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EFFECTS OF PRETREATMENT AND DRYING TEMPERATURE ON ANTIOXIDANTS AND ANTINUTRIENTS OF *Justicia insularis* AND *Jatropha tanjorensis* LEAVES

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Abstract: Antioxidants and antinutrient contents of leafy vegetables may be influenced by post-harvest processing. The present study was carried out to assess the effect of pretreatments (P) and drying temperatures (T) on antioxidants and antinutrients of *Justicia insularis* and *Jatropha tanjorensis* leaves. Four groups of samples were prepared, namely: fresh sample (FR), un-blanching and dried sample (UB), sample blanching in hot water and dried (BHW) and sample blanching in salt water and dried (BSW). Drying was conducted at 40 °C, 50 °C and 60 °C using hot air oven. Antioxidants and antinutrients of the samples were determined. The data collected were subjected to a two-way Analysis of Variance (ANOVA) as well as Dunnett t-test at 5% probability level. Based on the results, BHW between 50 °C and 60 °C increased the antioxidant activity in both vegetables. BHW 60 °C was able to reduce oxalate and phytate contents to reasonable level while BSW 50 °C drastically decreased hydrogen cyanide and tannin contents. However, the overall, individualistic, and interactive effects of P and T had significant ($P_{cal} < 0.05$) influence on majority of antioxidant activity and antinutrient contents in both vegetables.

Key words: *Justicia insularis*, *Jatropha tanjorensis*, pretreatment,
drying temperature, antioxidants, antinutrients

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INTRODUCTION

Vegetables are succulent part(s) of plant which could be eaten raw, cooked with other dishes, as an appetizer or salad [1]. Some of them include pumpkin, cabbage, carrot, garden egg, *Justicia insularis* and *Jatropha tanjorensis*, etc. They are key ingredients in many processed foods and pharmaceutical products [2]. *Justicia insularis*, commonly called “hunter’s weed” is an herb of the family *Acanthaceae* with angular stem, and swollen aerial root at basal part of the stem [3]. The leaves are used as food ingredient especially for infants and as healing balm in some parts of West Africa [4]. Similarly, *Jatropha tanjorensis*, otherwise known as “hospital-too-far”, belongs to the family of *Euphorbiaceae* and is widely grown in the tropics [5]. The leaves are used as food ingredient and also employed in the traditional treatment of many diseases such as anaemia, diabetes and cardiovascular diseases [4]. Both vegetables contain essential food nutrients such as vitamins, minerals, fibre, carbohydrate, protein, natural antioxidants as well as traces of antinutrients [6,4,5]. However, antioxidants are free radical scavengers (e.g. carotenoids, folic acid, tocopherols, phenolic compounds, etc) that protect the harmful effects of reactive oxygen species such as hydroxyl radicals and peroxides [7]. Excess of these free radicals in the human body can lead to development of chronic diseases such as cancer, arteriosclerosis, nephritis, diabetes mellitus, etc [8]. Hence, natural antioxidants play a vital role in maintaining and preserving good health [9]. Conversely, antinutrients are natural or synthetic compounds found in a variety of plants that can inhibit the absorption of food nutrients in humans [10], e.g. oxalate, hydrogen cyanide, tannin, phytate, saponins, flavonoid, alkaloids, amongst others. Since both vegetables are perishable and the nutritional contents are very essential, preservation of the plant leaves is necessary. Several methods of preserving vegetables in terms of antioxidant retention or increase and reduction of antinutrients to minute level have been reported [11, 12, 13]. It has been found that pretreatment methods (e.g. blanching, cooking, boiling, etc.), blanching time, drying temperature could significantly affect antioxidants and antinutrients in fruits and vegetables [14, 15, 16; 17, 18, 19, 20, 21]. In an effort to preserve or retain antioxidant contents, and to reduce antinutrients to safe level, it was necessary to investigate the most suitable pretreatment and drying temperature combination that will give the product the desired quality. However, in this study the antioxidants considered were phenolic compounds while that of antinutrients were oxalate, hydrogen cyanide, tannin and phytate. The major objective of this study is to evaluate the effects of processing temperature and pretreatments on antioxidant and antinutrient content of *Justicia insularis* and *Jatropha tanjorensis* leaves.

