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Impact of Processing on Nutraceutical Potential of Helianthus Tuberosus

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Abstract

Helianthus tuberosus, a kind of herbaceous perennial tuber has high amount of soluble fibres and biologically active components that possesses strong antioxidant activity, anti-inflammatory, antifungal, antimicrobial, anti-diabetic, anti-obesity and anticancer activities. The present study was undertaken to explore the influence of blanching and autoclaving processing methods on nutraceutical potential of *Helianthus tuberosus* (*Ht*). Soluble fibres content (inulin and fructo-oligosaccharides) and antioxidant properties (total phenols content, total flavonoids content and DPPH radical scavenging activity) were performed with slight modification in standard protocol. The study results revealed that blanched-*Ht* extract had significantly decrease inulin (21.53 ± 0.16 g/100ml) and fructo-oligosaccharides content (4.28 ± 0.17 g/100g) followed by autoclaving (17.43 ± 0.25 g/100ml and 3.76 ± 0.19 g/100g) when compared with unprocessed-*Ht* extract (23.29 ± 0.16 g/100ml and 5.31 ± 0.45 g/100g) at $p<0.05$ level. Unlike this, blanched-*Ht* extract had significantly higher total phenols content (9.36 ± 0.12 mgGAE/100g), total flavonoids content (3.30 ± 0.36 mgQE/100g) and ascorbic acid (17.71 ± 0.81) followed by autoclaving (8.9 ± 0.1 6mgGAE/100g, 4.38 ± 0.22 mgQE/100g and 14.36 ± 0.3 1mg/100g) as compared to unprocessed-*Ht* extract (7.91 ± 0.09 mgGAE/100g, 3.30 ± 0.28 mgQE/100g and 21.83 ± 0.64 g/100g). Likewise, blanched-Ht extract exhibits highest antioxidant capacity with IC_{50} value (21.07 µg/ml) followed by autoclaved-*Ht* extract (23.1 µg/ml) when compared with unprocessed-*Ht* extract (26.2 µg/ml). Hence, the present study suggests that blanched *Ht* aqueous extract would be appropriate to possess pharmaceutical properties due to high nutraceutical content.

Keywords

Fructo-oligosaccharides; *Helianthus tuberosus*; Inulin; Total flavonoids content; Total phenols content

It is commonly known that oxidative stress caused by free radicals and their derivatives is responsible for disturbing redox homeostasis [1]. It is also one of the primary factors involved in the development of chronic metabolic disorders and degenerative diseases. Reactive oxygen species are a group of unstable molecules that are generated in all cells during normal physiological and biochemical processes. These radicals may cause DNA damage, leading to mutagenic changes and cell death [2]. An extremely important role in the fight against damage caused by free radicals play nutraceutical derived from diet. However, some epidemiological studies stated the protective association between nutraceutical and chronic ailments. Nutraceuticals, a combination of nutrition and pharmaceutical are the naturally occurring compounds derived from foods and associated with improving health, delaying the aging process, increasing life expectancy and supporting the structure and function of body [3].

Helianthus tuberosus L. (Jerusalem artichoke) belongs to family *Asteraceae*, is a herbaceous perennial tuber that is cultivated worldwide in the temperate regions [4]. It contains good amount of nutrients and excellent amount of soluble dietary fibres (inulin and fructo-oligosaccharides) instead of starch. It has high amount of biologically active components including sesquiterpenes, flavonoids, isoflavonoids, phenols, phenolic acids, glycoalkaloids, phytic acids, coumarins, organic acids, polyacetylenes, and their derivatives. The tubers are also rich source of naturally occurring isomers of caffeoylquinic acid namely neochlorogenic acid, chlorogenic acid, crypto chlorogenic acid and 4 isomeric di-caffeoylquinic acids [5]. Bioactive compounds in tubers are secondary metabolites associated with various pharmacological activities, such as cholagogue, aphrodisiac, aperient, stomachic, diuretics, and tonic effects. Moreover, it also possesses strong antioxidant activity, anti-inflammatory, antifungal, antimicrobial, anti-diabetic, anti-obesity and anticancer activities, which may be associated with its highest level of phenolic content [6]. Hence, in the light of the above research facts, the present investigation was undertaken to assess the impact of processing on nutraceutical profile of *Helianthus tuberosus*.

