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Phytochemical Profile and Scavenging Capacity Of Blanched *Cnidoscolus Aconitifolius* (Mill.) I.M. Johnst and *Telfairia Occidentalis* Hook. Fil. Leaves

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ABSTRACT

The application of blanching to activate and inactivate key enzymes, increase vital phytochemicals, and reduce undesirable anti nutrients present in *Cnidoscolus aconitifolius* and *Telfaira occidentalis* is of enormous potential health benefits and applications in food and pharmaceutical industries. This study aimed to investigate the phytochemicals, and antioxidant properties of blanched *Cnidoscolus aconitifolius* and *Telfairia occidentalis.* The blanching pretreatment involves immersing the leaves of *C. aconitifolius* and *T. occidentalis* in 80°C water for 3 minutes, followed by a quick cooling process. Phytochemicals and enzymes were extracted and analyzed using spectrophotometry to measure absorbance at different wavelengths. The concentration of phenol (37.00±1.00) and saponin (21.98±3.75) were significantly ($p \leq 0.05$) increased in blanched *T. occidentalis*, and statistically similar in *C. aconitifolius*. Blanching reduced the flavonoid content for *T. occidentalis* (46.32±1.55) and *C. aconitifolius* (42.42±3.03), while their anthocyanin content remains significantly similar when compared with their respective un-blanched samples. Tannin content was significantly reduced in blanched *T. occidentalis* (52.04±3.58), and a non-significant reduction was observed for *C. aconitifolius* (47.70±1.47). The DPPH and FRAP radical scavenging assay at various concentrations (IC_{25}, IC_{50}, IC_{75}, IC_{100}) revealed an increasing distinct trends in *C. aconitifolius* and *T. occidentalis* under blanched and un-blanched treatments. For peroxidase, no significant difference was seen between the un-blanched and blanched leaves of the two plant species. Blanching significantly increased polyphenol oxidase for *C. aconitifolius* (73.80±4.51), while *T. occidentalis* (31.91±1.75) was reduced when compared with their respective counterparts. This study established the effectiveness of the novel blanching techniques in enhancing various attributes of phytochemicals and antioxidant present in *C. aconitifolius* and *T. occidentalis.*

Key Words
Cnidoscolus aconitifolius, Telfairia occidentalis, blanching, enzymes, phytochemicals.

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INTRODUCTION

Indigenous plants serve as rich repertoire of natural drugs for pharmaceutical research and development [1]. Many indigenous medicinal plants are useful as spices, food, and highly important to the wellbeing of individuals and diverse communities [2]. They are also good sources of medicinal treatments for many years, while they play essential roles in the sustainability of 80 % of the world's health care system most especially for developing countries [3]. The World Health Organization had specified the need to know the composition of bioactive botanical substances considered for medicinal purposes [4].

C. aconitifolius (Family-Euphorbiaceae) has many native and common names due to its transcending traditional and medicinal uses: in Southwest Nigeria, it is natively known as 'Efo Jerusalem' while some refer to it as 'Efo Iyana Ipaja'. People in the Niger Delta area of Nigeria, call it 'Hospital Too Far'; while the common name is 'tree spinach' and generally known as 'Chaya'. It is an evergreen leafy vegetable with alternate palmate leaves, which grow up to 6 m in height and with milky sap. The leaves are large, 32 cm x 30 cm wide on succulent petiole. Its native origin is from the Maya region of Guatemala, South–East Mexico, Belize, during pre-Cambrian period [4] and due to its ease of cultivation and potential productivity, it has worldwide distribution including the tropical environment [5]. The leaves and shoot are administered as laxative, diuretic, circulatory stimulants, to stimulate lactation and to harden fingernails. It is exceptionally high in fiber (31.165 %), calcium (50 mg/g), iron (10 mg/g), potassium (20 mg/g) and vitamin C (892.025 mg/100 g) [6], thereby providing other wide range of health benefits such as anti-diabetic activity, cardio protective activity, antimicrobials, anti-inflammatory, hematopoietic property and anticancer properties [7].