Fig. 1. *Justicia insularis* plant

Fig. 2. *Jatropha tanjorensis* plant

MATERIAL AND METHODS

Preparation of Fresh and Dried Samples

About 200 g each of *Justicia insularis* and *Jatropha tanjorensis* leaves were sourced from a local farmer in Uyo, Akwa Ibom State. The leaves were carefully washed with distilled water to remove the dirt, and wiped with a lint free towel. They were divided into four groups, namely group I, II, III and IV. Group I was regarded as fresh sample. Group II was blanched in hot water where it was placed in a sieve and immersed in a thermostatic water bath at 95 °C for 2 minutes. The samples were then subjected to running water at 4 °C for 2 minutes. They were later exposed to air for 20 minutes and wiped with a lint free towel. Group III was blanched in 10% brine solution and followed by the same procedures carried out in Group II. Group IV was untreated. Each of the group was divided into three (3) sets before subjected to oven drying using a hot air dryer Genlab model DC 125 in Food Engineering Laboratory, University of Uyo, Uyo, Nigeria, at temperature of 40°C, 50°C and 60°C, respectively in duplicate. The range of temperature and brine solution used in this experiment was as found in literature [22, 23]. Each dried sample was ground into powder, bottled and labelled and was used for analysis.

Preparation of Sample Extract

The mixture of the sample (3.0 g) and 20 ml of 95% ethanol was mechanically shaken for 2 hours (Griffin Flash shaker, London) and centrifuged at 3,650 rpm for 35 minutes at 4°C (Anke DL-6000 B, China). The residue was re-extracted under the same conditions and both supernatants were collected and adjusted to 40 ml with 95% ethanol. All extracts were filtered through Whatman N°1 filter paper and stored at 4°C until analysis, [24].

Determination of Antioxidant Activity

The antioxidant activity was assessed using the following assays

Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) was determined using the procedure described by Bakar, Mohamed, Rahmat & Fry, [25]. Folin–Ciocalteu reagent (2.25 ml) was diluted ten-fold with distilled water and the extract (300 µl) added. Na₂CO₃ solution (2.25 ml) was mixed with the set up and was allowed to react for 5 mins at room temperature. After incubation for 90 mins at room temperature, absorbance was read at 725 nm using spectrophotometer. The concentration was expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g db).

Determination of 2, 2-Diphenyl-1-Picryl Hyrazyl (DPPH) Radical Scavenging Activity

The DPPH radical scavenging activity was determined based on the method described by Gulluce, Sahin, Sokmen, Ozer, Daferera, Sokmen, Polissiou, Adiguzel & Ozkan, [26].

A mixture of aqueous sample extract (0.1 ml) and 0.004% methanolic solution of DPPH (3 ml), was prepared. Absorbance of the mixture was read at 517 nm after 30 mins using spectrophotometer. The capacity to scavenge the DPPH radical was found using Equation 1:

$$\text{DPPH scavenging activity (\%)} = \left(\frac{A_w - A_e}{A_w} \right) \times 100 \quad (1)$$

where A_w = absorbance without extract and A_e = absorbance with extract.

Determination of Cupric Ion Reducing Antioxidant Capacity (CUPRAC)

CUPRAC was determined using the method described by Sethi, Joshi, Arora, Bhowmik, Sharma & Kumar, [27]. A mixture of sample extract (0.1 ml) and distilled water (1 ml) was made. This was followed by addition of CuCl_2 , neocuproine and ammonium acetate buffer (pH 7) ($\text{CH}_3\text{COONH}_4$) solution in 1:1:1 to obtain total reaction mixture of 4.1 ml. After 30 minutes of incubation at room temperature, absorbance was read at 450 nm using spectrophotometer. The result was expressed as $\mu\text{mol Trolox / g dry sample}$.

Determination of Ferric Reducing/Antioxidant Power (FRAP)

FRAP was determined using the method described by Butsat and Siriamornpun [28]. The FRAP solution is a mixture of 10 mM tripyridyltriazine solution, 40 mM HCl, 20 mM FeCl_3 solution, 300 mM acetate buffer and pH 3.6. The fresh solution was heated to 37 °C before use. A mixture of 100 μl of sample extract (100 μl) and FRAP solution (1.9 ml) was prepared. The absorbance of the mixture was read at 593 nm after 60 mins using spectrophotometer. The concentration of the sample was extrapolated from the standard curve prepared with known concentrations of FeSO_4 in $\mu\text{mol FeSO}_4/\text{g dry sample}$.

Determination of Antinutrients

The following antinutrients were determined:

Determination of Oxalate Content

The oxalate content of the ground sample was determined using titration method as described by Association of Official and Analytical Chemists [AOAC] [29].

A mixture of ground sample (5 g), distilled water (95 ml) and 5.0 ml 6N HCl in a beaker was heated in a water bath at 50 °C for 2 hours. Then, distilled water (126 ml) was added to the digest. The filtrate (50 ml) with methyl red indicator (few drops) in a beaker was evaporated. Ammonium hydroxide (5 ml) was added to the resulting filtrate, and heated to 90 °C, while 5% calcium chloride (10 ml) solution was added, stirred and kept overnight at 5 °C. The precipitate obtained from centrifugation and filtration was washed with 10 ml of 20% v/v H_2SO_4 and diluted with 125 ml distilled water.

