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Research Article

IN VITRO ASSESSMENT OF TEMPERATURE DEPENDENT METHANOLIC LEAF EXTRACTS OF RAPHANUS SATIVUS VAR. LONGIPINNATUS L. BY ABTS ANTIOXIDANT AND METAL CHELATING ACTIVITIES

Meera Indracanti[1]* and Sivakumar ChV[2]

[1]Department of Biotechnology, Institute of Biotechnology, University of Gondar, Gondar, Amhara, Ethiopia, [2]K N Biosciences India Pvt. Ltd., Hyderabad, India
***Corresponding author: meerauog@gmail.com**
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ABSTRACT

Radish (*Raphanus sativus* L. var. *longipinnatus*) is an important member of crucifer family widely cultivated as an important vegetable crop throughout the world including India. Different parts of radish including leaves are consumed as raw salads or in the cooked form and used in household remedy for different diseases including liver diseases and so on. The current research study was performed to evaluate the antioxidant, metal chelating potential and other biological activities of methanolic leaf extracts at room temperature (ME_{RT}) and boiling temperature (ME_{BT}) of *Raphanus sativus* var. *longipinnatus*. The phenolic content of the leaf extracts was found to be more in ME_{BT} (29.8± 1.98 µg/mg). ME_{BT} extract showed a significant increase in activity in a dose-dependent manner in all the assays over ME_{RT} extract. The antioxidant potential IC_{50} values of extracts were assayed. The antioxidant and metal chelating potential of white radish leaves was influenced by temperature dependent methanol extraction method.

Keywords: ABTS, chelating activity, methanol extract, radish, *Raphanus sativus*.

INTRODUCTION

Epidemiological studies reveal the plant family of Cruciferae (Brassicaceae) contains many vegetables of economic importance have established an opposite relationship flanked by antioxidant property and certain diseases such as cancer, diabetes, emphysema, cardiovascular diseases, organ dysfunction and so on (Potter and Steinmetz,1996). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been widely caught up as key mediators in the development of these chronic diseases (Kehrer, 1993) generated in the body due to metabolic process and environmental stress. Familiar ROS include superoxide anion (O_2), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and reactive hydroxyl (OH) radicals.

RNS includes nitric oxide (NO) and peroxynitrite anion (ONOO) (Joyce, 1987). Ingestion of antioxidant substances strengthens the defences against ROS and RNS which prevents oxidative damage. Plants with medicinal properties have been studied due to their antioxidant activities, less or no side effects and economic feasibility. Flavonoids and phenolic compounds are widely reported to exercise multiple biological properties, including antioxidant, free radical scavenging abilities, metal chelating, anticarcinogenicity (Shahidi and Wanasundara, 1992; Miller, 1996). The antioxidant action of the dietary phenolics were greater to that of the essential vitamins holding high redox potential that can perform interruption free radical mediated reactions by donating hydrogen from the phenolic hydroxyl groups (Parr and Bolwell, 2000). *Raphanus sativus* var. *longipinnatus* (white radish) is originally from Europe, Asia and widely distributed in India, grown-up mainly for eating, popular part for consumption is the napiform taproot, although the entire plant is edible and the aerial part can be used as a leafy vegetable. Roots are extensively used in Indian traditional medicine to maintain a healthy liver and to promote digestion (Nadkarni, 1976; Kapoor, 1990). Leaves have been used as laxative, stimulant and appetizer in herbal medicine. Root, stem and leaf showed broad spectrum of antibacterial activity against food-borne pathogens and drug resistant strains (Beevi *et al.,* 2009). Presence of glucosinolates and their degradation products, isothiocyanates in the plant creates interest as ingredient for production of healthy food. The sprout extracts have shown inhibition of cell proliferation and induce cancer cells to undergo apoptosis (Papi *et al.,* 2008). Radish leaves contain sulfonium diateroisomers of S-adenosylmethionine (AdoMet), an allosteric enzyme effector, methyl group donor for most biological transmethylation reactions and a precursor of spermine biosynthesis, spermidine, and ethylene (Rosa and Lule, 2004).

Studies on crude methanolic extracts of several culinary plants including *R. sativus* var. *longipinnatus L*, showed highest potency among the studied common vegetable and found to contain sinapic acid esters and flavonoids as main phenolic components (Takaya *et al.*, 2003). Nevertheless, information regarding the polyphenolics content, metal chelating property and the antioxidant property of methanolic leaf extract at different temperature of *R. sativus var. longipinnatus* is lacking. In the present study, phenolics from leaves *R. sativus* and the extracts was used for analysis of antioxidant property, metal chelating property and total phenolic content determination.

