosinolate content. Glucosinolates are hydrolysed by the endogenous enzyme myrosinase and their degradation products are reported to have anticancer activity. The FRAP assay (ferric reducing ability of plasma) is a simple test that has been employed in this study to detect the free radical scavenging activity of *Brassica oleracea* extract. The present paper discusses the antioxidant and antibacterial activity of *Brassica oleracea* extract.

INTRODUCTION: Herbal products encompass a variety of preparations of plant origin that may be categorized as food, dietary supplements and herbal products [1]. Various dietary products act as antioxidants for example β carotene, green tea etc. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions produce free radicals, which cause major endogenous damages (damage to DNA, oxidation of amino acids and lipids) in the biological system.

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Free radicals are constantly generated for specific metabolic requirement (wound repair, host defense, blood homeostasis etc) and quenched by an efficient antioxidant network (some Vitamins like C and E, some minerals like selenium and manganese and certain enzymes like glutathione) in the body[2].

Antioxidants are either naturally produced in situ, or externally supplied through foods and/or supplements. Endogenous and exogenous antioxidants act as "free radical scavengers" by preventing and repairing damages caused by ROS and RNS, and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases [3].

Free radicals are highly reactive molecules having unpaired electrons produced by radiation or as by-products of metabolic processes [4]. To gain stability, free radicals capture electrons quickly from donor

compounds and the attacked compound becomes a free radical itself, which continues to attack other compounds thereby generating free radicals. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. Antioxidants are often reducing agents for example thiols, ascorbic acid or polyphenols [5]. As oxidative stress is important in many human diseases (Atherosclerosis, Asthama etc), the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases[4]. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease[5].

The Brassicaceae family comprises many commonly consumed vegetables, condiments, forages and oil containing plants, such as cabbage, broccoli, cauliflower, Brussels sprouts and rape[6]. They are rich in glucosinolates. Glucosinolates (alkyl-N-hydroximine sulphate esters with a β-D thioglucopyranosid group attached to the hydroximine carbon in Z-configuration relative to the sulphate group) have been reported to have detrimental activity against various types of cancers such as breast, lung and colon [6]. These are also reported to have antibacterial and fungistatic activity (8). Over 120 different glucosinolates have been identified to this date [6].

Glucosinolates may breakdown by the action of the endogenous enzyme myrosinase (thioglucoside glucohydrolase) to form isothiocyanates, nitriles thiocyanates, indoles and oxazolidinethiones. Isothiocyanates and indoles in particular have been implicated to have anticarcinogenic properties [7]. There are clear indications that they block tumour initiation by modulating the activities of Phase I and Phase II biotransformation enzymes and increase the antioxidant effect and suppress tumors by forcing tumor cells to go for apoptosis [6].

MATERIALS AND METHOD:

Plant Material:

Brassica oleracea was purchased from a local grocer. The leaves were washed, air dried in shade and ground to a fine powder using an electric grinder [9].

Preparation of extract:

The powder thus obtained was used for extraction purposes. Extraction was performed by following 2 methods:

1 Soxhlet Extraction: 8 gm of powder was extracted by soxhlet apparatus with 80% methanol (250ml) [6]. After extraction, the solvent was evaporated and extracts were preserved at 4^0C. For phytochemical screening, 1mg extract was dissolved in 1 ml distilled water[9]

2. Cold Maceration: 30 gm of powdered cabbage in 150 ml of 80% methanol was kept for 3 days on a rotary shaker. The supernatant obtained was utilized for phytochemical screening [9].

FRAP Assay or Reducing Power Assay:

The ferric reducing property of the extract was determined by taking 1ml of different dilutions of standard solutions of Ascorbic acid (10 -100 µg/ml). Methanolic extract adjusted to linearity range (500µg/ml) was taken in 10ml volumetric flasks and mixed with 2.5ml of potassium buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was incubated at 50◦C for 20min. Then 2.5ml of 10% trichloroacetic acid was added to the mixture to stop the reaction. To the 2.5ml of above solution 2.5ml of distill water was added followed by addition of 0.5ml of 0.1% of $FeCl_3$. This was allowed to stand for 30 min at RT. Absorbance was measured at 700 nm. The absorbance obtained was converted to ascorbic acid equivalent in mg/gm of dry material (AAE/g) using the ascorbic acid standard curve[5].

Antibacterial Activity of *Brassica oleracea* extract:

Agar disc diffusion method was used to study the antibacterial activity of *Brassica oleracea*. 2% methanol and 0.2 % DMSO here used as control[10]. Different concentration of the extracts was prepared by reconstituting with methanol[11] After solidification of agar, the filter paper discs (5 mm in diameter) impregnated with the extracts were placed on clinical isolates of *E.coli* seeded plates [12]. Different concentration of extracts ranging from 10-100 mg was utilized to study the antibacterial activity of the extract against the test organism.

HPLC:

The Glucosinolates in the hot and cold cabbage extract were analyzed using high performance liquid chromatography. The HPLC method for determining desulfated glucosinolates provides a simple means for obtaining information on the glucosinolate profiles. The desulfoglucosinolates were separated using a AminoPak C18 reverse phase column with a flow rate of 1.4 ml/min. Elution of desulfoglucosinolates from the HPLC column was performed by gradient system of methanol/water (90:10, v/v,) and was detected by a UV detector at a wavelength of 254 nm[6].