T. occidentalis (fluted pumpkin) (Family-Cucurbitaceae) is native to West Africa and predominantly grown in Sierra Leone, Ghana, and Nigeria. *T. occidentalis* in the Southern region of Nigeria is known as "apiroko" in Yoruba language, while the Igbo, Efik, Edo and Urhobo speaking tribe refer to it as "ugu", "ubong" umeke" and Umee respectively [8, 9]. The plant has been domesticated by the Igbo tribe speaking people of Nigeria around 800 years ago. In traditional Igbo culture, the leaves were often used as a leafy green vegetable, while the seeds were eaten as a snack or ground into flour. The leaves are entire, leaf base; cordate, leaf venation; palmate, leaf apex; acute, and leaf surface is smooth and glossy. The stems are smooth and green, and can grow up to 15 feet long. The roots are long and tuberous, and are often harvested for food [9, 10]. The flowers are small and yellow, and the fruit is green and contains many small black seeds. The leaves are abundant in fat (18 %), protein (29 %), vitamins (20 %), and also a rich source of phosphorus, calcium, zinc, iron, and copper [11]. Phytochemical screening has confirmed the presence of saponins, alkaloids, tannins, and phenolics [10].

Globally, there is a significant demand for quality shelf-stable foods, minimally processed with optimized quality, for quick-cooking dishes. The food industries have focused their research on technologies that can help to preserve the wholesomeness of leafy and cut vegetables. This calls for the modification of practices (such as time, temperature of blanching, size of the plant materials and physiological properties of the plant) to enhance food quality and reduce adverse effects in areas of decreased nutritional value, changes in colour, texture, and loss of flavor. Hot water blanching pretreatment is one of the most commercially used blanching method in food industry because it is less expensive and simple to use. Its numerous objectives involve inactivation of enzymes such as polyphenoloxidases (PPO) and peroxidase (POD) [12], microorganisms destruction [13], removal of intercellular air from the tissues, [14, 15], extend shelf life [16], and increased extraction efficiency of bioactive compounds [17].

The enormous versatility of *C. aconitifolius* and *T. occidentalis* as a primary base for food, traditional medicine and drug development necessitates the need for better understanding and harnessing its functional bioactive properties. This present study, therefore aimed at describing the relative advantages of blanching on the phytochemical constituents, and scavenging capacity in *C. aconitifolius* and *T. occidentalis.*

MATERIALS and METHOD

Plant materials and growth

One hundred (100) matured Seeds of *C. aconitifolius* and *T. occidentalis* were respectively collected from farmers in Ibarapa East local government farm settlement, Oyo state, Nigeria (N 7^{o} 31.839, E 3^{o} 25.754) and Omu Panu farm in Osogbo local government area, Osun state,

Figure 1. *C. aconitifolius* (Mill.) I.M. Johnst.

Figure 2. *T. occidentalis* Hook.fil.

Nigeria (N 7º 46.147, E 4º 36.701). The seeds were planted in the same soil in the Department of Botany botanical garden, Lagos state University, Nigeria. Complete randomized block design was used as our experimental design with three (3) replicates each, and grown for four (4) months to attain maturity. Matured leaves that were rich in antioxidants, phytochemicals and minerals were harvested, thoroughly washed under running water, dewatered, and further allowed to remain in distilled water till the following day.

Blanching and Sample preparation

The use of hot water blanching is one of the tentative advances in leaf blanching technology. The soaked matured leaves were removed, drained and kept in hot water (80°C, 3 minutes). The leaf samples were immediately cooled for 30 seconds inside granulated ice block to stop emerging heat transfer, maintain texture and prevent excessive colour change. A known weight of about 100 grams was weighed and dried in a controlled oven at 60°C, with relative humidity of 18.25 % and 1.0 m/s for 6 hours to remove excess moisture. It was later pulverized into fine particles, stored in an airtight container and refrigerated at -20°C.

Phytochemicals extraction

To carry out the extraction process, the pulverized samples were extracted with 70 % ethanol with a Soxhlet extractor (6 hours) and filtered with Whatman filter paper. The filtrates were stored in a reagent bottle, well covered to prevent light exposure and refrigerated (4°C). The extraction was in a triplicate term (n= 3).