Finally, aliquot solution (125 ml) was heated and titrated against 0.05 N standardized KMnO_4 solution to a faint pink colour.

Determination of Hydrogen cyanide (HCN)

Hydrogen cyanide in the ground sample was determined using alkaline picrate *method* (Nwokoro, Ogbonna & Okpala [30]. The mixture of ground sample (5 g) and in 50 ml distilled water in conical flask was corked, and the extract left overnight and later filtered.

Different concentrations of KCN solution containing 5 - 50 mg cyanide and 1 N HCl (25 ml) were used to prepare cyanide standard curve. A mixture of filtrate (1 ml) and alkaline picrate (4 ml) in a test tube was incubated in a bath for 15 minutes to develop reddish brown colour. The absorbance was read using spectrophotometer at 490 nm. The blank absorbance containing only 1.0 ml distilled water and 4.0 ml alkaline picrate solution was also read. The hydrocyanide content was extrapolated from the standard cyanide curve and expressed in mg /100 g dry sample.

Determination of Tannin

The tannin content was determined using Folin Denis Reagent as described by Makkar, Blummel, Borowy & Becker [31]. Furthermore, a mixture of ground sample (2 g) and 70% acetone (10 ml) in a corked bottle was placed in ice bath centrifuge at 4°C for 6 min. The 0.2 ml of cold –stored supernatant solution was pipetted into distilled water (0.8 ml). Standard solution of tannin acid ($\text{C}_{76}\text{H}_{52}\text{O}_{46}$) was also prepared. Folin reagent (0.5 ml) was added to both sample and standard followed by 20 % Na_2CO_3 (2.5 ml). The solution was incubated at room temperature for 40 minutes. Then, the absorbance was read at 725 nm. The concentration of tannin was estimated from the standard tannic acid curve and expressed in mg /100 g dry sample.

Determination of Phytate Content

Phytate content was determined using the method described by AOAC [29]. Moreover, a mixture of finely ground sample (2.0 g) and 0.2 N HCl (20 ml) was filtered. The mixture of filtrate (0.5 ml) and ferric ammonium iron (III) sulphate (1 ml) solution in a test tube was boiled in a water bath for 30 minutes, cooled and centrifuged. The supernatant (1.0 ml) was mixed with 2, 2-bipyridine ($\text{C}_{10}\text{H}_8\text{N}_2$) solution (1.5 ml) and the absorbance measured using spectrophotometer at 519 nm. The concentration of phytate was obtained by extrapolation from a standard curve prepared from standard phytic acid ($\text{C}_6\text{H}_{18}\text{O}_{24}\text{P}_6$) solution, and expressed in mg /100 g dry sample.

Statistical Analysis

Data were collected in duplicate, Analysis of Variance (ANOVA) and Dunnett t-test done using Statistical Package for Social Scientists (SPSS) version 20 at 5% probability level was used to assess significant mean difference (MD) between the control (fresh) and pretreatments. The results were presented in bar charts, tables, mean, standard deviation and coefficient of determination.

RESULTS AND DISCUSSION

Antioxidant Activity

The summary and multiple comparisons of antioxidant activity of fresh leaves with pretreated and dried leaves are presented in Table 1.

Table 1. Summary and multiple comparisons of mean value of antioxidant activity of *Justicia insularis* and *Jatropha tanjorensis* fresh leaves with their mean values at different pretreatments and drying temperatures

