



A COMPARATIVE STUDY ON IMPACT OF BLANCHING AND AUTOCLAVING ON NUTRACEUTICAL PROFILE OF *HELIANTHUS TUBEROSUS* L. (JERUSALEM ARTICHOKE)

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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 profile of *Helianthus tuberosus* (*Ht*). Soluble fibres content (inulin and fructo-oligosaccharides) and antioxidant profile (total phenols content, total flavonoids content, ascorbic acid and DPPH radical scavenging activity as well as FRAP 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 (8.93 ± 0.16 mgGAE/100g, 4.38 ± 0.22 mgQE/100g and 14.36 ± 0.31 mg/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 ($21.07 \mu\text{g/ml}$) followed by autoclaved-*Ht* extract ($23.1 \mu\text{g/ml}$) when compared with unprocessed-*Ht* extract ($26.2 \mu\text{g/ml}$). The FRAP activity of Unprocessed-*Ht* was 16.40 ± 0.33 which was significantly increased by 57.5% (Blanched-*Ht*) and 15.5% (Autoclaved-*Ht*). Hence, the present study suggests that blanched *Ht* aqueous extract would be appropriate to possess pharmaceutical properties due to high nutraceutical content.

1.Introduction

It is commonly known that oxidative stress caused by free radicals and their derivatives is responsible for disturbing redox homeostasis (Hybertson et al. 2011). 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 (Redza-Dutordoir and Averill-Bates, 2016). 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 (Nasri et al. 2014).

Helianthus tuberosus L. (Jerusalem artichoke) belongs to family *Asteraceae*, is a herbaceous perennial tuber that is cultivated worldwide in the temperate regions (Slimestad et al. 2010). 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 (Kapusta et al. 2013). 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 (Zhang and Hye-Young, 2015). 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*.

2. Materials and methods

2.1. 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 ($95^{\circ}\text{C}\pm 2$) for 2 minutes whereas in autoclaving, tubers were kept at 121°C for 15 min and cooled for 10 min at room temperature. Blanched and autoclaved tubers were exposed directly to sunlight until samples reached constant weight.

20g of powdered tuber was kept in 200ml conical flask and 100ml 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 min for continuous agitation at 150 rpm for thorough mixing. The extracts were filtered by using muslin cloth followed by Whatman filter paper No. 42 (125mm) and kept in amber colored screw capped bottle at 18°C for further analysis.

2.2. Determination of inulin

2.2.1. Free fructose content (F_f)

150 μl tuber extract was pipetted into 10ml volumetric flask containing 20 mmol L^{-1} citrate buffer 6 (5ml) 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 350nm using a UV-Vis spectrophotometer.

2.2.2. Total fructose content (F_{tot})

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

$$I = k(F_{tot} - F_f) \quad (1)$$

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 (Saengkanuk *et al.* 2011; Srinameb *et al.* 2015).

2.3.Determination of fructo-oligosaccharides (FOS)

Total sugars were estimated by phenol sulphuric method (Agrawal *et al.* 2015). Whereas, reducing sugars was estimated by Di-Nitro Salicylic Method (DNS) (Dangeti *et al.*, 2013). The FOS content was calculated as non-reducing sugars which derived from total sugars subtracted by reducing sugars (Ngampanya *et al.* 2016).

2.4.Preliminary phytochemical screening of *Helianthus tuberosus*

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 (Shinoda test), Terpenoids (Salkowski test), glycosides (Legal's test), steroids (Libermann Burchard test), phenols (Ferric Chloride) and anthroquinones (Borntrager's reaction) (Gul *et al.* 2017).

2.5.Determination of antioxidant potential

2.5.1.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 1:10g/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) (Kim *et al.* 2019).