Quantification of total phenol content

This was achieved using the Folin-Ciocaltue assay following the procedure of Agbor *et al.*, [18]. A known volume of 300 µL of the stored filtrates was measured and poured in a test tube containing the mixture (10 % Folin-Ciocaltue reagent (1.5 ml), 7.5 % sodium carbonate (1.2 ml). It was further incubated at 45°C for 45 min. The spectrophotometric absorbance was read at wave length = 765 nm, and the construction of calibration curve was done using Gallic acid. Total phenol content was expressed as GAE/g DM (mg Gallic acid equivalent per 100 g).

Quantification of total tannin content

Quantification of total tannin content was done following Folin-Denis colorimetric method [19]. Five (5) g of the extract was weighed and poured into a test tube containing fifty (50) ml of distilled water and shaken vigorously. It was allowed to stand for thirty (30) minutes at 28°C, and filtered. Two (2) ml of the filtrates was pipetted inside a 50 ml volumetric flask, while another two (2) ml of distilled was also dispensed into another 50 ml volumetric flask (reagent blank). Tannin solution (tannic acid) measuring 2 ml was added to the two separate volumetric flask, and immediately followed by adding sodium carbonate (2.5 ml). The separate flask was filled up (50 ml) with distilled water and immediately incubated (28°C, 90 min). Spectrophotometric absorbance was measured at 765 nm, while the reagent blank was used for zero calibration.

Quantification of flavonoid content

Aluminium trichloride ($AlCl_3$) colorimetric procedure according to Liu *et al*., [20] was used. A volume of 500 µl of stored extract was measured and transferred to a test tube containing 2.5 ml distilled water. Five percent (5 %) sodium nitrite solution (150 µl) was added and allowed to remain for 5 minutes. Another 300 µl of aluminium chloride was added and kept to remain for 1 minute, after which the addition of one (1) ml of 1 M (NaOH) sodium hydroxide occurred. The total mixture was later diluted with distilled water (550 µl) and shaken vigourously using an orbital shaker. The spectrophotometric absorbance of the total mixture was carefully read at 510 nm. The standard used was Quercetin, and flavonoid content expressed in quercetin equivalent (CEQ/100 g).

Quantification of anthocyanin content

This was carried following the protocol of Wrolstad [21]. One (1) ml of the leaf extract was respectively added to nine (9) ml of pH buffers (1.0 and 4.5). Spectrophotometric absorbance was respectively taken at 20 nm for pH buffer 1.0 and 700 nm for pH buffer 4.5. Results were recorded as Cyanidin 3-glucoside equivalent per g dry matter (mg C3GE/g DM). Total anthocyanin content was calculated using the formula below

$$Total\ anthocyanin\left(\mu g.\cdot mL\right)=\frac{\left(A\times MW\times DF\right)}{\varepsilon\times L}$$

$$A = (A510 - A700)_{pH1.0} - (A510 - A700)_{pH4.5}$$

A=Absorbance, MW = anthocyanin molecular weight of 449.2, ε =Cyanidin-3-glucoside molar absorbance (26,900), L = cell path-length (1.0 cm), DF = dilution factor.

Quantification of saponin content

This was done according to the modified protocol of Mhada *et al*., [22]. One (1) ml of extract was measured and added to 3.5 ml of Liebermann-Burchard reagent containing 16.7 % acetic anhydride in concentrated sulfuric acid, and vortexed immediately using a vortex mixer for one (1) minute. It was later allowed to remain for thirty (30) minutes at room temperature. Spectrophotometric absorbance was read at 528 nm. Standard calibration curve was prepared using Oleanolic acid.

Enzyme activity

Quantification of Polyphenol Oxidase and Peroxidase enzyme

Magangana *et al*., [23] protocol with slight modification was used to achieve this. Ten (10) mL of cold extraction buffer (0.05 M/L EDTA, 60 g/L polyvinyl polypyrrolidone and 0.1 M phosphate buffer (pH 7), using ratio 1:1:1) was pipetted to one (1) g of the pulverized leaf samples, and thoroughly shaken (30 s) using a vortex mixer before incubation for ten (10) minutes at room temperature. It was then kept in the dark (120 minutes, 4°C), and centrifuged at 4000 g/rpm for twenty-five (25 min) at 4 °C. The supernatant was pipetted into test tubes and used as crude enzyme extract.