MATERIALS AND METHODS

Preparation of extracts

Raphanus sativus var. *longipinnatus* was obtained fresh from the local vegetable market in Dehradun (India) and was processed on the same day itself. The leaves were separated washed thoroughly with distilled water and air-dried. The dried samples were ground to powder using laboratory blender, 1:20 ratio of powder and solvent was transferred into reflux apparatus and maintained for six hours at boiling temperature (ME_{BT}). Another set of 1:20 ratio mixture was allowed on magnetic stirrer at RT (ME_{RT}) for 24 hours and both were evaporated to dryness under vacuum on a rotary evaporator at 40^{o}C. The obtained gummy dried residues at different temperature were subsequently dissolved in methanol for total polyphenolics and other biological activities. All the extracts were filtered through a Whattman filter No1 and were stored at -20°C until use.

Assay of phenolic content

The total phenolic contents (TC) of the leaf extracts of radish were determined by Folin-Ciocalteu reagent (Singleton *et al.*, 1999) using spectrophotometric method. Methanolic extract of leaf samples (1 mg/ml) was prepared and reaction mixture consisted 0.5 ml of leaf extract, 2.5ml of 10% Folin-Ciocalteu's reagent and 2.5 ml 7.5% $NaHCO_3$. Blank was simultaneously prepared, containing methanol in place of extract. The samples, blank and standard were incubated at RT for 20 min. The absorbance was determined using double beam spectrophotometer at λ_{max}=765 nm. The samples were prepared in triplicate for each analysis and the average value was calculated. The gallic acid standard of different concentration was prepared and calibration curve was graphed and phenolic content of extracts was expressed in gallic acid equivalents.

Reducing potential assay

The reducing potential of *R sativus* var. *longipinnatus L.* was assayed using method of Oyaizu (1986). Varying concentrations of extract (10-50 µg/ml) in the test tube was mixed with 2.5 ml of 0.2 M phosphate buffer (pH= 6.6) and 2.5 ml of 1% potassium ferricyanide $[K_3Fe(CN)_6]$ solution. The mixture was incubated at 50ºC for 20 min. 2.5 ml of 10% trichloroacetic acid was added to mixture and centrifuged at 3000 rpm for 15 min. The upper layer of supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ and the absorbance was measured using double beam spectrophotometer at 700 nm. L-Ascrobic acid was used as reference standard and phosphate buffer as blank. Increased absorbance of the reaction mixture indicated increased reducing power.

Ferrous ion chelating property

The Ferrous ions chelating effect was determined by method of Dinis *et al*. (1994). The reaction mixture was prepared by adding 2 ml of various concentrations (50-250 µg/ml) of methanolic extract obtained at two different temperature and 0.05 ml of 2 mM of $FeCl_2$. The reaction tube was kept at RT for 2 min and 0.2 ml of 5mM of Ferrozine was added. The mixture was shaken and placed for 10 min at RT. Absorbance of the solution was obtained using double beam spectrophotometer at 562 nm. The percentage inhibition of ferrozine Fe^{2+} complex formation was calculated by using formula:

$$\text{Metal chelating effect (\%)} = [(\text{A control} - \text{A sample}) / \text{A control}] \times 100.$$

Antioxidant assays

Phosphomolybdenum reduction

The phosphomolybdenum reduction capacity of extract was evaluated as per procedure of Prieto *et al*. (1999). The 0.4 ml extract at concentration of 2 mg/ml was mixed with 4 ml of reagent. The reagent was prepared using 0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate. The tubes were covered and incubated in water bath at 90ºC for 45 min, brought down the temperature to RT and absorbance was measured using double beam spectrophotometer at 695nm. The blank solution was prepared by adding 4 ml reagent solution and an appropriate volume of the solvent used for the extract. The mean of the 5 readings was used and expressed as mg ascorbic acid equivalents (AAE)/g extract.

ABTS radical cation decolorization assay

$ABTS^{\cdot+}$ radical stock solution was prepared by reacting 7mM ABTS (2,2'-azino-bis- (3-ethylbenzothiazoline -6- sulfonic acid) and 2.45 mM potassium persulphate ($K_2S_2O_8$) in a ratio of 1:0.5 and allowed the mixture to stand in the dark at room temperature for 12–16 h before use. The stock solution was diluted with solvent and PBS (pH 7.4) to give absorbance of 0.7 ($\pm$0.02) at 734 nm at RT. The radical decoloration assay was performed in a volume of 1ml of diluted $ABTS^{\cdot+}$ solution

and 20 µl volume of extract or standard compound (final concentration of 10-50 µg/ml) and absorbance reading was recorded at RT accurately 1 min after initial mixing up to 20 min. Respective solvent blank was run and determinations were carried at least five times of standard and samples. The percentage inhibition of radical absorbance at 734 nm was recorded and IC_{50} values calculated.

