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Deterioration of Antioxidant and Antidiabetic Activity of Seven Taruls Through Boiling

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Abstract
The underground edible rhizomes and tubers are commonly known as 'Taruls' in Darjeeling Himalaya and are mostly available during winter season. These vegetables are consumed as raw or processed through boiling for making them more palatable by local people of Darjeeling Himalaya. The methanolic extracts of raw and boiled (ten minutes) taruls were screened for their *in vitro* antioxidant potential by DPPH and $ABTS^{+}$ free-radicals, reducing power, metal chelating, nitric oxide, superoxide, hydroxyl radical scavenging capacity and anti-lipid peroxidation assays along with measuring *in vitro* antidiabetic activity by α-glucosidase and α-amylase inhibition. Our present study demonstrated that in majority of experimental assays, the performance of *Dioscorea alata* (ghar tarul) was best followed by *Ipomoea batatas* (red and white cultivar of sakarkanda) and *D. hamiltonii* (ban tarul), which showed stronger free-radical scavenging activity, high degree of different polyphenol content as well as antidiabetic capacity except for the iron-induced metal chelation where *Sechium edule* (squash-jara) was excellent performer. Therefore it can be stated that the extracts of different taruls might help in preventing or slowing the progress of various oxidative stress induced diseases. Our study also showed that in every antioxidant assays the scavenging activity reduced with boiling. As boiling decreases bioactive potency, it can be suggested that some alternative strategies should be developed during post-processing through which valuable phytochemicals of taruls might be preserved.

Keywords: Antioxidant, antidiabetic, phytochemicals, taruls

INTRODUCTION

Naturally occurring secondary plant metabolites which have attracted large attention from the scientific community for their antioxidant attributes and their implications in a variety of biological mechanism to prevent degenerative processes are also responsible for plant food colour, flavour and taste (Kaur and Kapoor, 2001). Fruits and vegetables are a good source of these secondary metabolites. Several epidemiological studies have supported the protective effects of fruits and vegetables consumption against the risk of numerous age related diseases due to the presence of secondary metabolites (Siddhuraju and Becker, 2007). Most vegetables are normally cooked before being consumed. It is well known that cooking stimulates significant changes in chemical composition, influencing the concentration as well as bioavailability of bioactive compounds from vegetables. However, both positive and negative effects have been reported depending in morphological and nutritional characteristics of vegetables species (Makris and Rossiter, 2001; Dewanto *et al*., 2002; Ismail *et al*., 2004; Jiratanan and Liu, 2004; Zang and Hamauzu, 2004). In addition, length of heating time might have special effect on the antioxidant properties of vegetables (Hunter and Fletcher, 2002; Zhang and Hamauzu, 2004). During vegetable processing, qualitative changes of phytochemicals and their leaching into surrounding water may influence the antioxidant property of the vegetables (Podsedek, 2007). Though, flavonoids and some phenolics are quite stable at high temperature and remain unaltered over long periods of storage (Vallejo *et al*., 2002), many antioxidant compounds like ascorbic acids and carotenoids are very sensitive to heat and lost their properties during different vegetable processing steps (Zhang and Hamauzu, 2004). In 2002, Hunter and Fletcher found that antioxidant activity of vegetables was influenced by storage under different conditions like freezing, canning and jarring etc. According to Lin and Chang (2005), antioxidant

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property of broccoli increased under different cooking treatments. In another study Turkmen (2005) examined the antioxidant activity of different selected green vegetables and found that antioxidant activity increased or remain unchanged after different cooking treatments (i.e. steaming, boiling and microwave cooking). In the same year, Oboh (2005) noticed that antioxidant property was decreased by blanching of vegetables.

In Darjeeling Himalaya vegetables are consumed as raw or by cooking like boiling or frying. Among them underground rhizomes and tubers are popularly called 'Taruls' and are mostly available during winter season. The festival named 'Maghi Sankranti' was organized in the month of January particularly for the purpose of commercializing diversified edible underground species called 'Taruls' grown in Darjeeling Hills. These plant parts are used as raw as well as boiled. The heat treatments might influence the efficacy and concentration of constituents of the underexplored vegetables found in Darjeeling Himalaya. However, literature data on the effect of cooking on nutritional properties of vegetables of this region are still missing. Therefore, the aim of the present study was to evaluate the effect of the domestic cooking practices i.e. boiling on phytochemical constituents and antioxidants as well as antidiabetic activity of seven vegetables.