Polyphenol oxidase (PPO) was quantified following the procedure of Gonzalez *et al*., [24]. Three (3) ml of the pipetted supernatant was added to an already prepared reaction mixture (0.3 mL (0.1 M) catechol solution and 2.5 ml potassium phosphate buffer (0.1 M, pH 6),), after which an enzyme extract (0.2 mL) was added to the total reaction mixture to start the reaction process. Spectrophotometric absorbance was read at 420 nm at 25°C for 3 minutes, while the reaction mixture without extract enzyme was used for blank.

Peroxidase (POD) was measured following the methodology of Meighani *et al*., [25]. A well calibrated pipette was used to transfer 0.1 ml of guaiacol (0.045 M), 0.15 ml hydrogen peroxide solution, and 2.73 ml Na_3PO_4 (sodium phosphate buffer) (0.1 M, pH 6), inside a 3 ml cuvette. The stored supernatant (0.02 mL) was further added to initiate the reaction, spectrophotometric absorbance taken at 470 nm wavelength over a 2 min period, and the extraction solution (0.1 mL of guaiacol (0.045 M), 2.73 ml of sodium phosphate (Na_3PO_4) buffer (0.1 M, pH 6), and 0.15 mL of hydrogen peroxide solution) without the extract enzyme was used as blank.

Determination of Antioxidant activity

DPPH radical scavenging assay

This was done following the method of Karioti *et al*., [26] with slight modification. Briefly, one (1) ml of already prepared 0.1 mM DPPH in ethanol was added to one (1) ml of the extract at different concentration (IC_{25}, IC_{50}, IC_{75}, IC_{100}). The mixture was vortex, and absorbance taken at 517 nm using UV-Visible Spectrophotometer. Percentage DPPH scavenging effect was determined following the equation.

$$\text{Percentage DPPH Scavenging effect}(\%) = \frac{Ao - A1}{Ao} \times 100$$

A_0 = spectrophotometric absorbance of the control, A_1 = spectrophotometric absorbance of the extract, IC_{100} = concentration of the compounds that caused 100 % inhibition of DPPH radical formation.

Ferric Ion Reducing Antioxidant Power (FRAP)

It was determined according to the method of Benzie and Strain [27] with slight modification. One (1) ml of extract was added to ten (10) ml of 50 % methanol and immediately placed in ice cold water for 3 minutes. It was then centrifuged at 4000 rpm (4°C) for five (5) minutes. The supernatant (150 µl) was carefully pipetted and added to 2850 µl of FRAP reagent (25 ml acetate buffer (pH 3.5, 310 mM), 2.5 ml $FeCl_3$ solution (20 mM $FeCl_3 6H_2O$ in water), and 2.5 ml of TPTZ (2, 4, 6-Tris (2-pyridyl)-s-triazine) (10 mM TPTZ 40 mM HCl). The mixture was immediately vortexed (60 s), and incubated (room temperature, 45 minutes). Spectrophotometric absorbance was read at 593 nm.

STATISTICAL ANALYSIS

All analyzed phytochemicals, enzymes, and antioxidant measurements were tested before analysis of variance (ANOVA). For post-hoc analysis, data with normal distribution were subjected to Duncan's Multiple Range Test (DMRT) to separate the means. The result of all studied variables was presented as mean value±standard deviation (SD). Probability value (P-value 0.05) was used as standard significant difference among parameters. Evaluating the relationships between phytochemicals, enzymes activities, and total antioxidant potency in the samples was done using a two-tailed Pearson's correlation coefficient using SPSS 23 statistical package. The statistical analysis conducted had a 95 % confidence level.