P	T (°C)	<i>Justicia insularis</i>			
		TPC (mg GAE / g db)	DPPH (%)	CUPRAC(μ mol Trolox /g db)	FRAP(μmol FeSO ₄ /g db)
FR		9.81±0.03 ^j	29.20 ± 0.11 ^a	185 ± 8.49 ^c	260 ± 14.14 ^d
UB	40	8.62 ± 0.01 ^a	31.45 ± 0.35 ^b	212 ± 32.83 ^d	280 ± 7.07 ^e
	50	9.07 ± 0.03 ^b	32.25 ± 0.21 ^c	267 ± 4.24 ^e	308 ± 5.66 ^f
	60	8.47 ± 0.01 ^c	31.15 ± 0.07 ^d	280 ± 5.66 ^f	312 ± 2.83 ^g
BHW	40	9.94 ± 0.02 ^d	30.23 ± 0.08 ^e	345 ± 7.07 ^g	331 ± 4.24 ^h
	50	9.98 ± 0.04 ^e	34.70 ± 0.14 ^f	481 ± 2.83 ^h	355 ± 32.83 ⁱ
	60	8.65 ± 0.03 ^f	30.30 ± 0.21 ^g	492 ± 4.28 ⁱ	353 ± 4.24 ^j
BSW	40	8.29 ± 0.03 ^g	28.40 ± 0.04 ^h	181 ± 2.83 ^c	234 ± 1.41 ^k
	50	7.18 ± 0.01 ^h	27.60 ± 0.42 ⁱ	235 ± 1.48 ^j	247 ± 4.95 ^d
	60	7.16 ± 0.03 ⁱ	27.30 ± 0.28 ^j	223 ± 3.5 ^k	258 ± 2.47 ^d
<i>Jatropha tanjorensis</i>					
FR		7.81±0.03 ^g	18.25 ± 0.35 ^a	255 ± 5.66 ^a	311 ± 11.31 ^c
UB	40	7.35 ± 0.04 ^a	19.10 ± 0.14 ^b	379 ± 7.07 ^b	330 ± 4.24 ^d
	50	8.02 ± 0.08 ^g	20.20 ± 0.06 ^c	387 ± 2.83 ^c	340 ± 43.54 ^e
	60	7.89 ± 0.01 ^g	19.30 ± 0.04 ^d	383 ± 4.24 ^d	421 ± 1.41 ^f
BHW	40	8.96 ± 0.01 ^b	20.30 ± 0.01 ^e	398 ± 1.41 ^e	356 ± 4.95 ^g
	50	8.97 ± 0.02 ^c	22.40 ± 0.05 ^f	403 ± 4.24 ^f	444 ± 5.66 ^h
	60	9.97 ± 0.02 ^d	21.50 ± 0.14 ^g	412 ± 8.49 ^g	546 ± 2.83 ⁱ
BSW	40	7.75 ± 0.07 ^g	17.50 ± 0.07 ^h	269 ± 1.41 ^a	300 ± 2.83 ^c
	50	7.55 ± 0.16 ^c	17.80 ± 0.05 ⁱ	302 ± 2.12 ^h	410 ± 7.07 ^j
	60	6.97 ± 0.04 ^f	16.40 ± 0.06 ^j	293 ± 0.71 ⁱ	434 ± 1.41 ^k

Note: P=pretreatment, T=temperature (°C), FR=fresh sample, UB = un-blanching and dried sample, BHW=sample blanching in hot water and dried, and BSW=sample blanching in salt water and dried. All data are the mean ± standard deviation of duplicate. Mean with the same superscript in the same column are statistically the same as the fresh sample (ANOVA, $P_{\text{tab}} < 0.05$, Dunnett t-test).

From Table 1., the fresh samples of *Justicia insularis* and *Jatropha tanjorensis* leaves contained 9.81 and 7.81 mg GAE / g db of total phenolic content (TPC), 29.20 and 18.25% of DPPH scavenging activity (DPPH), 185 and 255 μmoles Trolox / g db of CUPRAC, and 260 and 311 μmole FeSO₄ /g db of FRAP, respectively. The values for TPC were low when compared to values reported in literature for leafy vegetables [16], however, the DPPH was within range. These are just some of the ways in which each plant differs from each other, no two plants are exactly the same, even the same specie but different cultivars are different, hence need for characterization. The effects of each of the pretreatments used on this study the TPC are as follows:

There was increase with the BHW samples of up to about 1.73 and 1.325 % at 50 and 40 °C of drying temperature respectively, similar to reports of Bamide *et al.* [16]. This must have been due to the composition of the TPC of this plant.