2.5.2.Determination of total flavonoids content

Total flavonoids content were determined by using aluminium chloride colorimetric assay. A volume of 125 μ l of tuber extract is added to 75 μ l of a 5% NaNO₂ solution. The mixture was allowed to stand for 6 min. 150 μ l of aluminium trichloride (10%) was added in it and incubated for 5 min, followed by the addition of 750 μ l of NaOH (1M). The final volume of the solution was adjusted to 2500 μ l with distilled water. After 15 min of incubation of mixture turned to pink and the absorbance was measured at 510nm using spectrophotometer. The total flavonoids content was expressed as mg of quercetin equivalent (mgQE/g dry mass) (Aryal *et al.* 2019).

2.5.3.Ascorbic acid content

10g tuber powder was ground in 40ml of metaphosphoric acid to stabilize ascorbic acid content of the sample. The content was made upto 100ml by using 6% metaphosphoric acid. 20ml 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 15seconds marked the completion of titration (Dinesh *et al.* 2015).

2.5.4.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 μ g/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. IC₅₀ value was also determined by graph (Sandiya and Munniappan, 2015).

2.5.5. FRAP (ferric reducing antioxidant powder) radical scavenging activity

FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+} -TPTZ complex with an absorption maximum at 750nm. This reaction is pH-dependent (optimum pH 3.6). 0.1ml extract is added to 3.0ml FRAP reagent (10 parts 300mM sodium acetate buffer at pH 3.6, 1 part 10mM TPTZ (2, 4, 6- tripyridyl-s-triazine) in 40mM HCl and 1 part 20mM FeCl_3) and the reaction mixture is kept in a water bath at 50°C for

20min. The absorbance was measured at 595nm. FeSO_4 (100 to 1000 $\mu\text{M ml}^{-1}$) was used as a positive control (Jung et al. 2011).

2.6. Statistical analysis

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

3. Results and discussions

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	-	+	+

HtAqE: *Helianthus tuberosus* Aqueous Extract. - Absence, + Present, ++ Fairly Good, +++ Good, ++++ Very Good, +++++ Excellent.

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, (2017) 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. According to Gul et al. (2017), 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*.

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

Parameters	Unprocessed- <i>Ht</i> Aqueous Extract	Processed- <i>Ht</i> 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%↓

Values are Mean±SD of triplicate determinations. *Ht*: *Helianthus tuberosus*

*denotes significant difference when compared with unprocessed-JATF at p<0.05 level

^a denotes significant difference and ^{NS} shows non-significant difference when autoclaved-*Ht* aqueous extract compared with blanched-*Ht* aqueous extract

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-*Ht*AqE was 23.19±0.16 which agrees with El-Kholy and Mahrous, (2015) who reported that *Helianthus tuberosus* had 21.46g/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. On the other hand, autoclaving resulted significantly decrease in inulin content by 19.4% when compared with blanched-*Ht* extract. Likewise, Takeuchi and

Nagashima, (2011) revealed that Jerusalem artichoke chips treated for 120 seconds lost 20-30% inulin in hot water. 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. (2015) who stated that *Helianthus tuberosus* had

6.71±0.06g/100g fructo-oligosaccharides content and also revealed that blanching reported significantly decrease in inulin (26.14±0.87g/100g) and fructo-oligosaccharides content (4.97±0.005g/100g) when compared with unblanched *Helianthus tuberosus* (33.81±1.44g/100g and 7.35±0.07g/100g). The loss of inulin and fructo-oligosaccharides content during thermal treatment is associated with its solubility in the hot water (Vendrell-Pascuas et al. 2000).

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. (2018) who reported that *Helianthus tuberosus* had 76.84±4.96mgGA/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).