Materials and Methods

Collection of plant material and extraction procedure of *Helianthus tuberosus*

Freshly cultivated *Helianthus tuberosus* were collected from Indian Institute of Vegetable Research (IIVR), Varanasi. The tubers were selected considering the absence of any visual damage and infection as well as the uniform size, colour and maturity. Sorted and cleaned tubers were stump trimmed off and weighed. The tubers were peeled, washed with tap water and cut into thin slices and then blanched with hot water (95°C ± 2) for 2 minutes whereas in autoclaving, tubers were kept at 121°C for 15 minutes and cooled for 10 minutes at room temperature. Blanched and autoclaved tubers were exposed directly to sunlight until samples reached constant weight.

20 g of powdered tuber was kept in 200 ml conical flask and 100 ml of distilled water was added. The mouth of the conical flask was covered with the aluminium foil and kept in a reciprocating shaker for 25 minutes for continuous agitation at 150 rpm for thorough mixing. The extracts were filtered by using muslin cloth followed by Whatman filter paper No. 42 (125 mm) and kept in amber colored screw capped bottle at 18°C for further analysis.

Determination of inulin

Free fructose content (F_f)

150 µl tuber extract was pipetted into 10 ml volumetric flask containing 20 mmol L^{-1} citrate buffer 6 (5 ml) and diluted with water up to 10ml. After 5 min, 150 µl of 100 mmol L^{-1} potassium iodide was added, and mixture was left for an additional 5 min. The absorption of solution was measured at 350 nm using a UV-Vis spectrophotometer.

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Total fructose content (F_{tot})

0.50 ml of tuber extract was acidified with HCL (0.2 mol L^{-1}) in a final volume of 25 ml and kept for acid hydrolysis at 97 ± 2°C for 45 min. The solution was then adjusted to 7 pH with NaOH before dilution with water to 25 ml. The absorption of neutral hydrolysate was measured at 350 nm using a UV-Vis spectrophotometer.

The inulin content was calculated using the following equation:

$$I = k(F_{tot}\text{-}F_f)$$

Where I is inulin content, F_{tot} is total fructose content, F_f is free fructose content, k is 0.995, it is a correction factor for the glucose part of water and inulin loss during hydrolysis [7,8].

Determination of fructo-oligosaccharides (FOS)

Total sugars were estimated by Di-Nitro Salicylic Method (DNS) [9]. Whereas, reducing sugars was estimated by phenol sulphuric method [10]. The FOS content was calculated as non-reducing sugars which derived from total sugars subtracted by reducing sugars [11].

Preliminary phytochemical screening of *Helianthus tuberous*

The filtrate of unprocessed and processed tuber were tested for the presence of various bioactive compounds namely saponins (Froth Test), tannins (Ferric chloride test), alkaloids (Mayer's reagents), flavonoids (Shonoda test), Terpenoids (Salkowski test), glycosides (Legal's test), steroids (Libermann Burchard test), phenols (Ferric Chloride) and anthroquinones (Borntrager's reaction) [12].

Determination of antioxidant potential

Determination of total phenols content

Total phenols content were determined by Folin-Ciocalteu Reagent using gallic acid as a standard phenolic compound. A dilute extract of tuber (0.5 ml of 1:10 g/ml) or Gallic acid was mixed with Folin-Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and the mixture was stirred vigorously. 4ml of aqueous sodium carbonate (1M) was added after 3 minutes and then allowed to stand for 2 h with intermittent shaking. After that, the absorbance was measured at 765 nm in spectrophotometer against blank consisting of all the reaction agents except the extract. Total phenols content values are expressed in terms of Gallic acid equivalent (mgGAE/g of dry mass) [13].

Determination of total flavonoids content

Total flavonoids content were determined by using aluminium chloride colorimetric assay. A volume of 125 µl of tuber extract is added to 75 µl of a 5% $NaNO_2$ solution. The mixture was allowed to stand for 6 min. 150 µl of aluminium trichloride (10%) was added in it and incubated for 5 min, followed by the addition of 750 µl of NaOH (1 M). The final volume of the solution was adjusted to 2500 µl with distilled water. After 15 min of incubation of mixture turned to pink and the absorbance was measured at 510 nm using spectrophotometer. The total flavonoids content was expressed as mg of quercetin equivalent (mgQE/g dry mass) [14].

Ascorbic acid content

10 g tuber powder was ground in 40 ml of metaphosphoric acid to stabilize ascorbic acid content of the sample. The content was made upto 100 ml by using 6% metaphosphoric acid. 20 ml of standard ascorbic acid solution was taken in a conical flask and titrated against 2, 6 -dichlorophenol indophenols solution. Faint pink color resisting for at least 15 seconds marked the completion of titration [15].

