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Review Article

Blanching and Processing Effect on Functional Properties of Lizard Plant (*Hottuynia cordata* Thunb.)

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Abstract

The present study is an attempt to put an insight into a medicinal plant *Houttuynia cordata* Thunb., which is indigenous to North-East India and China. The plant is used as a medicinal salad for lowering blood sugar level in parts of North-Eastern parts of India. The study provides detail information about the chemical composition and functional properties (total chlorophyll, total polyphenols, total flavonoids, antioxidant activity and anti-glycation activity) of plant before and after processing (dehydration and pressure cooking). The plant parts were subjected to blanching for 2 minutes and its effect on functional properties were determined. As different parts of the lizard plant were processed (drying and pressure cooking) and its effect on functional properties determined. The total chlorophyll (2.61 mg/g db), total polyphenols (30.72 mg GAE/g db) and total flavonoids (12.91 mg/g db) content of fresh gande in 80% acetone extract and 80% ethanol extract determined spectrophotometrically lowered significantly ($p<0.05$) after blanching. The dehydrated leaves showed highest retention in chlorophyll content followed byalkaline blanched samples. The effect of processing on antioxidant and anti-glycation activity of plant extract was compared with fresh plant parts extract. DPPH radical scavenging activity or IC_{50} value of fresh leaves extract was 285.69 µg/ml, followed by rhizomes (309 µg/ml) and stem (367.87 µg/ml). Similarly, the fluorescence value of glycated material showed highest inhibition to glycation by fresh leaves extract (93.82%). Processing conditions significantly ($p<0.05$) decreased the activity i.e. higher concentration was required for 50% inhibition and lower inhibition for glycation. The study showed that total polyphenol and the flavonoid content of HC were the highest in controlled sample. The results imply that these plants are potential sources of natural antioxidants which have free radical scavenging activity and might be used for reducing oxidative stress in diabetes.

Keywords: Lizard Plant; *Hottuynia cordata*; Blanching

Introduction

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Lizard plant (*Houttuynia cordata*) a medicinal herb plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [1].

Lizard plant (Gande) is an important medicinal plant widely distributed in East Asia. Gande is a flowering plant native to Japan, southern China and Southeast Asia, where it grows in moist shady places. The shoots are eaten as a vegetable and aerial parts are used in traditional Chinese medicine. Gande was first described by the Swedish naturalist Carl Peter Thunberg in his Flora Japonica in 1784 from material collected in Japan. It is also common in the wetter and warmer parts of China, where it is valued for its many uses [2]. The interest in finding natural sources of antioxidants, in particular, of plant origin, has witnessed a recent upsurge. Numerous crude extracts and purified natural compounds from plants have antioxidant and radical-scavenging activities [3]. The chemical components of lizard plant comprise 6 major types: volatile oils, flavonoids, alkaloids, fatty acids, sterols, and polyphenolic acids [4].

The popular Japanese beverage 'dokudami cha' or Gande tea is known to be very effective in chronic earaches. This 'poison blocking herb' is known for its miraculous properties since a long time ago. In China the herb is referred to as the 'fishy smell herb' owing to the foul fishy smell the leaves emit when pressed hard. The Vietnamese call it 'fish-mint' for similar reasons [2].

Total polyphenols

Polyphenols are structural class of natural, synthetic and semi synthetic organic chemicals characterized by the presence of large multiples of phenol structural units (right). The number and characteristics of these phenol structures underlie the unique physical,

chemical and biological (metabolic, toxic, therapeutic etc.) properties of particular members of the polyphenol class. The word phenol which refers to a chemical structure formed by attaching to an aromatic benzenoid (phenyl) ring, a hydroxyl (-OH) group akin to that found in alcohols (hence the –ol suffix). The term polyphenol appears to have been in use since 1894 [5].

Phenolic acids are known to acts as antioxidants by donating hydrogen or electrons. In addition, their stable radical intermediates prevents the oxidation of various food ingredients, particularly fatty acids and oils [6]. Plant polyphenols have been implicated for disease resistant by a number of researchers [7]. Much of the literature refers to the single group of plant phenolics, the flavonoids, which have application as antibiotic, anti-ulcer and anti-inflammatory agents [8]. Polyphenols also have several industrial applications such as in the production of paints, paper and cosmetics, as tanning agents and in the food industry as additives [8].