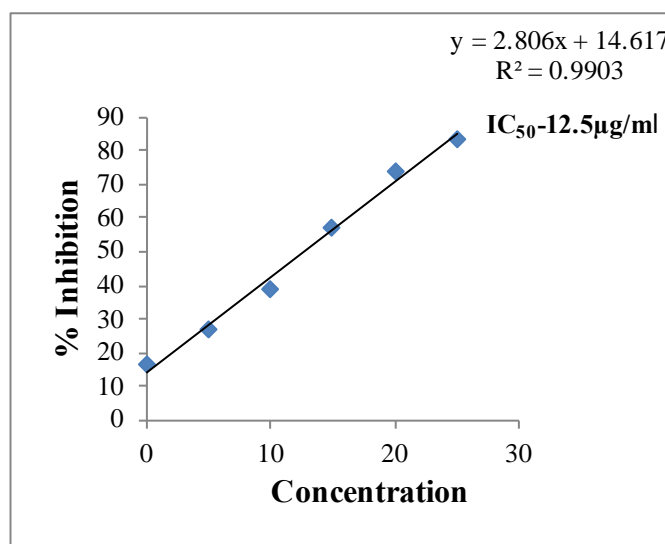


Figure 1.a. DPPH radical scavenging activity of ascorbic acid

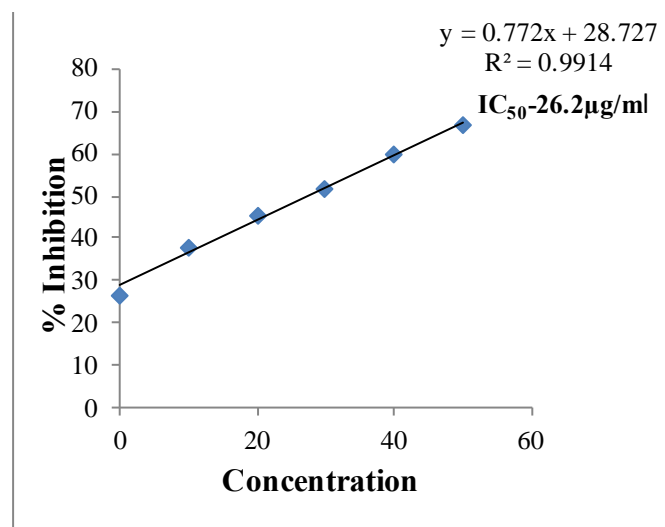


Figure 1.b. DPPH radical scavenging activity of unprocessed-*Ht* methanol extract