RESULTS AND DISCUSSION

The results obtained are shown in Fig.1-6 and Table 1 and 2.

Phenolic content

The boiling temperature methanolic extract had higher phenolic content than root temperature methanolic extract. Phenol antioxidant index is a measure of quality antioxidant property of the vegetable (Roberta *et al.,* 1999). The phenolic compounds exhibit scavenging efficiency of free radicals and reactive oxygen species (Elliot, 1999).

Reductive potential

The boiling point of methanolic extract showed increased reducing capacity with increase in concentration than room temperature extract. An assortment of mechanisms such as reducing potential, prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, radical scavenging activity have been explained as antioxidant activates (Prior and Cao, 2000). There is direct correlation between antioxidant activities and reducing capacity and reducing property are related to presence of reductones which have been shown antioxidant activity by breaking free radical chain by giving hydrogen atom (Diplock, 1997).

Metal chelating activity

Decreased colour intensity was observed with increase in extract concentration. The standard EDTA exhibited 80.9% activity at 100 µg/ml concentration. Ferrozine can complex with Fe^{2+} ions and form colour solution, but in presence of chelating compounds complex formation disrupted and resulted decrease in red colour intensity. It has been claimed that metal chelating activity is one of the antioxidant activity mechanism which can reduce the catalyzing transition metal in lipid peroxidation (Pin-Der-Duh, 1998). Chelating agents form s-bonds with metal are effective because they reduce redox potential required to stabilize oxidized form of metal ion.

Phsophomolybdenum assay

The reduction of Mo (IV) to Mo (V) by the extract and subsequent development of colour solution with maximum absorbance at 695 nm was the base of the assay. The antioxidant capacity of the extracts through this assay was found to decreasing in the order ME_{BT}>ME_{RT}.

ABTS assay

The IC_{50} values of the extracts were 660µg/ml (ME_{BT}) and 1041 µg/ml (ME_{RT}) respectively. The correlation between polyphenolic content of extracts and its antioxidant activity is well accepted (Senevirathne, 2006).

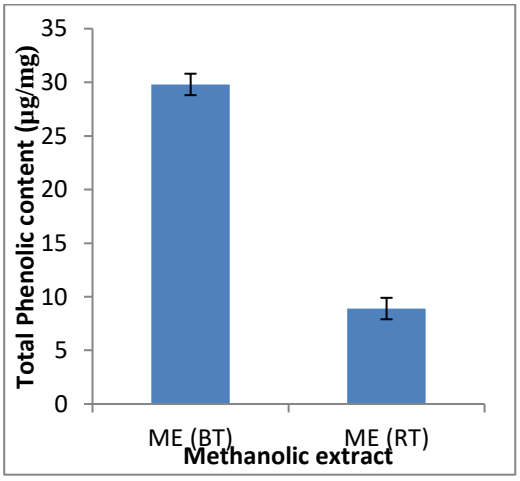

Fig.1. Phenolic content expressed as GAE/mg of extract.

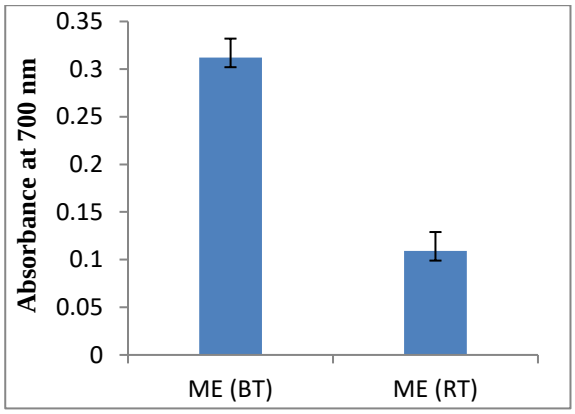

Fig. 2. Reducing ability of ME_{BT} and ME_{RT}.