MATERIALS AND METHODS

Plant samples collection and identification

Plant specimens (Figure 1) viz. ghar tarul [*Dioscorea alata* L.], ban tarul [*D. hamiltonii* Hook. f.], lal and seto sakarkanda [*Ipomoea batatas* (L.) Poir., red and white cultivars], simal tarul [*Manihot esculenta* Crantz], squash jara [*Sechium edule* (Jacq.) Sw.] and pindalu [*Xanthosoma brasiliense* (Desf.) Engl.] were collected from natural forests and cultivated fields in collaboration with farmers and suppliers of the specimens during reproductive season. Finally these plants represent our voucher specimens and are deposited in the 'NBU Herbarium' of Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal for identification and recorded against the accession no 7890, 9545, 7889, 7890, 9576, 7887 and 9598.

Preparation of samples

Seven Taruls were collected from Takdah Basti. Vegetables were washed with tap water after removing manually the non-edible parts. Vegetables were dried with paper towel and were cut into almost equal sized small pieces or slices, and mixed well. These processed samples were subjected to boiling for ten minutes followed by measurement of different phytochemicals and antioxidant as well as antidiabetic activity, keeping one portion as control (uncooked). Cooking conditions were determined with a preliminary experiment for each vegetable.

Preparation of methanolic plant extracts

Taruls were processed after peeling and were separately crushed with mortar and pestle. Under a Soxhlet extractor, crushed fruits were individually extracted with methanol for 8h. The methanol was completely removed by vacuum rotary evaporator at 50°C. These crude extracts were freeze-dried. The powder was stored at 4°C and used for further investigation. The extractive value of the plant materials were calculated on dry weight basis from the formula given below:

$$\text{Percent extractive value (yield \%)} = \frac{\text{Weight of dry extract}}{\text{Weight taken for extraction}} \times 100$$

Animal material

Goat liver, used for anti-lipid peroxidation assay, were collected from slaughter house immediately after slay and experiment was conducted within one hour after collection.

Determination of *in vitro* antioxidant activity

DPPH radical scavenging assay

Radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-1- picrylhydrazyl) was determined spectrophotometrically. The changes in color of DPPH free-radical (from deep-violet to

light-yellow) were measured at 517 nm wave length in presence of antioxidants. Radical scavenging activity of extracts was measured by standard method proposed by Blois (1958). Two micro-liters of each sample, prepared at various concentrations were added to 2 ml of 0.2 mM DPPH solution. The mixture was shaken and allowed to stand for 30 min at 20° C in dark condition and then the absorbance was measured at 517 nm with UV-VIS spectrophotometer (Systronics, 2201). The percentage inhibition activity was calculated by the following equation:

DPPH scavenging effect (%) = $[(A_{control} - A_{sample}) / A_{control} \times 100]$

Where, $A_{control}$ is the initial concentration of the stable DPPH radical without the test compound and A_{sample} is the absorbance of the remaining concentration of DPPH in the presence of methanol. IC_{50} values (mg/ml) were determined from a plotted graph of scavenging activity against the concentrations of the extracts; where IC_{50} is defined as the total amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%.

$ABTS^{\cdot}$ radical scavenging assay

The free radical-scavenging activity was determined by 2,2 azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) $ABTS^{\cdot}$ radical cation decolorization assay was described by Re *et al.* (1999). $ABTS^{\cdot}$ was dissolved in water to a 7 µM concentration. $ABTS^{\cdot}$ radical cation ($ABTS^{+}$) was produced by reacting $ABTS^{\cdot}$ stock solution with 2.45 µM potassium persulfate (final concentration) and kept in the dark at room temperature for 12–16h before use. The radical was stable in this form for more than two days when stored in the dark at room temperature. For the study of infusion, the samples containing the $ABTS^{\cdot}$ solution were diluted with redistilled water to an absorbance of 0.700 (±0.02) at 734 nm and equilibrated at 30° C. A reagent blank reading was taken. After addition of 3.0 ml of diluted $ABTS^{\cdot}$ solution (734 nm) to 30 µl of plant extracts, the absorbance reading was taken exactly 6 min after initial mixing. The IC_{50} value was calculated by the same procedure mentioned above.