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Test}}{\text{Standard}} \text{ X Concentration of standard}$$

DPPH radical scavenging activity

The ability of the aqueous extracts to scavenge free radicals was determined against a very stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) determined Spectrometric method. Aliquots of the sample extract at different concentrations 20-200 µg/ml were added to 1 mm aqueous solutions of DPPH. Each mixture was vortexes vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage. IC_{50} value was also determined by graph [16]. DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \text{ X } 100$$

Statistical analysis

The results obtained were expressed as Mean ± SD and student t-test of three determinations and also statistically analysed to ascertain its significance at $p \leq 0.05$ levels.

Results and Discussion

The preliminary phytochemical analysis revealed that unprocessed-HtAqE contained saponins, tannins, alkaloids, flavonoids, terpenoids, glycosides and phenols except steroids and anthroquinones as depicted in Table 1. However, anthroquinones were found to be positive in processed-HtAqE. Though, blanched-*Ht* aqueous extract contained very good number of secondary metabolites followed by autoclaved-*Ht* aqueous extract. The present study is comparable with Krishnapriya and Suganthi stated that aqueous and methanolic extracts of *Colocasia esculenta* tubers showed the presence of alkaloids, glycosides, terpenoids, flavonoids, phenols and the absence of tannins, quinones and steroids [17]. According to Gul et *al.*, saponins, glycosides, alkaloids, phenols and flavonoids were found to be positive and tannins was found to be negative in methanol and ethanol extract of *Ephedra intermedia* [12].

Table 2 shows the nutraceutical profile of (inulin, fructo-oligosaccharides (FOS), Total phenols content (TPC), total flavonoids content (TFC) and ascorbic acid content) of unprocessed and processed-*Ht* aqueous extract. The data showed that inulin content (g/100ml) of unprocessed-HtAqE was 23.19 ± 0.16 which agrees with El-Kholy and Mahrous, who reported that *Helianthus tuberosus* had

Table 1: Effect of processing on phytochemical screening of *Helianthus tuberosus* aqueous extract.

Phytochemicals	Unprocessed-JAT	Processed-HtAqE	
		Blanching	Autoclaving
Saponins	+	++++	+++
Tannins	+	++	+
Alkaloids	+	+++	++
Flavonoids	++	+++	++
Terpenoids	+	+++	++
Glycosides	+	++	+
Steroids	_	_	_
Phenols	++++	+++++	++++
Anthroquinones	_	+	+

Table 2: Effect of processing methods on nutraceutical profile of *Helianthus tuberosus* aqueous extract.

Parameters	Unprocessed-*Ht* Aqueous Extarct	Processed-*Ht* Aqueous Extract	
		Blanching	Autoclaving
Inulin (g/100ml)	23.29±0.16	21.53±0.07* (7.29%↓)	17.43±0.25*a (25.16%↓)
			19.04%↓
Fructo-oligosaccharides (g/100g)	5.31±0.45	4.28±0.17* (19.41%↓)	3.76±0.19*a (29.14%↓)
			12.14%↓
Total Phenols Content (mgGAE/g)	8.10±0.09	9.36±0.12* (15.5%↑)	8.93±0.16*NS (10.24%↑)
			4.59%↓
Total Flavonoids Content (mgQE/g)	3.30±0.28	4.94±0.36* (49.6%↑)	4.11±0.22*NS (24.5%↑)
			16.8%↓
Ascorbic Acid (mg/100g)	21.83±0.64	17.71±0.81* (18.8%↑)	14.36±0.31*a (34.2%↑)
			18.91%↓