Some phenolic compounds were seen to appreciate on blanching while some depreciate at varying degrees on blanching. Irondi *et al.*[32] reported that every other phenolic compound identified in their study depreciated during blanching but quercetin increased by 245 % and the least affected being the ellagic acid. Therefore, *Justicia insularis* may have been made of more of quercetin which may have been adversely affected at a higher temperature (60 °C) of drying. The increase in TPC may also be due to the release of the phenolic acids that were bound in the cell walls of the leaves. The pretreatment that affected TPC most adversely was the BSW, it shows the highest loss of about 27.01 % which may have been due to the fact that the salt solution may have reacted with the TPC to formed complex / other compounds making the TPC not available, making them sensitive to salt water. *Jatropha tanjorensis* followed a trend similar to that of *Justicia insularis*, with a maximum increase of 27.66% during BHW pretreatment and a highest loss of 10.76 % during BSW pretreatment. UB samples showed the least loss, this must have been due to fact the degradation wasn't much prior to drying. The little loss during UB may have been due to the drying temperature. The effects of temperature on TPC are seen to be minimal at 50 °C, followed by 40 °C and maximum at 60 °C. The DPPH scavenging activity was seen to increase for both leaves (Table 1 and 2) during the UB and BHW and worst during BSW. The increase in DPPH activity does not agree with the studies of Irondi *et al.* [32]. Several studies have reported the loss of DPPH during blanching due to leaching which is logical. However, this present study shows that every plant is unique. Report [17] has that blanching time and drying temperature could either reduce or enhance antioxidant activity in dried vegetables depending on the vegetable. The highest increase of CUPRAC (492 µmoles Trolox /g) in dried *Justicia insularis* samples was obtained at BHW60 °C while the least increase (181 µmoles Trolox /g db) was obtained at BSW 40 °C. In the same vein, CUPRAC in dried *Jatropha tanjorensis* leaves at BSW 40 °C recorded the least increase (269 µmoles Trolox /g db) while the highest increase (412 µmoles Trolox /g db) was found at BHW60 °C. Apart from that, FRAP in both dried vegetables increased as the drying temperature increased from 40 °C to 60 °C except at BHW where the content increased to 331 µmole FeSO₄ /g db initially and reduced infinitesimally from 355 to 353 µmoles FeSO₄ /g db in dried *Justicia insularis* samples. The highest FRAP of 355 and 546 µmoles FeSO₄ /g db were obtained at BHW50 °C and BHW60 °C, while the least amount of 234 and 300 µmoles FeSO₄ /g db were found at BSW 40 °C, in both dried *Justicia insularis* and *Jatropha tanjorensis* leaves, respectively. Steam blanching of kaffir lime leaf was found to increase TPC and FRAP from 20.10 ± 0.14 to 22.18 ± 0.06 mg GAE / g and 447 ± 9.01 to 628 ± 27.02 µmole FeSO₄ /g (Ratseewo *et al.*, 2016). Blanching of *Andrographis paniculata* and heat-pump dehumidified drying at 40 to 60°C was observed to increase total phenolics [33]. Bamidele *et al.* [16] reported that 5 minutes blanching time was found to increase TPC of several vegetables (from 280.6 to 980.6 mg GAE / 100 g db) and other antioxidant activities (from 25.1 – 95.1 mg GAE / 100 g db). A combination of freeze/ microwave drying was observed to significantly increase the antioxidant activity (DPPH scavenging activity and FRAP) [13].

A study by Pierre *et al.*, [20] showed an increase in total reducing power but decrease in DPPH scavenging activity of *Moringa oleifer* leaves after blanching and drying in an electric oven.

Polyphenol content and DPPH of gurum seed oil were found to increase from 22.6 to 25.3 mg GAE / kg oil and 59.2% to 64.7% after pretreatment, respectively [12]. The results of the present study are in line with similar reported studies [17, 34, 35].

However, by comparing both fresh leaves with pretreated and dried leaves, it is observed that all the mean values of TPC at different pretreatments and drying temperatures had statistical significant difference ($P_{\text{tab}} < 0.05$) for dried *Justicia insularis* leaves when compared with the fresh leaves. However, the pretreatment/ drying temperatures, for instance, UB50 °C and 60 °C and BSW 40 °C did not have any statistical significant difference on TPC when compared with the fresh leaves of *Jatropha tanjorensis* while the rest had statistical significant difference. Besides, all the mean values of DPPH in both vegetables had statistical significant mean differences. Furthermore, the mean values of CUPRAC in dried *Justicia insularis* and *Jatropha tanjorensis* leaves were not different from those ones obtained at BSW 40 °C. Conversely, the mean values of FRAP in dried *Justicia insularis* and *Jatropha tanjorensis* leaves were all different from their corresponding fresh contents except those obtained at BSW 50 °C and 60 °C, and BSW 40 °C.

Effect of Pretreatments and Drying Temperatures on Antioxidant Activity

The effects of pretreatments and drying temperatures on antioxidant activity of *Justicia insularis* and *Jatropha tanjorensis* leaves are given on Table 2.

Table 2. Summary of ANOVA results showing the effect of pretreatments and drying temperatures on antioxidant activity of *Justicia insularis* and *Jatropha tanjorensis* leaves

Sample Leaves	Antioxidant Activity	Sources of Variance	R ²	F-Value	Sig. (P _{cal})
<i>Justicia insularis</i>	TPC (mg GAE / g)	T	1.000	9060.7*	0.000
		P		1818.4*	0.000
		T × P		748.4*	0.000
	DPPH (%)	T	0.994	543.0*	0.000
		P		109.0*	0.000
		T × P		72.97*	0.000
	CUPRAC (μmoles Trolox /g)	T	0.999	5039.8*	0.000
		P		804.8*	0.000
		T × P		104.1*	0.000
<i>Jatropha tanjorensis</i>	TPC (mg GAE / g)	T	0.997	813.04*	0.000
		P		62.90*	0.000
		T × P		2.52	0.115
	DPPH (%)	T	0.999	1309.30*	0.000
		P		22.28*	0.000
		T × P		126.03*	0.000
	CUPRAC (μmoles Trolox /g)	T	0.996	3983.70*	0.000
		P		379.96*	0.000
		T × P		113.74*	0.000
	FRAP (μmoles FeSO ₄ /g)	T	0.998	1190.81*	0.000
		P		21.76*	0.002
		T × P		7.01*	0.008
				691.02*	0.000
				1649.43*	0.000
				132.34*	0.000

Note: R² = coefficient of determination, F-value =variance ratio and
P_{cal} = calculated value of probability distribution.