Superoxide anion scavenging activity

The superoxide anions generated by phenazine methosulphate (PMS) and reduced nicotinamide-adanine dinucleotide phosphate (NADPH), were detected by the reaction with 2,2′-di-*p*-nitrophenyl-5,5′-diphenyl-(3,3′-dimethoxy-4,4′-diphenylene) di-tetrazolium chloride, nitro-blue tetrazolium (NBT) (Nishikimi *et al.*, 1972). Reaction mixture contained 1 ml samples (different concentration), 1 ml of NBT solution (312 µM prepared in phosphate buffer, pH-7.4) and 1 ml of NADH solution (936 µM prepared in phosphate buffer, pH-7.4). Finally, the reaction was accelerated by adding 100 µl PMS solution (120 µM prepared in phosphate buffer, pH -7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min and absorbance at 560 nm was measured against methanol as control. Percentage inhibition and IC_{50} value was calculated using the same formula mentioned above.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was determined by using the 2-deoxyribose oxidation assay (Jung *et al.*, 2008). A solution (0.2 ml) of 10mM $FeSO_4$, $7H_2O$ and 10 mM ethylenediamine tetra-acetic acid (EDTA) was prepared in a screw capped test tube. Then, 0.2 ml of 10 mM 2-deoxyribose solution, 0.5 ml of each sample (different concentration) and 0.1M sodium phosphate buffer (pH 7.4) were added to give a total volume of 1.8 ml. Finally, 200 µl of 10 mM H_2O_2 solution were added to this reaction mixture and incubated at 37° C for 120 min. After incubation, 1 ml each of 2.8% trichoroacetic acid and 1.0% thiobarbituric acid were added to the reaction mixture. The sample was boiled at 100° C for 10 min, cooled in ice, and then its absorbance was measured with a spectrophotometer at 515nm. The IC_{50} value was calculated by the same process mentioned above.

Determination of reducing power

One milliliter of plant extract, 2.5 ml sodium

phosphate buffer (0.2 M, pH 6.6), and 2.5 ml potassium ferricyanide (1% w/v) were incubated at 50° C for 20 minutes. The tube was cooled in ice and 2.5 ml 10% trichloroacetic acid was added. The mixture was centrifuged at 3000 rpm for 10 minutes to collect the upper layer of solution (2.5 ml) and mixed with distilled water (2.5 ml) and 0.25 ml of $FeCl_3$ (0.1% w/v). Finally, the absorbance was measured at 700 nm against blank sample (Aiyegoro and Okoh, 2009).

Anti-lipid peroxidation (ALP) assay

The anti-lipid peroxidation activity of the extracts of plants was determined by the standard method followed by slight modification with the goat liver homogenate (Bauchet and Barrier, 1998). 2.8 ml of 10% goat liver homogenate, 0.1ml of 50 mM hydrated ferrous sulphate and 0.1 ml extract was mixed. This mixture was incubated for 30 minutes at 37° C. 1 ml of reaction mixture was taken with 2 ml 10% trichloroacetic acid (TCA) -0.67% thiobarbituric acid (TBA) in acetic acid (50%) for blocking the reaction. Then the mixture was boiled for 1hour at 100° C and centrifuged at 10,000 rpm for 5 min. Supernatant was taken for absorbance at 535 nm. ALP % was calculated by using the following formula:

$$\text{ALP percent} = \frac{\text{Abs. of } Fe^{2+} \text{ induced peroxidation} - \text{abs. of sample}}{\text{Abs. of } Fe^{2+} \text{ induced peroxidation} - \text{abs. of control}} \times 100.$$

Total phenol estimation

Total phenolic compounds of plant extracts were determined by Folin-Ciocalteu method (Folin and Ciocalteu, 1927). For the preparation of the calibration curve, 1 ml aliquot of 0.025, 0.05, 0.075, 0.1, 0.2 and 0.3 mg/ml methanolic gallic acid solution was mixed with 5 ml of Folin-Ciocalteu reagent (10 times diluted) and 4 ml sodium carbonate (75 g L^{-1}). The absorbance at 765 nm was measured after 1 hr. at 20° C and the calibration curve was drawn. 1 ml methanolic fruit extracts (50 mg/ml FWT) was mixed to the same reagent and the mixture was incubated for one hour in room temperature. After 1 hour the absorbance was measured at 765nm.