RESULTS

Phytochemicals quantification of the studied plant species

The concentration of Phytochemicals significantly varies in the blanched and unblanched conditions of the two studied plant species (Table 1). Blanching significantly ($p \leq 0.05$) reduced the flavonoid (42.42±3.03) content of *C. aconitifolius* when compared with the unblanched content. No significant difference was observed in the remaining phytochemical contents (phenol, tannin, anthocyanin, saponin, gallic acid) of blanched and un-blanched conditions when compared together. The phenol (37.00±1.00) and saponin (21.98±3.75) content of *T. occidentalis* were significantly increased by blanching, while tannin (52.04±3.58) and flavonoid (46.32±1.55) were significantly reduced when compared to their respective un-blanched conditions. Anthocyanin concentration remained significantly unchanged in blanched and un-blanched *C. aconitifolius* and *T. occidentalis.*

Table 1. Phytochemical Composition of blanched and unblanched *C.aconitifolius* and *T. occidentalis.*

Sample	Phenol GAE/g DM	Tannin GAE/g DM	Flavonoid CEQ/100 g	Anthocyanin mgC3GE/gDM	Saponin mg DM/100 g
C. aconitifolius (Un-blanched)	32.28±2.67^{b}	52.03±4.37^{b}	54.04±1.02^{a}	26.59±3.10^{a}	19.14 ±2.73^{ab}
C. aconitifolius (Blanched)	31.54±2.19^{b}	47.70±1.47^{b}	42.42±3.03^{b}	26.77±2.02^{a}	18.74±1.48^{b}
T. occidentalis (Un-blanched)	31.53±5.26^{b}	69.00±0.09^{a}	53.32±2.45^{a}	24.27±5.65^{b}	17.32±2.70^{b}
T. occidentalis (Blanched)	37.00±1.00^{a}	52.04±3.58^{b}	46.32±1.55^{b}	24.44±3.13^{b}	21.98±3.75^{a}

Results expressed as means±SD (3 replicate); Means presenting the same letters shows no significant difference (for same column) at p≤0.05 (LSD).

Table 2. Polyphenol Oxidase (PPO) and Peroxidase (POD) Enzyme Activities of *Cnidoscolus aconitifolius* and *Telfairia occidentalis.*

Sample	Polyphenol Oxidase (U/g FW)	Peroxidase (U/g FW)
C. aconitifolius (Un-blanched)	63.98±1.43^{b}	0.84±0.19^{b}
C. aconitifolius (Blanched)	73.80±4.51^{a}	0.94±0.36^{b}
T. occidentalis (Un-blanched)	47.83±2.37^{c}	1.32±0.36^{a}
T. occidentalis (Blanched)	31.91±1.75^{d}	1.38±0.26^{a}

Results expressed as means±SD (3 replicate); Means presenting the same letters shows no significant difference (for same column) at p≤0.05 (LSD).

Polyphenol Oxidase (PPO) and Peroxidase (POD) Enzyme Activities

Table 2 outlines the enzyme activities of *C. aconitifolius* and *T. occidentalis*, under un-blanched and blanched conditions. Blanching significantly (p≤0.05) increased the Polyphenol oxidase (73.80±4.51) activity of *C. aconitifolius,* while it decreases in *T. occidentalis* (31.91±1.75). Peroxidase activity were significantly similar under the two different conditions for *C. aconitifolius* and *T. occidentalis.*

DPPH (2,2-diphenyl-1-picryl hydrazyl) Scavenging assay of *C. aconitifolius* and *T. occidentalis*

The DPPH radical scavenging assay results conducted at various concentrations (IC_{25}, IC_{50}, IC_{75}, IC_{100}) on *C. aconitifolius* and *T. occidentalis* under blanched and un-blanched conditions revealed distinct increasing trends as concentration increases (Table 3). Blanching significantly (p≤0.05) reduced DPPH activity for *C. aconitifolius* at IC_{25} (25.64±2.21) concentration, while it remained significantly unchanged in the remaining concentration. Blanched DPPH activities for *T. occidentalis* at IC_{75} (64.56±4.87) concentration was significantly increased when compared with the un-blanched, while other DPPH activities were significantly similar.

Table 4: Ferric ion reducing antioxidant power (FRAP) Radical Scavenging assay of *C. aconitifolius* and *T. occidentalis*

Table 4 shows the results of the FRAP Radical Scavenging assay performed on *C. aconitifolius* and *T. occidentalis* at different concentrations (IC_{25}, IC_{50}, IC_{75}, IC_{100}). The assay revealed increasing activity as concentration increases, but showed no significant differences between blanched and un-blanched conditions when compared.

Pearson's correlations of *C. aconitifolius* and *T. occidentalis* (Considered variables: TPC, TTC, TFC, TAC, TSC, PPO, POD, DPPH, and FRAP).