Advanced glycation end product

Advanced glycation end products (AGEs) represent a complex group of compounds derived from the nonenzymatic glycation of proteins, lipids, and nucleic acids formed endogenously but also from exogenous supplies (also referred as glycotoxins) [9]. Tobacco smoke and certain foods rich in glycotoxins that are formed by specific cooking methods represent the two major exogenous sources of glycotoxins. AGEs are heterogeneous molecules that share some common characteristics including the formation of covalent cross-links between proteins, the effect of turning some foodstuffs a yellow-brown color (the 'browning' effect), and the ability to generate fluorescence. They have diverse chemical structures but have a common characteristic lysine residue in their molecule. Based on their properties, AGEs can be categorized as: (a) fluorescent and cross-linking AGEs, including pentosidine, crossline, 2-(2-furoyl)-4(5)-(2-furanyl)-1Himidazole, glyoxal-lysine dimer, and methylglyoxal-lysine dimer (MOLD); and (b) non-fluorescent and non-cross-linking agents such as N-carboxymethyl lysine (CML), N3-(carboxyethyl)lysine, and pyrraline [10].

Advanced glycation end products (AGEs) have been implicated in many age-related chronic diseases, diabetes related complications and in protein ageing. These products are associated with diabetic complications, neurodegenerative diseases, cancer and the normal ageing process [11]. The formation of AGEs begins with the autoxidation of glucose and further interactions with proteins will generate several intermediates including schiff's base, amadori products, hydroperoxides and carbonyl compounds.

Materials and Methods

Raw material

The raw material gande (*Houttuynia cordata*) for the study was collected from five localities of Dharan in one lot. The whole plant was thoroughly washed in cold water to remove adhering dirt before being used for the study.

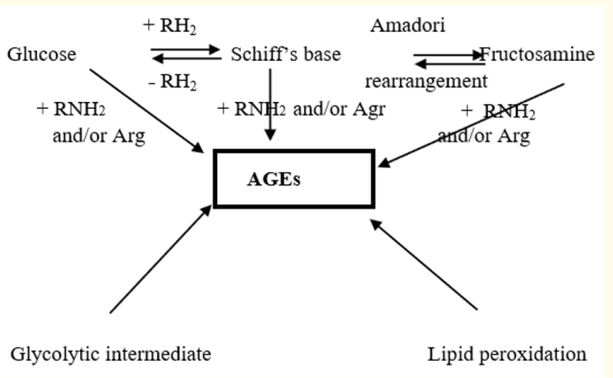

Figure a: Formation of reactive dicarbonyls and AGEs. (Source: Singh *et al.*, 2001).

Proximate analysis

The moisture content, crude protein, crude fat and crude fibre of gande was described in Rangana [12].

Adequacy of blanching

The cleaned gande leaves were tied in a muslin cloth and kept immersed in boiling water (100˚C) for two minutes and cooled immediately under running tap water and then drained. The blanched samples were grinded using motar and pestle (1:10 m/v). The solution was filtered and 10 ml of the filtered solution was taken in a test tube. One ml of 0.5% prepared gluaicol and one ml 0.5% hydrogen peroxide was added and allowed to stand for 4 minutes. The change in color was noted.

Pretreatments

The collected gande leaves were subjected to different pretreatments viz., blanching, alkaline blanching, dehydration with blanching and blanching with sulphitation.

Blanching with sulphitation

Potassium metabisulphate (KMS) 0.5% was prepared by adding 5 g in 100 ml distilled water. The leaves were immersed in the solution for 30 minutes and drained. Sulphitation was carried out after blanching.

Blanching with dehydration

Blanching was performed as described earlier and for alkaline blanching the pH of water was elevated to nine adding magnesium oxide. After blanching the sample was dried in cabinet drier at 50˚C for about 9 hours.

Chlorophyll

The total chlorophyll content of leaves after pretreatments was measured using the method as described in Rangana [12]. The

principle of estimation involves the absorption of light by aqueous acetone extracts of chlorophyll at a wavelength of 663nm (chlorophyll a) and at 645nm (chlorophyll b) and setting up simultaneous equation.

Preparation of extract

The freshly collected plant was cut small or crushed, 10g of the crushed plant material was placed in a close container with the solvent (80% ethanol) and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter had dissolved. Similar procedure was applied for dehydrated (50˚C) sample and pressure cooked (120˚C and 15 psi) sample.

The extract was then strained, the marc (the damp solid material) was pressed and the combined liquid was clarified by filtration or decantation after standing [13].

The extract obtained was then used for analysis of total polyphenols, total flavonoids, anti-oxidant activity and anti-advanced glycation activity.

Total polyphenols

Total polyphenols was determined as per Sadasivam and Manikam [14]. Two ml of extract was pipette out in a test tube in which 3ml distilled water was added. 0.5ml of Folin-Ciocalteau reagent added in the solution after 3 minutes, 2ml of 20% Na_2CO_3 solution then added in the solution. The tubes was placed in dark for 15-30 minutes and absorbance measured at 765nm against reagent blank [14].