21.46 g/100g of inulin content. Inulin content of blanched-*Ht* aqueous extract (21.53 ± 0.07) and autoclaved-*Ht* aqueous extract (17.43 ± 0.25) was significantly decreased by 7.29% and 25.16% at $p<0.05$ level when compared to unprocessed-*Ht* extract [18]. On the other hand, autoclaving resulted significantly decrease in inulin content by 19.4% when compared with blanched-*Ht* extract. Likewise, Takeuchi and Nagashima, revealed that Jerusalem artichoke chips treated for 120 seconds lost 20-30% inulin in hot water [19]. The fructo-oligosaccharides content (g/100g) results indicated that unprocessed-*Ht* aqueous extract had highest value (5.31 ± 0.45) while blanched-*Ht* extract and autoclaved-*Ht* extract had lowest value i.e. 4.27 ± 0.17 and 3.76 ± 0.19 which was significantly decreased by 19.41% and 29.14%. On the other hand, autoclaving resulted significantly decrease in fructo-oligosaccharides content by 12.4% when compared with blanched-*Ht* extract. The processed samples registered significant difference at $p<0.05$ level when compared to unprocessed-*Ht* aqueous extract. The present data is comparable with Khuenpet et *al.* who stated that *Helianthus tuberosus* had 6.71 ± 0.06 g/100g fructo-oligosaccharides content and also revealed that blanching reported significantly decrease in inulin (26.14 ± 0.87 g/100g) and fructo-oligosaccharides content (4.97 ± 0.005 g/100g) when compared with unblanched *Helianthus tuberosus* (33.81 ± 1.44 g/100g and 7.35 ± 0.07 g/100g) [20,21]. The loss of inulin and fructo-oligosaccharides content during thermal treatment is associated with its solubility in the hot water [22].

The data showed that total phenols (mgGAE/100g) and total flavonoids content (mgQE/100g) of unprocessed-*Ht* aqueous extract was 7.91 ± 0.09 and 3.30 ± 0.28 which agrees with Niziol-Lukaszewska et *al.* who reported that *Helianthus tuberosus* had 76.84 ± 4.96 mgGA/g (TPC) and 6.05 ± 0.32mgQE/g (TFC) content. TPC content of processed-*Ht* extract i.e. blanched (9.36 ± 0.12) and autoclaved (8.93 ± 0.16) was significantly increased ($p<0.05$ level) by 18.4% and 13.03% when compared with unprocessed-*Ht* aqueous extract (8.10 ± 0.09) [23]. On the other hand, autoclaving resulted insignificantly decrease in TPC content by 4.59% when compared with blanched-*Ht* extract. Similarly, TFC content of blanched-*Ht* aqueous extract (4.94 ± 0.36) and autoclaved-*Ht* extract (4.38 ± 0.22) was significantly increased by 49.6% and 32.7% at $p<0.05$ level as compared to unprocessed-*Ht* aqueous extract (3.30 ± 0.28). On the other hand, autoclaving resulted insignificantly decrease in TFC content by 11.3% when compared with blanched-*Ht* extract. The present data is comparable with Bembem and Sadana, who stated that the TPC content (mg/100g) of boiled (26.38) and pressure cooked (32.72) potato tuber was significantly increased ($p<0.05$) by 11% and 38% when compared with unprocessed tubers (23.75) [24]. Similarly, Kamalaja et *al.* reported that pressure cooked beans (577.13 ± 2.02) had higher TPC content as compared to unprocessed beans (501.4 ± 0.01) [25]. Data for TFC reported by Saetan et *al.* indicated that blanched *C. porrectum* herbal tea had significantly increased TFC value (mgCE/g) i.e. 57.05 ± 8.62 when compared with unblanched (45.32 ± 1.58) [26]. Thermal processing at low temperature releases more bound phenols due to breakdown of the cellular components of the tuber, thus increasing TPC during blanching. Secondary, the less obtained value in autoclaving is probably due to a degradation of some phenolic compounds at high temperature [27]. The ascorbic acid content (mg/100g) of unprocessed-*Ht* was 21.83 ± 0.64 which was comparable with Mahrous et *al.,* who reported that *Helianthus tuberosus* had 17.07 mg/100g of ascorbic acid. Ascorbic content of processed-*Ht* i.e. blanched (17.71 ± 0.81) and autoclaved (14.36 ± 0.31) was significantly decreased by 18.8% and 34.2% at $p<0.05$ level when compared with unprocessed-*Ht* (21.83 ± 0.64) [28]. On the other hand, autoclaving resulted significantly decrease in ascorbic acid content by 18.91% when compared with blanched-*Ht* extract. Likewise, Sinha et *al.* reported that steamed sweet potato had significantly lower ascorbic acid content i.e. 15.85 ± 0.35 mg/100g when compared with unprocessed (21.23 ± 1.22 mg/100g) [29]. The loss in ascorbic acid content during processing might be due to its sensitivity towards water, heat and air [30].