Total flavonoids estimation

Spectrophotometric aluminum chloride method was used for flavonoids determination (Sultana *et al.*, 2009). Each methanolic fruit extracts (0.5 ml of 100mg/ml) were separately diluted with 4 ml double distilled water. Then the diluted fruits extracts were mixed with 5% (0.3 ml) $NaNO_2$ and 10% aluminum chloride were then added with reaction mixture. After 6 min. 2ml (1.0 M) NaOH and 2.4 ml double distilled water was added and mixed well. There after absorbance was measured at 510 nm in spectrophotometer. Standard solution of quercet in (0-500 mg L^{-1}) was used as calibration curve.

In vitro methods employed in antidiabetic studies

Inhibition of α-amylase enzyme

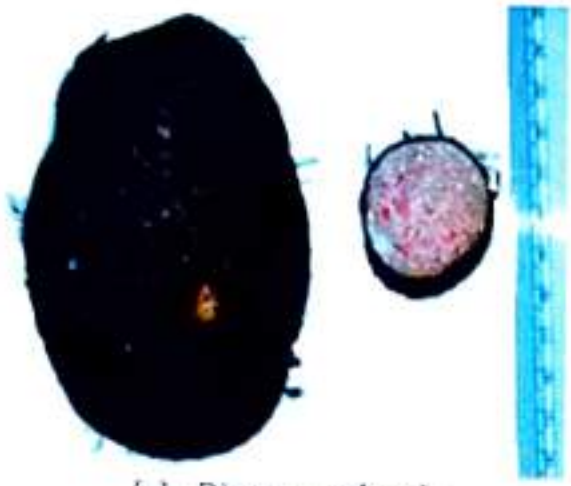
α-Amylase was premixed with the aqueous extract (methanolic extract was reconstituted with water) at various concentrations (50-250 µg/ml) and starch (0.5% w/v) as a substrate was added to start the reaction. This was carried out at 37°C for 5 min and terminated by addition of 2 ml of DNS (3,5-dinitrosalicylic acid) reagent. The reaction mixture was heated for 15 min at 100°C and diluted with 10 ml of distilled water in an ice bath (Heidari *et al.*, 2005). α-Amylase activity was determined by measuring concentration of α-amylase inhibitor to inhibit 50% of its activity under the assay conditions.

$$\% \text{ Inhibition} = (A_{540\ \text{control}} - A_{540\ \text{sample}}) / (A_{540\ \text{control}}) \times 100,$$

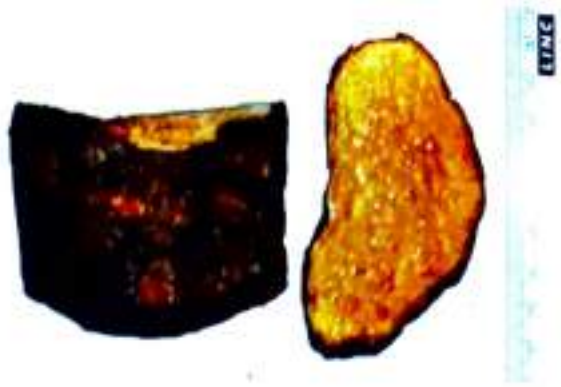
Where $A_{540\ \text{control}}$ = Absorbance of control at 540 nm and $A_{540\ \text{sample}}$ = Absorbance of sample at 540 nm.

Inhibition of α-glucosidase enzyme

α-Glucosidase inhibitory activities were assayed according to Oki *et al.*, (1999) with slight modifications. The reaction was initiated with 0.05 ml each of the samples at different concentrations in 0.2 mM phosphate buffer (pH 6.8), followed by incubation at 37°C for 15 min, after which 0.1 ml of enzyme solution was immediately added to the



[a] *Dioscorea alata* L.