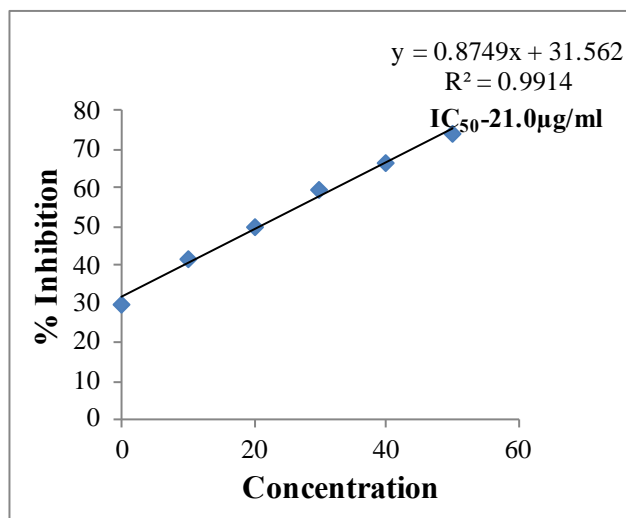


Figure 1.c. DPPH radical scavenging activity of blanched-*Ht* methanol extract

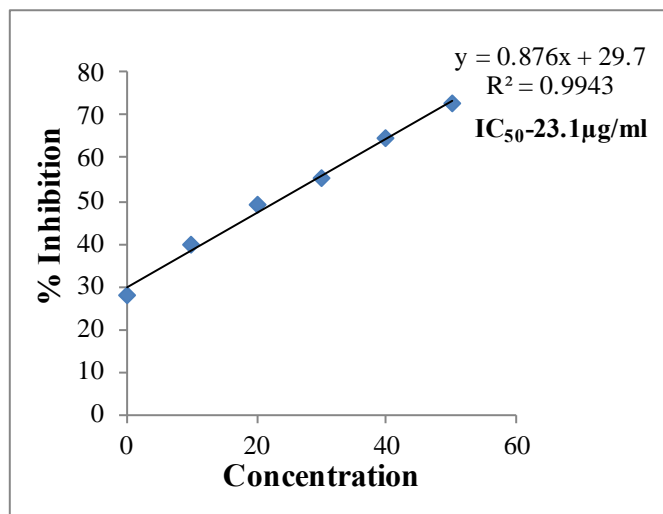


Figure 1.d. DPPH radical scavenging activity of autoclaved-*Ht* methanol extract

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, (2013) 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). Similarly, Kamalaja et al. (2018) reported that pressure cooked beans (577.13 ± 2.02) had higher TPC content as compared to unprocessed beans (501.4 ± 0.01). Data for TFC reported by Saetan et al. (2016) 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). Thermal processing

at low temperature releases more bound phenols due to breakdown of the cellular

Secondary, the less obtained value in autoclaving is probably due to a degradation of some phenolic compounds at high temperature (Dewanto et al. 2002). The ascorbic acid content (mg/100g) of unprocessed-*Ht* was 21.83 ± 0.64 which was comparable with Mahrous et al. (2016) who reported that *Helianthus tuberosus* had 17.07mg/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). 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. (2015) 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). The loss in ascorbic acid content during processing might be due to its sensitivity towards water, heat and air (El-Ishaq and Obirinakem, 2015).

The DPPH radical scavenging activity for ascorbic acid, unprocessed and processed-*Ht* methanolic extract is shown in Figure 2(a, b, c and d). 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 (Willis et al. 2019). 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 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 ($21.07 \mu\text{g/ml}$) followed

components of the tuber, thus increasing TPC during blanching.

by autoclaved-*Ht* extract ($23.1 \mu\text{g/ml}$) while unprocessed-*Ht* extract showed lowest scavenging activity $26.2 \mu\text{g/ml}$ when compared to control ($12.5 \mu\text{g/ml}$). The present study is comparable with Oboh, (2005) who reported that blanched *Telfairia occidentalis* had highest free radical scavenging (16.4%) when compared with unprocessed (20.0%). The decrease free radical scavenging during autoclaving occur due to the loss of functional groups as a result of polymerization reactions arising at high temperature while the increase scavenging during blanching is associated with high level of phenolic compounds (Carciochi et al. 2016).

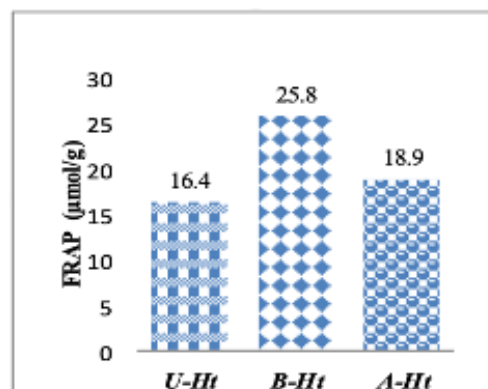


Figure 2. FRAP activity of *Ht* aqueous extract

Figure 2 depicts antioxidant estimation for FRAP ($\mu\text{mol/g}$) of unprocessed (U) and blanched (B) and autoclaved (A) *Helianthus tuberosus* aqueous extract. The FRAP activity of U-*Ht* was 16.40 ± 0.33 which was significantly increased by 57.5% (B-*Ht*) and 15.5% (A-*Ht*). Similar study was reported by Halvorsen, et al. (2006) that blanching of vegetables resulted in increased FRAP value. Sreeramulu and Raghunath, (2010) reported that unprocessed *Tryphonium trilobatum*, *Solanum tuberosum* and *Ipomoes batatas* had 2891.47 ± 310.24 , 704.73 ± 102.28 and

422.56±315.34mg/100g FRAP values which were higher than the present data.

4.Conclusions

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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