The result shows that the best significant (p≤0.05) correlation is between total saponin and total phenol content (.828**) (Table 5). A strong positive relationship also existed between DPPH and total saponin content. The

Table 3. DPPH Radical Scavenging assay of *C. aconitifolius* and *T. occidentalis.*

Sample	DPPH 25 µg	DPPH 50 µg	DPPH 75 µg	DPPH 100 µg
C. aconitifolius (Un-blanched)	37.17±1.01^{a}	55.20±2.65^{a}	65.61±3.16^{a}	84.16±2.74^{a}
C. aconitifolius (Blanched)	25.64±2.21^{b}	52.64±4.05^{a}	63.95±1.62^{a}	84.31±5.29^{a}
T. occidentalis (Un-blanched)	25.49±2.30^{b}	53.24±3.54^{a}	59.43±3.24^{b}	83.86±2.55^{a}
T. occidentalis (Blanched)	26.85±1.49^{b}	53.09±3.69^{a}	64.56±4.87^{a}	83.26±2.69^{a}

Results expressed as means±SD (3 replicate); Means presenting the same letters shows no significant difference (for same column) at p≤0.05 (LSD)

Table 4. FRAP Radical Scavenging assay of *C. aconitifolius* and *T. occidentalis.*

Sample	FRAP 25 µg	FRAP 50 µg	FRAP 75 µg	FRAP 100 µg
C. aconitifolius (Un-blanched)	0.15±0.01	0.36±0.00	0.38±0.00	0.42±0.00
C. aconitifolius (Blanched)	0.15±0.00	0.37±0.00	0.39±0.00	0.48±0.00
T. occidentalis (Un-blanched)	0.14±0.00	0.33±0.00	0.42±0.00	0.48±0.00
T. occidentalis (Blanched)	0.34±0.00	0.38±0.00	0.52±0.00	0.62±0.00

Results expressed as means±SD (3 replicate); Means presenting the same letters shows no significant difference (for same column) at p≤0.05 (LSD).

FRAP scavenging activities at IC_{75} and IC_{100} concentration had a strong correlation with DPPH at IC_{25}. Polyphenol oxidase exhibited a linear relationship with DPPH at IC_{75} concentration. While some of the variables exhibit positive correlations among themselves, suggesting a consistent linear connection, others showed a relatively weaker negative correlation implying a moderate inverse relationship.

DISCUSSIONS

Food processing technologies in industries across the globe have focused their attention on minimizing nutrient loss *vis a vis* producing good quality product for their consumers. Therefore, a correlation between polyphenol-oxidases (PPO), peroxidase (POD), phenol, tannin, flavonoid, anthocyanin and saponin, as well as the radical scavenging activities of 2,2-diphenyl-1-picryl hydrazyl (DPPH) and Ferric ion reducing antioxidant power (FRAP) under blanched condition reveals the integrity of blanching to salvage and enhance the nutrient quality of these leafy vegetables.

In the phytochemical extraction, significant increase was observed in total phenol content of the blanched condition of *T. occidentalis* in comparison to the unblanched counterpart. Contrary to this report, the studies of Ahmed and Ali [28] and Fang et al., [29] recorded a significant loss in the total phenolic content of fresh cauliflower and purple yam respectively. Nevertheless, our results are in accordance with the report of Oboh [30] who recorded an increase in the phenolic content of green leafy vegetables consumed in Nigeria after blanching for 5 minutes. Whereas, for *C. aconitifolius,* the total phenol content remained significantly similar. The large surface area, water temperature and processing time for *T. occidentalis* may be accounted as some of the major contributing factors for increased phenol content. Leaching could also be implicated in our study as some phenolic compounds are soluble in water [31, 32].

Saponins are high-molecular-weight glycosides with several biological properties [33]. Just like phenol, blanching significantly increased the saponin content for *T. occidentalis.* Saponins have proven to be useful in different industries especially in the preparation of detergents, soaps, fire extinguishers, beer, shampoos, and cosmetics [34]. This finding is of immense significance and importance to industries that requires improved saponin production for utilization.