From Table 2, the overall effects of pretreatment (P) and drying temperature (T) on antioxidant activity of both dried vegetables is mostly notable with the values of R^2 between 0.994 – 1.000. The amount of distinctive influence of P and T revealed that $P_{cal} < 0.05$ for all the antioxidant activities examined in both vegetables. Thus, F-values were all statistically significant. The interactive effect of $T \times P$ on antioxidant activities in both vegetables was also significant, except FRAP in dried *Justicia insularis* samples with $P_{cal} = 0.115$.

Antinutrient Contents

The summary and multiple comparisons of antinutrient content of fresh leaves with pretreated and dried leaves are presented in Table 3.

Table 3. Summary and multiple comparisons of antinutrient content of fresh leaves with pretreated and dried leaves of both *Justicia insularis* and *Jatropha tanjorensis*

P	T	<i>Justicia insularis</i>				<i>Jatropha tanjorensis</i>			
		Oxalate (mg/100 g db)	HCN (mg/100 g db)	Tannin (mg/100 g db)	Phytate (mg/100 g db)	Oxalate (mg/100 g db)	HCN (mg/100 g db)	Tannin (mg/100 g db)	Phytate (mg/100 g db)
FR		0.22 ± 0.03 ^a	0.65 ± 0.04 ^b	2.11 ± 0.01 ^c	4.35 ± 0.04 ^d	0.18 ± 0.01 ^a	0.10 ± 0.01 ^c	1.19 ± 0.01 ^e	1.24 ± 0.04 ^a
UB	40 °C	0.19 ± 0.04 ^a	0.62 ± 0.01 ^b	2.09 ± 0.02 ^c	4.22 ± 0.01 ^e	0.16 ± 0.03 ^a	0.08 ± 0.02 ^c	1.15 ± 0.03 ^e	1.11 ± 0.03 ^b
	50 °C	0.17 ± 0.02 ^a	0.57 ± 0.03 ^c	2.04 ± 0.03 ^d	3.91 ± 0.01 ^f	0.14 ± 0.02 ^b	0.06 ± 0.03 ^c	1.13 ± 0.01 ^f	1.10 ± 0.01 ^c
	60 °C	0.17 ± 0.01 ^a	0.45 ± 0.03 ^d	1.99 ± 0.03 ^e	4.01 ± 0.15 ^g	0.13 ± 0.01 ^c	0.04 ± 0.01 ^d	0.92 ± 0.01 ^g	0.99 ± 0.01 ^d
BHW	40 °C	0.14 ± 0.02 ^b	0.41 ± 0.01 ^e	1.03 ± 0.01 ^f	3.33 ± 0.47 ^h	0.09 ± 0.01 ^d	0.08 ± 0.02 ^c	0.93 ± 0.02 ^h	0.86 ± 0.03 ^e
	50 °C	0.09 ± 0.01 ^c	0.37 ± 0.02 ^f	1.02 ± 0.01 ^g	2.77 ± 0.07 ⁱ	0.07 ± 0.01 ^e	0.06 ± 0.03 ^c	1.03 ± 0.03 ⁱ	0.72 ± 0.02 ^f
	60 °C	0.07 ± 0.03 ^d	0.34 ± 0.03 ^g	0.86 ± 0.04 ^h	2.70 ± 0.01 ^j	0.05 ± 0.02 ^f	0.05 ± 0.01 ^e	0.97 ± 0.03 ^j	0.63 ± 0.02 ^g
BSW	40 °C	0.21 ± 0.01 ^a	0.44 ± 0.02 ^h	1.12 ± 0.03 ⁱ	3.51 ± 0.06 ^k	0.15 ± 0.04 ^a	0.07 ± 0.03 ^c	0.81 ± 0.01 ^k	0.93 ± 0.03 ^h
	50 °C	0.18 ± 0.01 ^a	0.31 ± 0.01 ⁱ	0.89 ± 0.01 ^j	3.48 ± 0.08 ^l	0.11 ± 0.03 ^b	0.03 ± 0.01 ^f	0.76 ± 0.02 ^l	0.86 ± 0.03 ⁱ
	60 °C	0.14 ± 0.02 ^a	0.33 ± 0.01 ^j	0.81 ± 0.03 ^k	2.98 ± 0.03 ^m	0.09 ± 0.02 ^b	0.05 ± 0.01 ^g	0.96 ± 0.01 ^m	0.85 ± 0.01 ^j

Note: P = pretreatment, T = temperature (°C), FR = fresh sample, UB = un-blanching and dried sample, BHW = sample blanching in hot water and dried, and BSW = sample blanching in salt water and dried. All data are the mean ± standard deviation of duplicate. Mean with the same superscript in the same column are statistically the same as the fresh sample (ANOVA, $P_{tab} < 0.05$, Dunnett t-test).