The phenolic content was then calculated from standard gallic acid curve and expressed as gallic acid equivalent.

Total flavonoids

Two ml of plant extract (leaves, stem and rhizomes) was pipette out in a test tube in which 0.2 ml of 5% $NaNO_3$ was mixed and stand for five minutes. 0.2 ml $AlCl_3$ was pipette out and mixed in the tubes, and stand for 5 minutes. Then 2 ml of 1N NaOH in the tubes added and volume made up to 5 ml, and absorbance measured after 15 minutes at 500 nm against a reagent blank.

The total flavonoids content was then as per standard equation.

Antioxidant activity by DPPH (2, 2-diphenyl 1-picrylhydrzyl)

DPPH radical-scavenging activity was performed by the method as described by Marinova and Batchvarov [15].

- **Dilute blank:** Two ml distilled water was diluted with 80% ethanol in a 25 ml volumetric flask and kept for 20 minutes at 20˚C.
- **DPPH solution:** 0.0024g DPPH was diluted in 100ml 80% ethanol (0.06 mM). The solution was kept at 20˚C for 20 minutes. 1.5 ml of prepared DPPH solution was mixed with 1.5 ml sample extract in a test tube, stored in dark for 30 minutes. And absorbance measured at 517 nm against diluted blank.
- **Control:** 1.5 ml diluted blank and 1.5 ml DPPH solution was mixed well in a test tube, stored in dark for 30 minutes. And absorbance measured at 517 nm against reagent blank.

The percentage
DPPH scavenging activity was calculated as follow

$$\text{DPPH scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

The % DPPH scavenging activity versus concentration of sample was plotted. The concentration of the sample necessary to decrease the DPPH concentration by 50% was obtained by interpolation from linear regression analysis and denoted as IC_{50} value (µg/ml). All determinations were carried out in triplicate.

Preparation of glycated materials

Glycated materials were prepared by the method described by Chompoo., *et al.* [16] with slight variation. The reaction mixture containing 10 mg/mL bovine serum albumin (BSA), 1000mM glucose, 0.5 mg/mL of test sample extract and 50mM phosphate buffer (pH 7.4) to a total volume of 4 mL, was incubated at 60˚C for 24 hours. A control containing all the reaction mixtures except the test sample was subjected to the same condition before measurement.

Anti-Advanced glycation activity

The anti-AGE activity by plant extract was determined by the method as described by Ahmad (2005) with slight modification. Two ml of plant extract at different concentration was incubated with Bovine Serum Albumin (BSA) (10 mg/ml) and glucose (500 mM) in 50mM phosphate buffer (pH 7.4) at 60˚C for 24 hours. The fluorescence (excitation 360 nm and emission at 450 nm) due to formation of AGEs was measured by fluorimetry. The AGEs inhibition was calculated as follow

$$\% \text{ AGE} = [C - (A-B)] \times 100/C$$

Where, A, B and C represent fluorescence of test sample (glucose, BSA, sample extract and phosphate buffer), test sample without BSA (glucose, sample extract and phosphate buffer) and control (glucose, BSA and phosphate buffer) respectively incubated at 60˚C for 24 hours.

Statistical analysis

The experiment was arranged as a Completely Randomized Design (CRD). Each variation in the individual processing was regarded as fixed factors. All of the experiments were conducted in triplicates and repeated twice. The data represents mean ± standard devia-

tion (sd). The results were determined using Excel, Microsoft Office, 2010. For significant analysis, the data were separated using Tukey's HSD range at P=0.05. All statistical analysis was performed using SPSS version 20.

Results

The effect of blanching, dehydration and pressure cooking on the functional properties of different parts (leaves, stem and rhizomes) of lizard plant collected from local gardens of Dharan were studied. The proximate composition of different parts of the plant as well as the variation in the contents of total phenols, total flavonoids, antioxidant activity and anti-glycation activity were studied.

Optimization of blanching time and blanching water pH

The collected lizard plant leaves were cleaned and blanched in boiling water (100 C) for different time periods. Adequate blanching time was optimized based on peroxidase enzyme test. Test for adequacy of blanching based on inactivation of peroxidase activity is shown in Table 1.

Blanching time (minutes)	Test results
0	positive
1	positive
2	negative
3	negative

Table 1: Test for adequacy of blanching.

Blanching for 1 minute showed positive result indicating the presence of peroxidase enzyme activity. While samples blanched for ≥ 2 minutes showed negative test results indicating that the optimum blanching time of 2 minute in boiling water.