Tannins have health promoting properties due to its antimutagenic, anticarcinogenic, antioxidant, and antimicrobial properties. They are also known as anti-nutrients because of their powerful iron chelating agents, as well as their capability of precipitating molecules and proteins [35, 36]. The significant decrease observed in tannin content for *T. occidentalis* corroborates with the work of Somsub *et al*., [37] where 21.0 to 35.2 % significant decrease was recorded in some selected Thai vegetables subjected to 100 °C hot water blanching treatment for 3 minutes. Tannins, phytates, and oxalates are regarded as heat-stable compounds [38, 39, 40, 41], and the major problem associated the leaching of tannin is the naturally accompanying removal of calcium, potassium, iron, magnesium, sodium, and phosphorus [42]. Hot water blanching pretreatment method may be highly recommended for the removal of anti-nutrients such as tannins in *T. occidentalis,* and a longer time exposure

Table 5. Pearson's correlations among 3 studied traits of *C. aconitifolius* and *T. occidentalis* (Considered variables: TPC, TTC, TFC, TAC, TSC, PPO, POD, DPPH, and FRAP).

	TPC	TTC	TFC	TAC	TSC	PPO	POD	DPPH 25 µg	DPPH 50 µg	DPPH 75 µg	DPPH 100 µg	FRAP 25 µg	FRAP 50 µg	FRAP 75 µg	FRAP 100 µg
TPC	1														
TTC mg/100g	-0.062	1													
TFC mg/100g	0.033	.608*	1												
TAC mg/100g	0.513	-0.092	0.148	1											
TSC mg/100g	.828**	-0.129	-0.108	0.534	1										
PPO	-0.448	-0.335	-0.099	0.417	-0.258	1									
POD	0.466	0.545	0.035	-0.191	0.292	-.810**	1								
DPPH 25 µg	0.130	-0.198	.587*	0.388	0.221	0.290	-0.555	1							
DPPH 50 µg	0.510	0.168	0.468	.772**	.601*	0.176	0.037	0.523	1						
DPPH 75 µg	.579*	-0.410	-0.012	.729**	.765**	0.206	-0.176	0.545	.673*	1					
DPPH 100 µg	0.483	0.174	0.267	.720**	0.561	0.264	0.054	0.286	.878**	0.525	1				
FRAP 25 µg	.673*	-0.212	-0.295	-0.160	0.568	-.799**	.608*	-0.206	-0.057	0.212	-0.101	1			
FRAP 50 µg	0.502	-.814**	-.580*	0.201	.598*	-0.075	-0.087	0.125	0.072	.616*	0.061	.649*	1		
FRAP 75 µg	.633*	-0.027	-0.311	-0.217	0.491	-.859**	.762**	-0.421	-0.118	0.048	-0.107	.963**	0.478	1	
FRAP 100 µg	.608*	-0.081	-0.440	-0.194	0.476	-.779**	.743**	-0.524	-0.156	0.039	-0.088	.932**	0.509	.985**	1

*Correlation shows significant difference at 0.05 level (2-tailed). **. Correlation shows significant difference 0.01 level (2-tailed).

for *C. aconitifolius* could lead to a significant reduction in her tannin content.

Flavonoid which is largely affected by heat in food were significantly reduced in the blanched conditions of *C. aconitifolius* and *T. occidentalis* when compared to the un-blanched. Porter [43] also recorded a 49.55 % decrease in the flavonoid content of a purple broccoli when subjected to hot water blanching treatment, which our result for flavonoid content is in accordance with. This study corroborates the report on flavonoid to be sensitive to degradation depending on the extent of structural variability exhibited by the C ring [44] which plays crucial roles in molecular stability, chemical reactivity and biological activity. Anthocyanin content were significantly similar for both blanched and un-blanched *C. aconitifolius* and *T. occidentalis.* This contradicts the report of Nurhuda *et a*l., [45] where a 39 % increase in anthocyanin concentration was recorded in rambutan peel after hot water blanching for 150 s at 100 °C. Hot

water blanching also reportedly caused significant increase in anthocyanins recovery for blueberry juice [46].