From Table 3, the fresh samples of *Justicia insularis* and *Jatropha tanjorensis* leaves were found to be 0.22 and 0.18 mg / 100 g db of oxalate, 0.65 and 0.10 mg / 100 g db of HCN, 2.11 and 1.19 mg / 100 g db of tannin, and 4.35 and 1.24 mg / 100 g db of phytate, respectively. Notably, the concentrations of all the antinutrients in *Justicia insularis* leaves were higher than that of *Jatropha tanjorensis* leaves and were also reduced at increased drying temperature from 40 °C to 60 °C. This study shows the reduction of antinutrients during all the pretreatment used in this study this is in agreement with some reported studies [36, 37, 14, 19, 38] while others reported of increase in antinutrients at higher temperatures [18, 39]. However, oxalate contents in the fresh *Justicia insularis* and *Jatropha tanjorensis* leaves were found to reduce to minimal contents of 0.07 mg / 100 g db and 0.05 mg / 100 g db, both at BHW60 °C, respectively. Similarly, BSW 50 °C produced the least HCN content of 0.31 and 0.03 mg / 100 g db for *Justicia insularis* and *Jatropha tanjorensis* leaves, respectively.

The minimum tannin contents of 0.81 and 0.76 mg / 100 g db were found at BSW60 °C and BSW50 °C for *Justicia insularis* and *Jatropha tanjorensis* leaves, respectively. The lowest phytate contents of 2.70 and 0.63 mg / 100 g db were obtained at BHW 60 °C for both *Justicia insularis* and *Jatropha tanjorensis* leaves, respectively. Generally, the reduction in antinutrients may be due to leaching during duration blanching. It was observed that BHW 60 °C favored reduction of oxalate and phytate contents while BSW50 °C drastically reduced HCN and tannin contents. However, by comparing both fresh leaves with pretreated and dried leaves, it is observed that the mean values of oxalate content of *Justicia insularis* leaves at BHW40 °C, BHW50 °C and BSW 60 °C were statistically different from that of the fresh sample at $P_{\text{tab}} < 0.05$ when compared while the rest with the same superscript in the same column did not have any statistical significant difference. Also, the mean value of oxalate content of fresh *Jatropha tanjorensis* leaves was not different from those ones obtained at UB 40 °C and BSW 40 °C. Similarly, the fresh HCN content of *Justicia insularis* leaves was the same as the one obtained at UB 40 °C. The rest showed statistical significant differences. However, all the mean values of HCN content of dried *Jatropha tanjorensis* leaves obtained at UB40 °C, UB50 °C, BHW40 °C, BHW50 °C and BSW40 °C were not significantly different from that of the fresh sample. Besides, the mean values of tannin content in dried *Justicia insularis* and *Jatropha tanjorensis* leaves obtained at UB40 °C were not different from their corresponding fresh contents while the rest recorded statistical significant mean differences. Furthermore, the mean values of phytate content in dried *Justicia insularis* and *Jatropha tanjorensis* leaves were all different from their corresponding fresh contents. It can be deduced that pretreatment and drying temperature could affect to some extent the antinutrients present in both vegetables under investigation.

Effect of Pretreatments and Drying Temperatures on Antinutrient Contents

The effects of pretreatments and drying temperatures on antinutrient contents of *Justicia insularis* and *Jatropha tanjorensis* leaves are given on Table 4 and Table 5.

Table 4. Summary of ANOVA results showing the effect of pretreatments and drying temperatures on antinutrient contents of *Justicia insularis* leaves

Sample Leaves	Antinutrient Content	Sources of Variance	R ²	F-Value	Sig. (P _{cal})
<i>Justicia insularis</i>	Oxalate (mg /100 g)	T	0.896	9.148*	0.007
		P		26.704*	0.000
		T × P		1.41	0.307
	HCN (mg /100 g)	T	0.979	47.54*	0.000
		P		152.54*	0.000
		T × P		7.10*	0.007
	Tannin (mg /100 g)	T	0.999	95.4*	0.000
		P		4083.4*	0.000
		T × P		17.69*	0.000
	Phytate (mg /100 g)	T	0.949	11.49*	0.003
		P		67.10*	0.000
		T × P		2.47	0.120