The leaves were subjected to plain water blanching and alkaline water (pH 9) blanching for two minutes. The total chlorophyll contents were analyzed. The total chlorophyll content in fresh sample was 2.6109 ± 0.216 mg/g dry matter (dm). Plain water blanching for 2 minutes showed lower retention of 49% (1.27 mg/g dm) in total chlorophyll compared to alkaline blanching (80%, 2.08 mg/g dm). Therefore, alkaline blanching for 2 minutes was used in the subsequent experiments.

Effect of blanching on total polyphenols and total flavonoids

The preliminarily treated lizard plants were subjected to blanching and its effects on total phenolics (TP) and total flavonoids (TF) contents were determined. The TP and TF contents of fresh, plain water balanced and alkaline blanched leaves of lizard plant are shown in Table 2.

Treatments	TP (mg/g GAE db)	TF (mg/g db)
Fresh	30.75 ± 0.17^{a}	12.91 ± 0.01^{a}
Plain water blanching	18.4 ± 1.21^{b}	4.02 ± 0.11^{b}
Water Blanch	20.82 ± 0.14^{c}	9.47 ± 0.15^{c}

Table 2: Effects of blanching on losses of lizard plant phenolics and flavonoids content.

The total phenolics content ranged from 30.75 to 18.4 mg GAE/g dry matter (dm) and total flavonoids ranged from 12.91-4.021 mg rutin/g dm in fresh, plain water blanched and alkaline water blanched samples. Fresh lizard plant had the highest value for both total phenolics and flavonoids contents, while the lowest for plain water blanched. The losses of TP and TF in plain water blanched and alkaline water blanched were 40.16% and 68.86% and 32.32 and 26.65% respectively.

Statistical analysis showed that both plain water blanching and alkaline blanching had a significant effect on TP and TF content of leaves of lizard plant. But alkaline water blanching showed significantly ($p<0.05$) higher retention in TP and TF contents compared to plain water blanching.

Values are the means of three determinations with standard deviation. Means having similar superscripts in a column are not significantly different ($p<0.05$) by LSD.

The findings of the study were similar to previous studies. A reduction of 75-80% TP was reported by Ghannam and Jaiswal [17] in york cabbage in conventional blanching for 14 minutes at 85-100˚C. A reduction of up to 45% TF was reported in 2 minutes water blanching (temperature, 80-90˚C) of york cabbage. Degradation of total phenolic content was reported to continue with increase in blanching temperature and time [17]. But in the present study, alkaline blanching showed lower reduction in phenolic content compared to conventional water blanching. Losses of polyphenols are attributed to the disruption of plant tissues due to heating effect, leading to polyphenols leaching out into blanching water environment [17]. Furthermore, reciprocal inter-conversion of insoluble phenolics into more soluble form can also occur, which may lead to additional losses in polyphenol [17].

Reports have suggested that reducing blanching time increases the retention of TP content. Also the blanching environment (hot water or steam), ratio of blanching water to the sample have a significant effect on polyphenols loss [17]. Alkaline blanching on other hand is found to have better retention of flavonoids content. The yellow flavonoids are pH sensitive, tending toward a deeper yellow in alkaline media [18]. This might also contribute towards better retention of total polyphenols in alkaline media.

Effect of processing on functional properties of different parts of lizard plant

The collected, washed and cleaned lizard plant parts were separated into leaves, stem and rhizomes. The functional properties such as total phenolics, total flavonoids, antioxidant activities and antiglycation activities of these different parts were determined. Further, the effect of dehydration at 50˚C in a cabinet drier and pressure cooking (15 lbs for 10 minutes) on these functional properties of leaves, stem and rhizomes were determined.

Total polyphenols (TP)

The total phenolic (TP) contents in different parts of the fresh plant (leaves, stem and rhizome) as well for both dehydrated and pressure cooked sample were determined and expressed as mg Gallic acid equivalent per gram dry matter. The effect of processing on TP content of leaves, stem and rhizomes of lizard plant are shown in Figure 1.

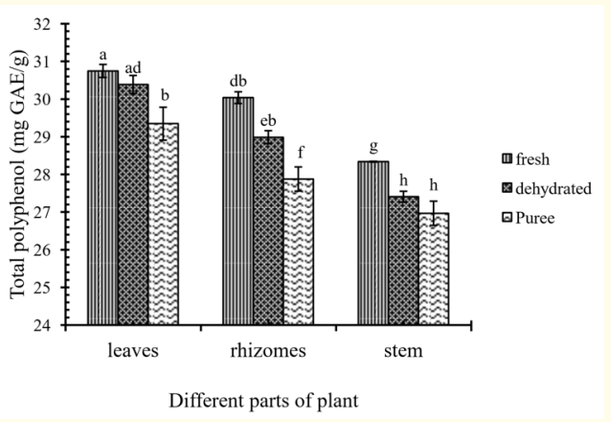

Figure 1: Effect of processing on total polyphenols content in different parts of plant.