RESULTS:

Antioxidant activity by FRAP assay:

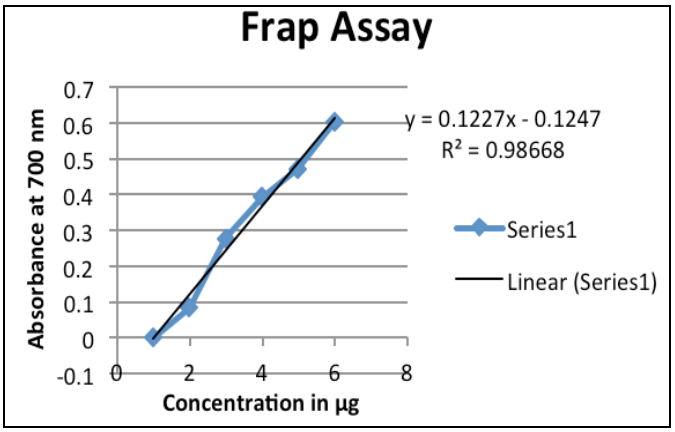

FIG. 1: GRAPH SHOWING ANTIOXIDANT ACTIVITY OF EXTRACT OF *BRASSICA OLERACEA.*

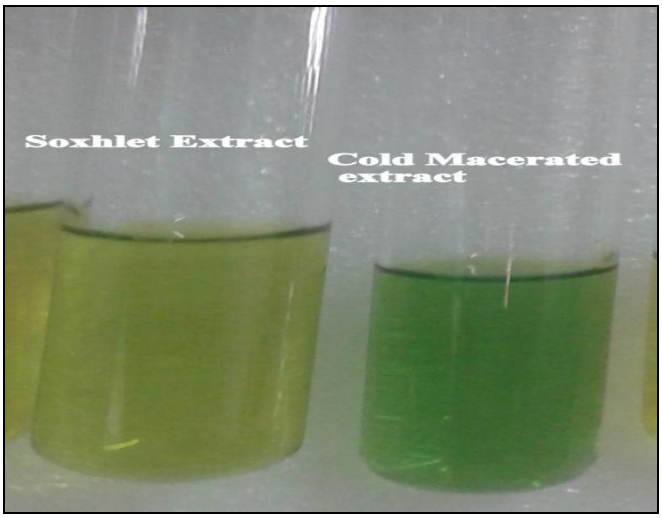

FIG. 2: ANTIOXIDANT ACTIVITY OF HOT AND COLD EXTRACT OF *BRASSICA OLERACEA*

At low pH (4-5), change in absorption at 700 nm was monitored for reduction of a ferric complex to the ferrous form, which gave an intense bluish green color. The change in absorbance was directly related to the combined or "total" reducing power of the electron-donating antioxidants present in the reaction mixture (as shown in **Fig. 2**) [5].

The antioxidant activity in soxhlet extract was found to be 1.295 ascorbic acid equivalent/gm of extract and 14.77 ascorbic acid equivalent/gm of extract in cold macerated extract (as shown by graph in **Fig. 1).**

Antibacterial Activity:

The antibacterial activity was investigated for soxhlet extracts and cold macerated extracts of *Brassica oleracea* by agar disc diffusion method. No antibacterial activity was detected in soxhlet extract, whereas a zone of inhibition is observed in cold extract at concentration of 80 mg (**Fig. 3** and **4**). The microorganisms utilized for this study were multidrug resistance *E.coli*, isolated from urine of urinary tract infected patients.

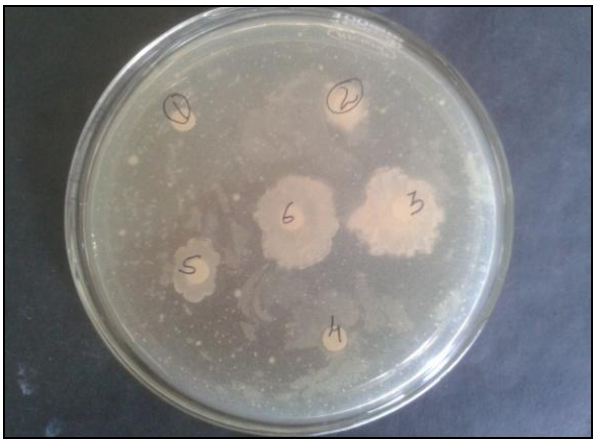

FIG. 3: ANTIBACTERIAL ACTIVITY OF *OLERACEA* COLD MACERATED EXTRACT

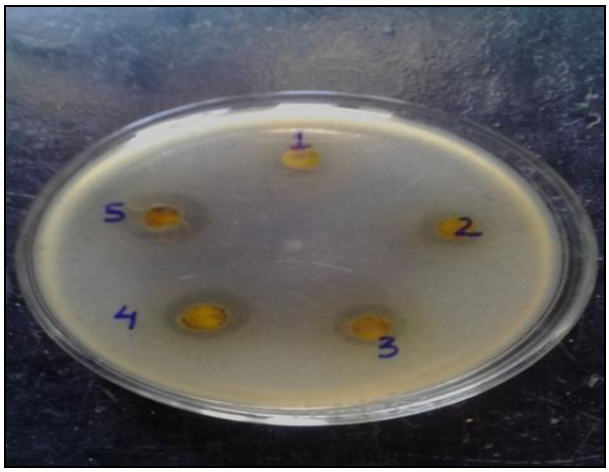

FIG. 4: ANTIBACTERIAL ACTIVITY OF *BRASSICA BRASSICA OLERACEA* SOXHLET EXTRACT

HPLC:

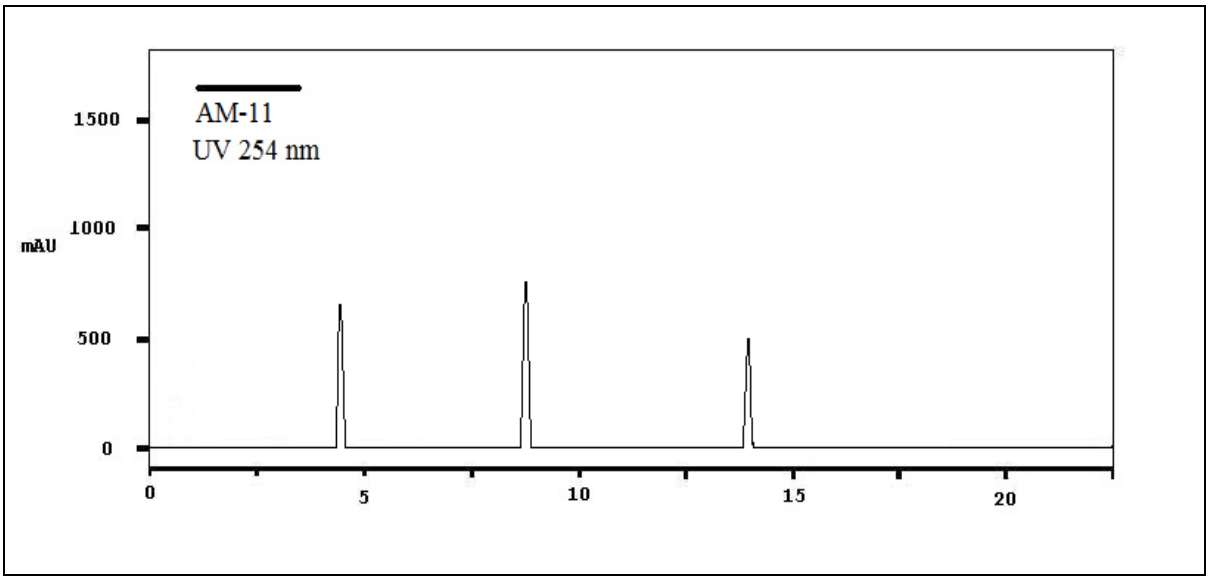

FIG. 5: PEAKS INDICATING PRESENCE OF DIFFERENT GLUCOSINOLATES IN *BRASSICA OLERACEA* EXTRACT

TABLE 1: IDENTIFIED GLUCOSINOLATES PRESENT IN *BRASSICA OLERACEA* EXTRACT

Peak Number	Retention Time in Min	Glucosinolate
1	4.63	Glucoiberin
2	8.95	Gluconapin
3	14.26	Glucobrassicin

DISCUSSION: The results obtained by FRAP assay indicate that methanolic soxhlet extract 1.295 mg equivalent of Ascorbic acid(AAE)/g and 14.77 mg AAE/g of cold macerated sample show antioxidant activity of extracts[5]. Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom.

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide ($Fe^{3+)}$ to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to convert ferric ions into ferrous form that has an absorption maximum at 700 nm. This conversion of ferric form into ferrous form serves as indicator for the antioxidant activity of the extract[13]. The results obtained clearly indicate that the reducing power of the methanolic extract increases by two folds correspondingly with the quantity (per mg AAE/g of extract) of sample[14]. Cold macerated extract showed more potent antioxidant activity than the soxhlet extract.

Experiment with hot and cold extracts of *Brassica oleracea* from 10-100 mg concentrations was done. In which, soxhlet extract did not yield any zone of inhibition whereas cold macerated extract have shown a zone of inhibition at 80 mg concentration thereby indicating presence of antibacterial activity.

CONCLUSION: It is well known that free radicals are one of the causes of several diseases. The result reveals that the *Brassica oleracea* extract possess significant antioxidant activity. The activity may be due to the presence of tannins and flavonoids found in the plant *Brassica oleracea.* This was earlier shown by phytochemical analysis [6]. HPLC analysis has proven the presence of Glucoiberin, Gluconapin and Glucobrassicin which are good antioxidants in measureable values (shown in **Table 1** and **Fig. 5**).

Hence, it can be said that the antioxidant activity is due to the aforementioned phytochemicals present in *Brassica oleracea*. This plant which is consumed as a vegetable can be included and recommended in a dietary regime as a potent natural antioxidant.

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