Table 5. Summary of ANOVA results showing the effect of pretreatments and drying temperatures on antinutrient contents of <i>Jatropha tanjorensis</i> leaves					
<i>Jatropha tanjorensis</i>	Oxalate (mg /100 g)	T	0.826	5.86*	0.023
		P		14.86*	0.001
		T × P		0.29	0.876
	HCN (mg /100 g)	T	0.531	3.05	0.097
		P		0.66	0.541
		T × P		0.70	0.613
	Tannin (mg /100 g)	T	0.986	1.38	0.300
		P		172.60*	0.000
		T × P		67.60*	0.000
	Phytate (mg /100 g)	T	0.989	62.50*	0.000
		P		327.84*	0.000
		T × P		8.80*	0.004

From Table 4 and Table 5, the overall effect of pretreatment (P) and drying temperature (T) on antinutrients of both dried vegetables is judged by the values of R^2 , which ranged from 0.531 to 0.999.

However, the gross effect of P and T on HCN content of *Jatropha tanjorensis* leaves was statistically insignificant, since $R^2 = 0.531$ (quite less than 1.00). This implies that the combination of P and T may likely have minute reduction in HCN content of dried *Jatropha tanjorensis* leaves. Besides, the amount of distinctive effect of P and T revealed that $P_{cal} < 0.05$, thus, F-values were statistically significant for all the antinutrients examined in both vegetables except tannin and HCN contents in *Jatropha tanjorensis* leaves. This means that within the drying temperature of 40 °C - 60 °C, variation in tannin and HCN contents in *Jatropha tanjorensis* leaves may be infinitesimal. The interactive effect (T × P) on antinutrients in both vegetables was also significant, except oxalate and phytate contents in dried *Justicia insularis* samples and oxalate content in dried *Jatropha tanjorensis* leaves with $P_{cal} > 0.05$. It therefore means that pretreated samples using appropriate method and dried at optimum temperature could possibly reduce these antinutrients.

CONCLUSIONS

This study was carried out to evaluate the effects of processing temperature and pretreatments on antioxidant and antinutrient content of *Justicia insularis* and *Jatropha tanjorensis* leaves. BHW pretreatment between 50 °C and 60 °C was found to increase the antioxidant activity in both vegetables. BHW at 60 °C was able to reduce oxalate and phytate contents to minimal level while BSW at 50 °C drastically decreased HCN and tannin contents. However, the overall, individualistic, and interactive effects of P and T had significant ($P_{cal} < 0.05$) influence on majority of antioxidant activity and antinutrient contents in both vegetables.

It could therefore be concluded that Blanching *Justicia insularis* and *Jatropha tanjorensis* leaves using hot water at 95°C for 2 mins and drying within the range of temperature in this study could possibly increase the antioxidant activity and also reduce antinutrients to some level.

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EFEKTI PREDTRETIRANJA I TEMPERATURE SUŠENJA NA ANTIOKSIDANTE I ANTINUTRIJENTE LISTA *Justicia insularis* I *Jatropha tanjorensis*

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Sažetak: Obrada posle berbe povrća može uticati na antioksidante i antinutrijente listova povrća. Ova studija sprovedena je da se proceni efekat predtretmana (P) i temperatura sušenja (T) na antioksidante i antinutrijente listova povrća *Justicia insularis* i *Jatropha tanjorensis*. Pripremljene su četiri grupe uzoraka: sveži uzorak (FR), neblanširani i osušeni uzorak (UB), uzorak blanširan u vrućoj vodi i osušen (BHV) i uzorak blanširan u slanoj vodi i osušen (BSV).

Sušenje listova povrća je izvedeno na temperaturama od 40°C, 50°C i 60°C u peći sa toplim vazduhom. Određeni su sadržaji antioksidanta i antinutrijenta u uzorku. Prikupljeni podaci su analizirani dvostrukom Analizom varijanse (ANOVA), I Dunnett t-testom na nivou verovatnoće od 5%.

Na osnovu rezultata, BHV između 50°C i 60°C povećao je antioksidativnu aktivnost u oba varijeteta listova povrća. BHV na 60°C je imao manji sadržaj oksalata i fitata na razumni nivo, dok je BSV 50°C drastično smanjio sadržaj hidrogen cijanida i tanina . Međutim, ukupni, individualni i interaktivni efekti P i T imaju značajan ($P_{cal} < 0,05$) uticaj na većinu antioksidativne aktivnosti i sadržaja antinutrijenata u oba ispitivana varijeteta povrća.

Ključne reči: *Justicia insularis, Jatropha tanjorensis, predtretman, temperatura sušenja, antioksidanti, antinutrijenti*

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