The DPPH radical scavenging activity for ascorbic acid, unprocessed and processed-*Ht* methanolc extract is shown in Figure 1a-1d. DPPH is a stable free radical that is deep purple in color. This assay measures the ability of biological samples to reduce 1,1-diphenyl-2-picryl hydrazyle radical to 1,1-diphenyl-2-picryl hydrazine, therefore a reduction in purple color indicates a reduction in free radicals [31]. The activity was estimated by comparing the % inhibition of DPPH radical formation by the extracts and ascorbic acid acted as positive control. It was found that the radical scavenging activity of control and samples extract increased with increasing concentration

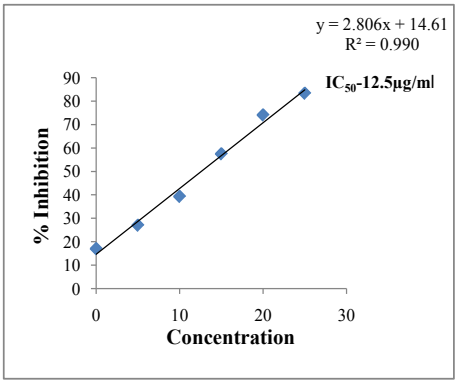

Figure 1a: DPPH radical scavenging activity of ascorbic acid.

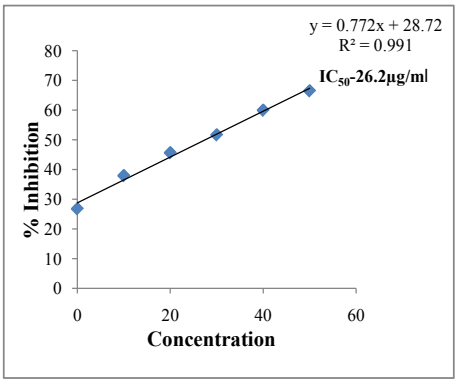

Figure 1b: DPPH radical scavenging activity of unprocessed-*Ht* methanolic extract.

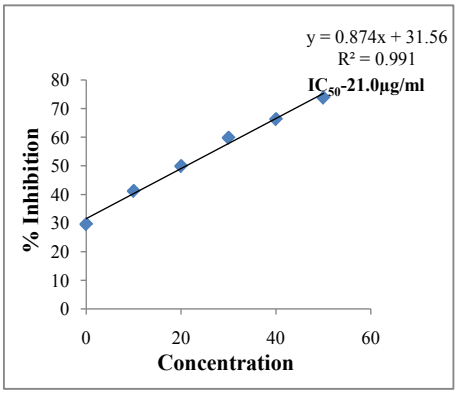

Figure 1c: DPPH radical scavenging activity of blanched-*Ht* methanolic extract.

and a lower value of IC_{50} value indicates higher antioxidant activity. The data indicated that blanched-*Ht* extract exhibits significantly highest antioxidant capacity with IC_{50}value (21.07 µg/ml) followed by autoclaved-*Ht* extract (23.1 µg/ml) while unprocessed-*Ht* extract showed lowest scavenging activity 26.2µg/ml when compared to control (12.5 µg/ml). The present study is comparable with Oboh, who reported that blanched *Telfairia occidentalis* had highest free radical scavenging (16.4%) when compared with unprocessed (20.0%) [32]. The decrease free radical scavenging during autoclaving occur due to the loss of functional groups as a result of polymerization reactions

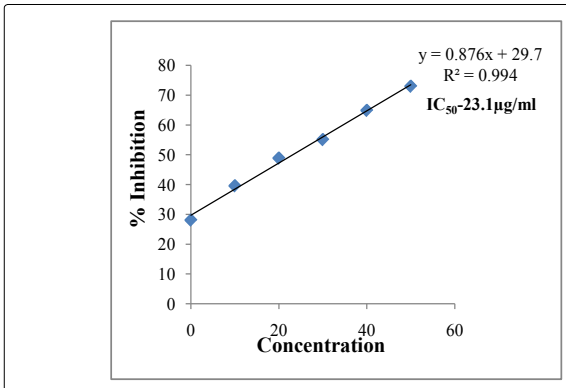

Figure 1d: DPPH radical scavenging activity of autoclaved-*Ht*methanolic extract.

arising at high temperature while the increase scavenging during blanching is associated with high level of phenolic compounds [33].

Conclusion

The present study uncovered the fact that blanching and autoclaving had significantly affected the nutraceutical profile of *Ht* aqueous extract. Blanching resulted significantly decrease in inulin, fructo-oligosaccharides and ascorbic acid content but less than autoclaving. Unlike this, it resulted significantly increase in total phenols and flavonoids content. Likewise, blanching exhibits high antioxidant capacity than autoclaving. Hence, in an overall consideration of these treatments the present study suggests that blanched tuber would be appropriate to possess pharmaceutical properties due to high nutraceutical content.

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