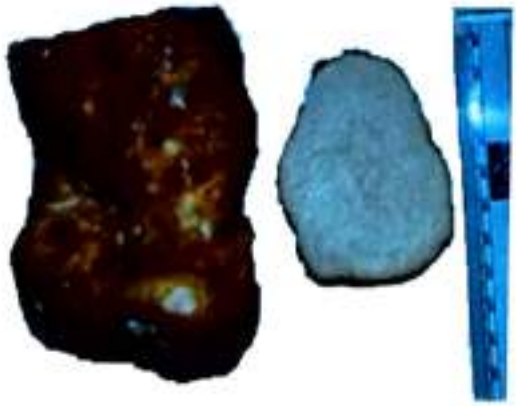


[b] *Dioscorea hamiltonii* Hook. f.

[c] *Ipomoea batatas* (L.) Poir. [Red]

[d] *I. batatas* (L.) Poir. [White]

[e] *Manihot esculenta* Crantz



[f] *Sechium edule* (Jacq.) Sw.

[g] *Xanthosoma brasiliense* (Desf.) Engl.

Figure 1. Taruls of Darjeeling Himalaya

mixture before mixing and incubation at 37°C. Then, 3 mM *p*-nitrophenylglucopyranoside (pNPG) (0.25 ml) was added, after which the reaction was stopped by the addition of 4 ml of 0.1 molar Na_2CO_3. α-Glucosidase inhibitory activity was determined by measuring the release of pNPG at 405 nm. The control contained all reagents without the tested sample. The reactions were conducted in triplicate. The α-glucosidase inhibitory activity was calculated as follows: Inhibitory ratio % = [1-(As- Ab)/ Ac] × 100;

where Ac, As, and Ab represent the absorbance levels of the control, sample, and blank respectively. The concentration of α-glucosidase inhibitor required to inhibit 50% of α-glucosidase activity under the assay conditions is defined as the IC_{50} value.

Statistical analysis

The data were pooled in triplicate and subjected to analysis of correlation co-efficient matrix using SPSS (Version 12.00) for drawing the relation between phytochemicals and antioxidant as well as antidiabetic attributes and MS Excel of Microsoft Office, 2007 was used for comparing the antioxidant attributes of different maturation stages of these fruits. The data analyzed by different group means were compared by Duncan's Multiple Range Test (DMRT) through DSAASTAT software ver. 1.022; $p<0.05$ was considered significant in all cases. Smith's Statistical Package (Version 2.5) was used for determining the IC_{50} values of antioxidants, antidiabetic activity and their standard error of estimates (SEE).

RESULTS

Data for the percentage yield of methanolic extracts of raw and boiled taruls are shown in Figure 2. Percentage yield of extracts of vegetables increased during thermal processing in *Ipomoea* spp. and decreased in most *Dioscorea* spp., *Manihot esculenta*, *Sechium edule* and *Xanthosoma brasiliense*. Figure 3 demonstrated the DPPH scavenging activities of different taruls of Darjeeling Himalaya. The IC_{50} values of DPPH scavenging activity of different taruls cooked by boiling showed significant differences. For all cases, heat treatment significantly decreased DPPH scavenging activities. Similar trends were also observed in case of scavenging capacity of other free radicals like $ABTS^+$ (Figure 4), metal chelating (Figure 5), reducing power ability (Figure 6), as well as hydroxyl radicals (Figure 7) after boiling treatment. Like antioxidant activity, an *in vitro* antidiabetic property which was parametrically assessed through α-glucosidase and α-amylase enzyme inhibition capacity was decreased with heat treatment (Figure 8 and Figure 9).

Total phenol content was highly correlated with DPPH scavenging activity whereas total flavonoids content was associated with $ABTS^+$ and hydroxyl radical scavenging property. On the other hand, both total phenol and flavonoid contents were accountable for α-amylase enzyme inhibition activity of taruls during heat treatment (Table 1), as revealed from high cohesiveness of both of the data variables. However, the quantity of bioactive phytochemicals responsible for antioxidant as well as antidiabetic activity namely total phenol (Figure 10), flavonoids (Figure 11) and ortho-dihydric phenol (Figure 12) were also reduced after boiling.