The TP content in fresh lizard plant ranged from 28.34 to 30.75 mg GAE/g dm. The TP content in dehydrated and pressure cooked samples were in the range of 27.41 to 30.39mg GAE/g dm and 26.97 to 29.35 mg GAE/g dm. The TP contents were calculated from standard curve (y=1.4914x + 0.3116) and was 30.75, 30.04 and 28.34 mg GAE/g dm for leaves, rhizomes and stem in fresh sample respectively. The TP contents after dehydration (50˚C) were 30.39, 28.99 and 27.41 mg GAE/g dm for leaves, rhizomes and stem respectively. In pressure cooked samples the TP contents were 29.35, 27.88 and 26.97 mg GAE/g dm for leaves, rhizomes and stem respectively.

Statistical analysis showed a significant difference in TP contents both with plant parts and with processing methods. The TP contents of leaves, rhizomes and stem were significantly different ($p<0.05$). The TP contents for every fresh part were higher than processed parts. The TP content of fresh leaves and dehydrated leaves were not different ($p>0.05$), but the values were significantly higher ($p<0.05$) to that of puree (29.35 mg GAE/g dm) made by pressure cooking of leaves. In rhizomes the TP contents of fresh parts were not significantly ($p<0.05$) affected by dehydration and pressure cooking. In stem the TP contents of fresh samples were affected significantly by dehydration and pressure cooking. Further, between parts and treatment, statistical analysis showed no significant difference ($p>0.05$) in pressure cooked and fresh rhizomes and dehydrated rhizomes. The TP contents decreased as per the following order:

Fresh leaves (30.75 mg GAE/g dm) > dehydrated leaves (30.75 mg GAE/g dm) > fresh rhizomes (30.04 mg GAE/g dm) > puree of leaves (29.35 mg GAE/g dm) > dehydrated rhizomes (28.99 mg GAE/g dm) > fresh stem (28.34 mg GAE/g dm) > puree of rhizomes (27.88 mg GAE/g dm) > dehydrated stem (27.41 mg GAE/g dm) > puree of stem (26.97 mg GAE/g dm).

The TP contents of fresh leaves were lowered by 1.17% and 4.55% after dehydration and pressure cooking respectively. In rhizomes TP contents of fresh parts decreased by 3.49% and 7.19% after dehydration and pressure cooking. In stem TP contents of fresh parts decreased by 3.28% and 4.83% after dehydration and pressure cooking.

The results showed higher decrease in TP contents in all pressure cooked samples. The rise in temperature and cooking time could be a factor for degradation of bioactive compounds present in the plant. A significant decrease in polyphenol content after drying (50˚C) in apricots was reported by Monica., *et al.* [19]. Similar results were reported by Larrauri., *et al.* [20] in red grape pomace peels dried at different temperatures (60, 100, and 140°C) which showed reduced levels of extractable polyphenols. The present study showed significant effect of high pressure and temperature processing on the polyphenol content of the plant but drying at 50˚C had least effect on the polyphenol content of the plant. High pressure (15 lbs/10 minutes) cooking significantly lowered the concentration of polyphenol content which might be due to high temperature and long time.

The TP contents (30.04 mg GAE/g dm) of fresh roots in present study were significantly higher to that reported by Tapan [21] in roots (24.60 mg GAE/g dm) of Houttuynia cordata. While TP contents reported by Wenguo., *et al*. [22] ranged from 1.9 to 10.26 mg GAE/g dry weight, which was lower than that determined in the present study. The difference in polyphenol content may be attributed towards climatic variation, altitude, soil type and various other factors.

Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups.

Therefore, the phenolic content of plants may contribute directly to their antioxidant action [23]. The total polyphenol values have a significant correlation with antioxidant property.