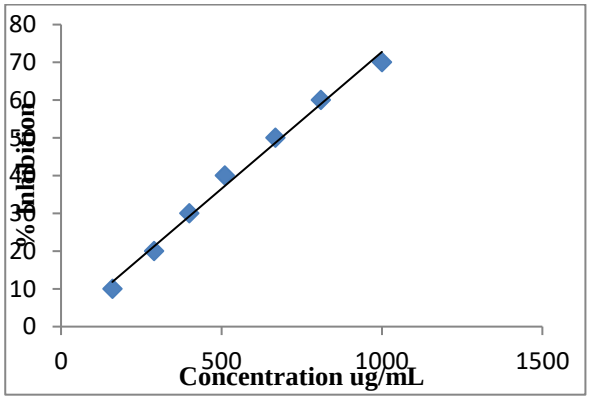

Fig. 3. Standard curve of metal chelating ability of ME_{BT}.

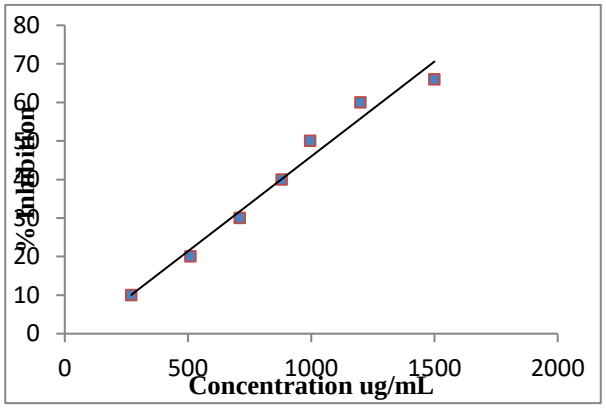

Fig. 4. Standard curve of metal chelating ability of ME_{RT}.

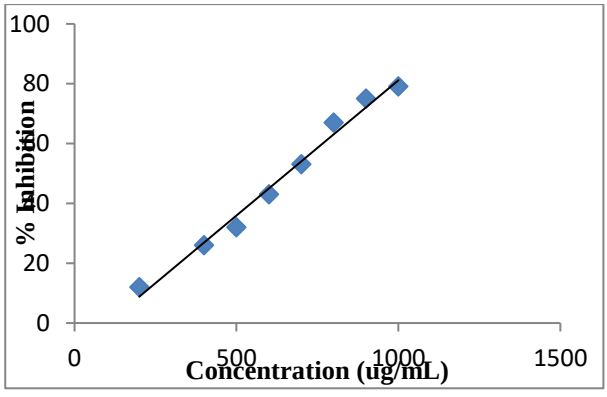

Fig. 5. Standard curve of ABTS assay of ME_{BT}

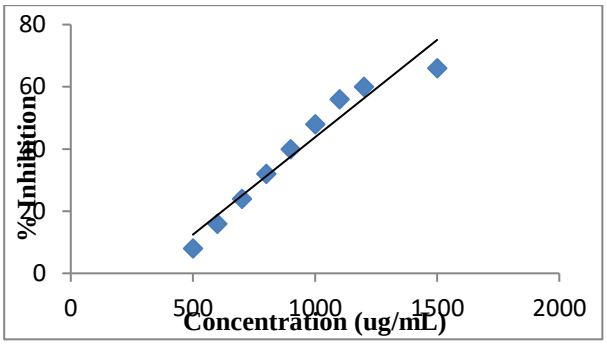

Fig. 6. ABTS assay standard curve of ME_{RT}

Table 1.Phenolic content and antioxidant capacity.

Extract	Total phenolic content (µg/mg)	Antioxidant capacity (%) equivalent to GA (phosphomolybdenum method)
ME_{BT}	39.8±0.5	20.5± 2.1
ME_{RT}	18.9 ± 0.9	11.9± 1.5

Values are means of triplicates ± SD.

Table 2. IC_{50} Values of different assays.

Extract	Metal chelating Assay IC_{50} (µg/ml)	ABTS Assay IC_{50} (µg/ml)
ME_{BT}	698±6.6	660.5±5.9
ME_{RT}	998±8.7	1041.6±10.8

IC_{50} value is calculated by linear regression analysis and expressed as mean ± SD (*n* =3).

CONCLUSION

The methanolic extracts obtained at boiling temperature and room temperature from *Raphanus sativus* var. *longipinnatus* L. leaves exhibited different levels of antioxidant activity in all assays. The boiling temperature of methanolic extract (ME_{BT}) showed high activity which is suitable for extraction of compounds from this plant. The boiling temperature of methanolic extract contains substantial amount of phenolics and being responsible for its marked antioxidant activity. These extracts have metal chelating

property with significant upmost activity found in the $ME_{BT.}$

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CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

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