DISCUSSION

The amount of the yield components that might be extracted from a particular plant depends on strength of the extraction procedure as well as possibility exists between sample-to-sample differences in extracted materials. From our findings, we noticed that the percentage yield of methanolic extracts of different plants enhanced 3 to 4 times after heat treatment. The increase in percentage yield of the extracts might be due to the fact that the heat treatment converts insoluble phenolic compounds into the soluble extractable forms (Jeong *et al.*, 2004). Phenolics are the main contributors to the total antioxidant capacity of fruits and vegetables (Heo *et al.*, 2007). Soler-Rivas *et al.*, (2009) also proved the effects of heat treatment on the duration of cooking. In our study, cooking treatments of underground parts significantly decreased phenolic contents. The reason might be

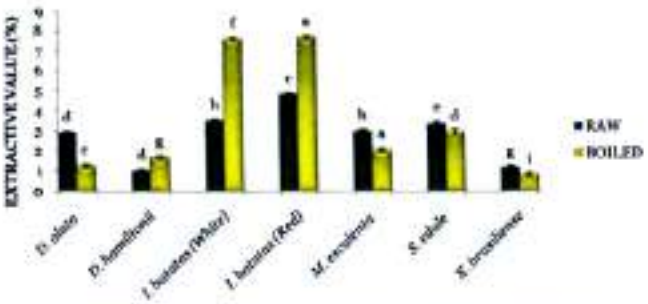


Figure 2. Extractive value (%) of different Taruls

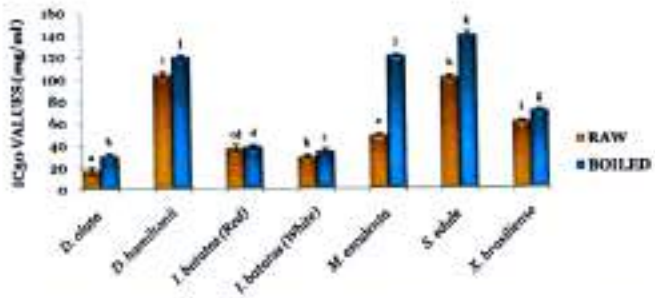


Figure 3. DPPH free radical scavenging activity (IC_{50} values mg/ml) of different Taruls

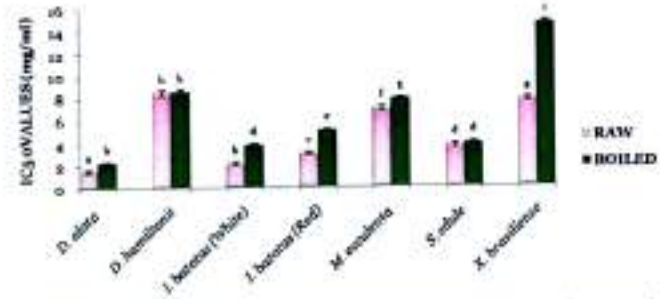


Figure 4. $ABTS^{+}$ radical scavenging activity (IC_{50} values mg/ml) of different Taruls