Total flavonoids (TF)

The total flavonoids content of fresh lizard plant parts and processed parts were determined and the values presented in Figure 2. The TF contents were calculated from standard curve (1 O.D. = 0.3987mg rutin/g dm). The TF contents ranged from 4.88 to 12.91mg/g dm in fresh sample, 3.91 to 10.47 mg rutin/g dm for dehydrated product and 2.72 to 9.79 mg rutin/g dm in puree. The mean value for fresh leaves, rhizomes and stem were 12.91, 7.558 and 4.88 mg rutin/g dm respectively. In dehydrated parts the TF contents were 10.47, 6.454 and 3.91 mg rutin/g dm for leaves, rhizomes and stem respectively. In puree the TF contents were 9.79, 5.44 and 2.72 mg rutin/g dm for leaves, rhizomes and stem respectively. The percentage distribution of TF and TP were similar in different parts of lizard plant. Higher value of flavonoids were determined in leaves followed by rhizomes and stem which might be due to higher risk factor to leaves and rhizomes compared to stem. Leaves being exposed to external factor may produce more of its secondary metabolites for its protective function.

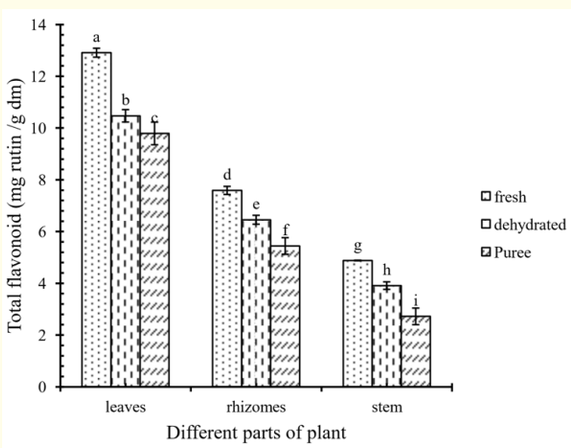

Figure 2: Effects of different processing on total flavonoid content in different parts of lizard plant.

Statistical analysis showed that TF contents of each part as well as treatments were significantly different ($p<0.05$). The TF contents of leaves of lizard plant significantly ($p<0.05$) decreased after both the treatments. The TF contents of rhizomes and stem also were affected significantly after drying and pressure cooking.

The TF contents were highest in fresh leaves (12.91 mg rutin/g dm) and minimum concentration in puree of stem (2.72 mg rutin/g dm). A difference of 62.19% in TF contents were found between the fresh leaves and fresh stem. Similar percentage (62.6%) difference was found in dehydrated leaves and dehydrated stem. In puree a difference of 72.21% was found in leaves and stem. The TF contents of leaves decreased by 18.9% and 24.16% after dehydration and pressure cooking of lizard plant. These values indicate that there was a significant effect of temperature on total flavonoids content of the plant. Pressure cooking (15 lbs/10 min) caused a significant decrease which might be due to leaching out of flavonoids and being carried away by steam.

The values showed in present study were comparable to those reported by Wenguo., *et al*. [22] where flavonoids contents were between 0.751 to12.4 mg rutin/g dm. The total flavonoid content showed the same distribution among the three parts as well as treatment.

Antioxidant activity

The plants rich in bioactive compounds (polyphenols, flavonoids etc.) acts as a chain breaking antioxidants which directly scavenge free radicals. The radical scavenging activity of cleaned and prepared fresh as well as processed lizard plant parts extract were determined by DPPH method. The ability of the plant parts to reduce the color concentration of DPPH by 50% was determined. And the required concentration or IC50 values were calculated by setting up a linear regression between concentration of plant extract and % inhibition of DPPH. The IC50 values of different plant parts are shown in Figure 4.

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The DPPH scavenging abilities (IC50 values) of fresh lizard plant parts ranged from 285.69 to 367.87µg/ml. In dehydrated parts it ranged from 289.94 to 372.60µg/ml. In pressure cooked parts IC50 value ranged from 298.97-384.31µg/ml. The lower IC50 values would reflect greater antioxidant activity of plant parts. The fresh leaves showed lowest IC50 value (285.69 ± 3.03 µg dry matter/ml) followed by fresh rhizomes (309.068 ± 1.838µg dry matter/ml) and highest by fresh stem (367.87 ± 0.934 µg dry matter/ml).The IC50 value for dehydrated leaves, rhizomes and stem were 289.94 ± 2.64, 317.56 ± 1.04 and 372.60 ± 1.55µg dry matter/ml respectively. In puree were 298.97 ± 1.159, 324.12 ± 2.48 and 384.31 ± 1.703µg/ml in leaves, rhizomes and stem respectively. The radical scavenging activity of plant extracts decreased in following order:

Fresh leaves > dehydrated leaves > puree of leaves > fresh rhizomes > dehydrated rhizomes > puree of rhizomes > fresh stem > dehydrated stem > puree of stem.