Polyphenol oxidase (PPO) and Peroxidase (POD) are known for catalytic enzymatic browning and texture improvement in vegetables and wounded fruits due to the reaction between phenols present in the plant tissues and atmospheric oxygen to produce quinones responsible for the formation of melanin [47, 48]. PPO and POD are one of the major goal during hot water blanching of many leafy and cut vegetables with a light-coloured flesh, since these enzymes are responsible for reducing the visual quality of the product. The significant increase recorded in the PPO of blanched *C. aconitifolius* depicts slight cellular disruption which led to the activation of PPO, leaf colour change and possibly the non-significant reduction in the phenolic content. Blanching was also unable to inactivate POD as a significant similarity was recorded between the blanched and unbleached samples of *C. aconitifolius.* However, the reverse was the case in blanched *T. occidentalis* where PPO was inactivated leading to the significant reduction recorded, while POD remained significantly similar and with high phenolic content. This blanching method is well suited for *T. occidentalis* and could be adopted as one of the best for the inactivation of these undesirable enzymes, and also increasing total phenolic content, while preserving colour and texture. Our results correspond to the report of Eissa *et al.*, [49] and Lespinard *et al.*, [50], where inactivation of PPO and POD in some species of mushroom after hot water blanching for 60-90 °C for 3 minutes were recorded. The findings of Ndiaye *et al.*, [51] on blanched mango slices also completely inactivated the activities of PPO and POD. Lin *et al.*, [52] observation on PPO and POD activity in *Rabdosia serra* (Maxim.) leaf after hot water blanching (90°C) for 90 seconds confirmed blanching to be the best strategy for inactivation of these unwanted enzymes *vis a vis* retaining high phenolic content.

Antioxidant activity and free radical scavenging capacity are prone to changes in vegetables when subjected to blanching and heat stress. The extent of changes may be attributed to degree of heat exposure, processing time and exposed surface area. In this study, FRAP helps to determine the inhibitory potentials of *C. aconitifolius* and *T. occidentalis* through reduction of Fe^{3+} -2,4,-tripyridyl-s-trazine (TPTZ) to the Fe^{2+}/ ferrous form in the presence of antioxidants. The extended absorbance at different concentration of the reaction mixture signify higher reducing strength. FRAP scavenging assay showed no significant differences in our study for both *C. aconitifolius* and *T. occidentalis* across all concentration (IC_{25}, IC_{50}, IC_{75}, IC_{100}).

Farvin and Jacobsen [53] reported that reducing potential of FRAP has a close relationship to total phenol content, act as electron donor, and also react with free radicals to form stable products that terminate radical chain reaction. This report justify the non-significant increase observed in FRAP activities of blanched *T. occidentalis* at all studied concentration and the high significant phenol content when compared to other samples.

The activity of DPPH radical scavenging assay across various concentration (IC_{25}, IC_{50}, IC_{75}, IC_{100}) in our study mostly showed that blanching had no significant effect on DPPH at 80 °C for 3 minutes. The exception recorded was in *C. aconitifolius* at IC_{25} concentration where a significant decrease was observed, and it correspond to the studies of Ahmed and Ali [28] and Bernaert *et al.*, [44] where the loss of DPPH radical scavenging activities in fresh cauliflower and leek was reported. *T. occidentalis* at IC_{75} showed a significant increase. The changes observed could be caused by the sensitivity of the studied vegetables to sequential modification or degradation from the hot water. Several authors have reported that changes observed in food free radical scavenging activity could be due to heat-induced synthesis, cell wall disruption caused by the release of antioxidant compounds, synthesized antioxidants, and inactivation of heat-induced oxidative enzymes caused by some chemical reactions [54, 55, 44].

CONCLUSION

This definitive finding have shown the immense benefit of blanching in obtaining high yield of several phytochemicals present in *T. occidentalis.* Based on our results, blanching could be recommended for improving the phenol and saponin content, as well as removal of anti-nutrient (tannins) present in *T. occidentalis.* Furthermore, this method could be exploited for the extraction of naturally occurring phytochemicals in pharmaceutical, food and cosmetics industries. Similar conclusions can also be made for catalytic enzymes like Polyphenol peroxidase (PPO) which was successfully inactivated under blanched *T. occidentalis*, while increasing phenol content, and preserving the leaf colour and texture. Whereas, a longer time exposure/other blanching methods for

C. aconitifolius is strongly recommended to bring about a significant reduction in the removal of the anti-nutrients present in it.

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