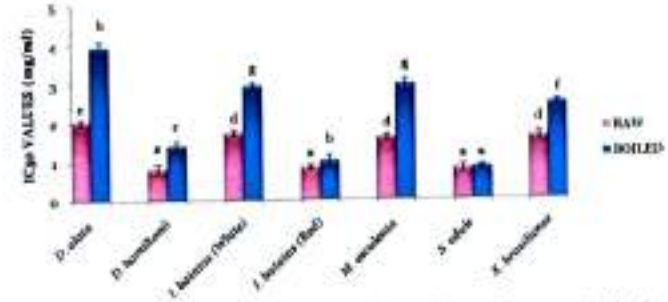


Figure 5. Metal chelating activity (IC_{50} values mg/ml) of different Taruls

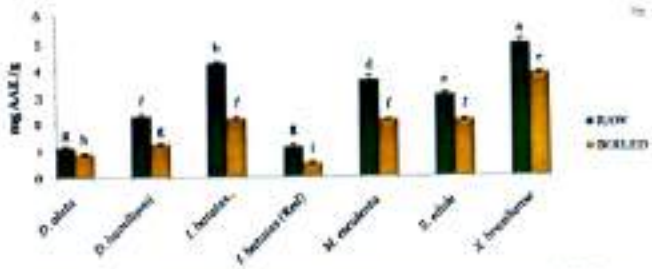


Figure 6. Reducing power capacity (mg AAE/g) of different Taruls

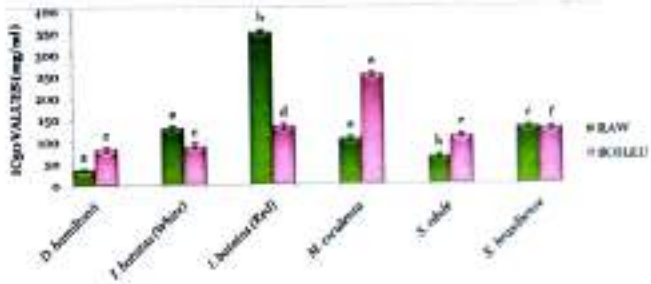


Figure 7. Hydroxyl radical scavenging activity (IC50 values mg/ml) of different Taruls

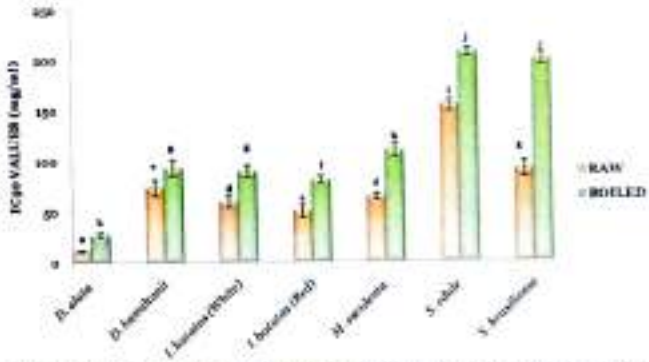


Figure 8. α-Glucosidase scavenging activity (IC50 values mg/ml) of different Taruls