Statistical analysis showed that antioxidant activity of every fresh parts (leaves, rhizomes and stem) as well as treated samples varied significantly ($p<0.05$). The DPPH scavenging ability of fresh leaves and dehydrated leaves were not different ($p>0.05$) but pressure cooking had a significant ($p<0.05$) effect on DPPH scavenging ability. Further analysis showed significant effect of dehydration and pressure cooking in rhizomes and stem ($p<0.05$).

The findings of present study on antioxidant activity or DPPH radical scavenging ability (306.25 µg dm/ml in fresh roots) were similar to those reported by Tapan [21] (317.75 ± 2.75 µg dm/ml in roots of lizard plant). Cheng-Hong Yang., *et al.* (2013) reported DPPH free radical scavenging activity of 266.75 ± 11.75 mg/ml in Houttuynia cordata, which is similar to the present study.

There is a strong correlation between total polyphenol and flavonoid content. The result of present study showed that ethanol extract of lizard plant leaves showed highest amount of phenolic compounds, exhibited the greatest antioxidant activity whereas stem containing low phenol content exhibited minimum radical scavenging activity. The results show that heat treatment had a significant effect on radical scavenging activity of plant. The antioxidant properties of plant are mainly attributed towards its polyphenol content. In our study, the parts having higher value of polyphenol and flavonoids showed higher scavenging ability i.e. less concentration was required to inhibit DPPH activity by 50%.

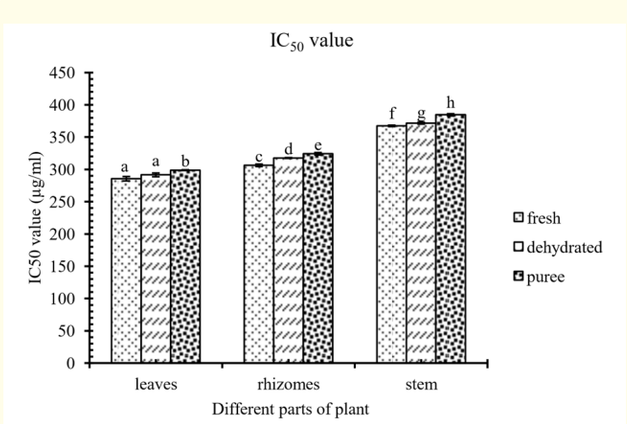

Figure 3: DPPH radical scavenging activity in different parts of lizard plant for different treatments.

The total antioxidant capacity determined through DPPH assay and total polyphenol was significantly correlated (R^2 =0.982 and R=0.991) in fresh parts of lizard plant. The value shows a positive linear correlation to total polyphenol and antioxidant activity. Higher polyphenol values in leaves showed high antioxidant activity i.e. lower concentration of the leaves were required for inhibition of DPPH activity.

Similar results for the correlations of antioxidant activity with the amount of total phenolics or flavonoids were reported in strawberry pressure cooked [24], huyou extract [25] and onion [26].

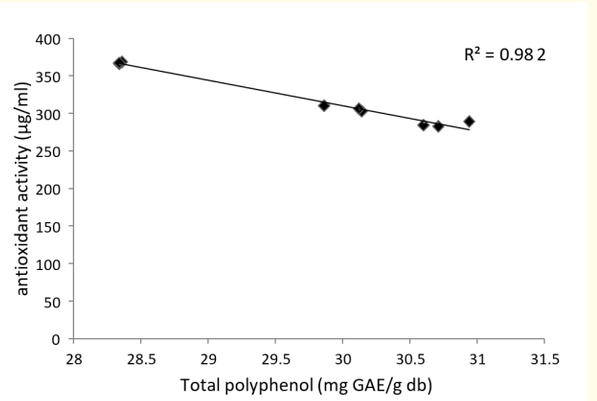

Figure 4: Linear regression between total phenolic content (mg gallic acid/g DW) and antioxidant capacity by DPPH assay (B).

Antiglycation activity/ advanced end product % inhibition

The collected lizard plant possessing functional properties were analyzed for percentage inhibition of glycated material. The percentage inhibition of glycated material by different fresh and processed plant parts (leaves, rhizomes and stem) were determined and are shown in Figure 5.

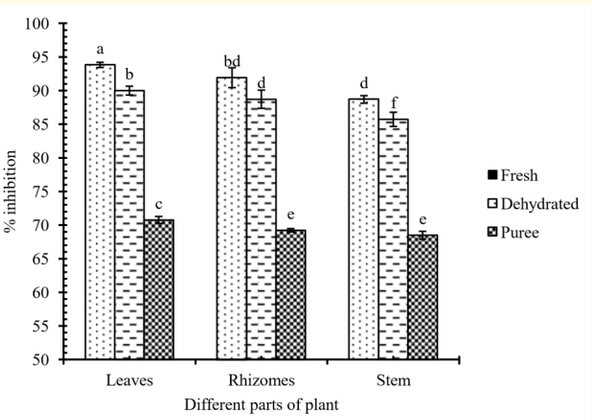

Figure 5: Antigylcation inhibition percentage of lizard plant.

The % inhibition to by fresh plant parts ranged from 88.69 to 93.82%. In dehydrated parts the % inhibition to glycation ranged from 85.73 to 91.91%. In puree % inhibition to glycation by different parts ranged from 68.49 to 70.76%. The fresh leaves showed highest inhibition of 93.82% compared to inhibition by other parts as well as treatment. The % inhibition to glycation decreased in following order:

dehydrated leaves (91.91%) > fresh rhizomes (90%) > dehydrated rhizomes (88.72%) > fresh stem (88.69%) > dehydrated stem (85.73%) > leaves puree (70.76%) > rhizomes puree (69.23%) > stem puree (68.49%). The lowest value for inhibition was in stem compared to all three treatments. As well the quality characteristic of puree of stem was not appreciable as that prepared from leaves.

Statistical analysis showed % inhibition to glycation by fresh leaves (93.82 ± 0.39%) were different ($p<0.05$) but the values for inhibition was not different ($p>0.05$) for fresh rhizomes (90 ± 1.48%) and fresh stem (88.69 ± 0.56%). Statistical analysis showed that dehydration and pressure cooking had a significant effect on % inhibition of fresh leaves. In rhizomes the effect of dehydration was not significant but pressure cooking decreased the % inhibition. In stem both dehydration and pressure cooking had a significant effect on AGE % inhibition. Further analysis showed the % inhibition by dehydrated rhizomes (88.72%) and fresh stem (88.69%) were not different. Similarly, the % inhibition by puree of rhizomes (69.23%) and puree of stem (68.49%) were not different.

The results obtained in present study were similar to that reported by Yoon and Shim, (2015) (decrease in AGE formation by 91-94.6% by methanol extract of lizard plant extract). Yoon and Shim (2015) reported that quercitrin and rutin, flavonoids in lizard plant could be effective in the inhibition of AGE formation by trapping methylglyoxyl (MGO). Methylglyoxal (MGO) results in intracellular AGEs formation owing to its high reactivity and its multiple origins under *in vivo* conditions [4]. Several studies have indicated that plant extracts rich in bioactive components were effective in reducing AGEs formation [27].

A study by Chi-Hao and Gow-Chin [28] showed that flavonoids content markedly reduced AGE activity from its early stage formation. The inhibition of free radicals generation derived from glycation process and subsequent inhibition of the protein modification was considered to be one of the mechanisms of anti-glycation effect [29].

Higher AGE formation inhibition were obtained from leaf extract of lizard plant and lower from rhizomes and stem which suggest a positive correlation with flavonoids content of each parts. A higher value of flavonoids content in leaves showed greater AGE formation inhibition.

Many researchers suggest that further studies on evaluating bioactive components or plant extracts as AGE formation inhibitors having potential effects for preventing diabetes-related complications and related diseases could be worthy [30]. Four herbal extracts (Anthemisnobilis, Crataegus oxyacantha, Houttuynia coradata, and Vitisvinifera) as well as their mixed extracts in a dose-dependent manner inhibited the generation of Maillard reaction products *in vitro* with a potency similar to that of aminoguanidine, a drug used for treating diabetic complications [31]. Moreover, AGE formation inhibition by lizard plant may not only be attributed towards flavonoids content but also many other bioactive compounds present in it, which are to be investigated [32].

Conclusion

The processing conditions were initially optimized on the basis of retention of total chlorophyll, total phenolics and total flavonoids content in plant extract. Then the plant parts were analyzed for its functional properties and processing effect on it. On the basis of work, the following conclusions were drawn.

- Optimum blanching time of 2 minutes and alkaline blanching showed highest retention of total chlorophyll content compared to other treatments with blanching. On the other hand, dehydration was significant in retaining total chlorophyll, total phenolics and total flavonoids content in plant extract.
- The total phenolics (TP) and total flavonoids (TF) content in fresh plant extract were highest in leaves followed by rhizomes and stem.
- Dehydration of different plant parts had insignificant ($p>0.05$) effect on TP and TC content. But pressure cooking showed significant reduction in TP and TF content.
- Antioxidant activity had negative correlation with TP content of plant i.e. low concentration of fresh leaves extract containing highest TP content was required to reduce DPPH activity.
- The plant possessing functional properties showed significant inihibition to advanced glycation end products.

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