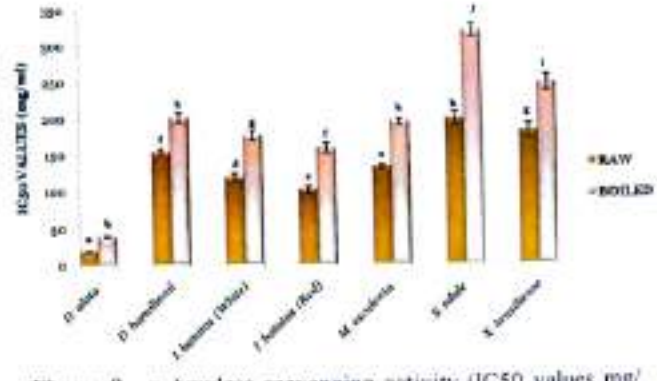


Figure 9. α-Amylase scavenging activity (IC50 values mg/ml) of different Taruls

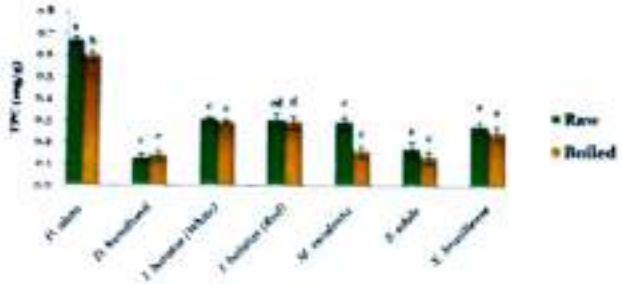


Figure 10. Total phenol content (TPC) (mg/g) of different Taruls

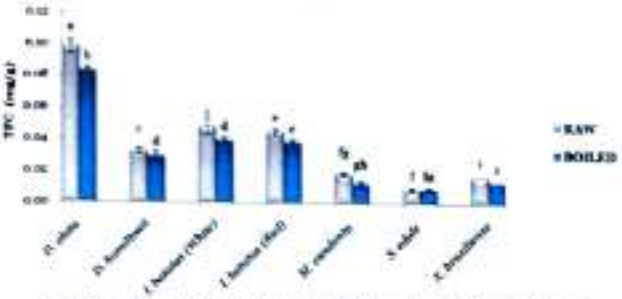


Figure 11. Total flavonoids content (TFC) (mg/g) of different Taruls

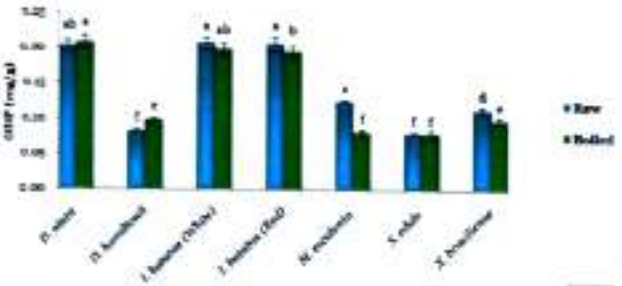


Figure 12. Ortho dihydric phenol content (ODP) (mg/g) of different Taruls

that heating sometimes destroyed the structure of phenolics and decreased their contents (Barros *et al.*, 2007). Like total phenolics, flavonoids also reduced in boiled vegetables. Flavonoid compounds might be leached in cooking water during boiling (Makris and Rossiter, 2001). Many parallel reports are available like Dietrych-Szostak (2006) who noticed that heat treatment of buckwheat significantly decreased the concentration of flavonoids. Similar loss in onions was also reported (Price *et al.*, 1997; Lombard *et al.*, 2005; Lee *et al.*, 2008). In the context of heat treatments, contradictory findings were also reported. Lombard *et al.* (2005) examined an increase of total flavonoids of onions. In 2008, Lee *et al.* noticed that steaming and baking didn't affect the flavonoids content significantly. Conversely, baking was found to increase quercetin conjugate and total flavonol content as these compounds were concentrated in the tissues (Lombard *et al.*, 2005). These contradictory results might be due to the diversity of food products used and the lack of the standardization of domestic processes.

Oboh *et al.*, (2013) noticed that heat treatment reduced the α-amylase as well as α-glucosidase inhibition activity of *Amaranthus cruentus* plant. Similarly, boiling treatment also destroyed the inhibition property of enzymes involved in glucose

Table 8.3 Correlation among phytochemical content of underground parts and various measurements of antioxidant activity

	DPPH	ABTS	OH	MC	RP	EV	TPC	TFC	ODP	GLU
DPPH										
ABTS	0.412									
OH	0.076	**0.562***								
MC	-0.288	0.008	-0.047							
RP	0.093	0.371	0.329	-0.067						
EV	0.079	-0.071	0.188	-0.106	-0.398					
TPC	**-0.584***	-0.519	-0.371	0.422	-0.475	-0.139				
TFC	-0.498	**-0.552***	**-0.543***	0.344	-0.526	-0.180	**0.963****			
ODP	**-0.89****	**-0.429***	-0.322	0.305	-0.349	-0.056	**0.636***	**0.609***		
GLU	**0.186**	**0.442**	**0.285**	**0.305**	-0.019	0.409	-0.320	-0.519	-0.159	
AMY	0.233	**0.245**	**0.213**	**0.291**	-0.021	0.366	**0.552***	**0.552***	-0.128	**0.933****

*. Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

metabolism by the plants' extracts, as revealed from our study.

Interestingly, a superior correlation was registered between antidiabetic activities with these bioactive secondary metabolites (Ghosal and Mandal, 2013). Arnous *et al.* reported a strong correlation between DPPH free radical scavenging ability and ferric ion reducing capacity of wines (Arnous *et al.*, 2002). Interestingly, a superior correlation was recorded in case of Taruls between α-amylase inhibition verses total phenol and flavonoids content (Table 8.3).

In conclusion, the present investigation clearly indicates that physicochemical as well as nutritional attributes of vegetables are extremely modified by domestic cooking and that the modifications of the evaluated parameters are strongly dependent upon the vegetable species. These cooking conditions would have promoted the overall decrease of antioxidant and antidiabetic values observed in case of Taruls is in agreement with the concept that processed vegetables have lower nutritional quality than the raw ones. Reduction in antioxidant activity might also rely on the fact that during boiling some polar antioxidants may solubilize in water, so we should also consume aqueous extracts for getting total benefits from Taruls.

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