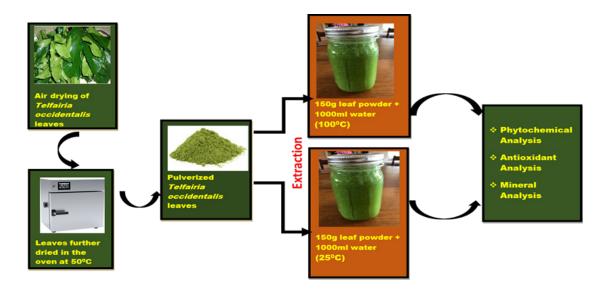
## RESEARCH ARTICLE

# Effect of extraction temperature on the phytochemicals, minerals and antioxidant properties of *Telfairia occidentalis* leaf extracts

## A. J. Awoniyi\*, O. A. Ogunmola, O.T. Babatunde and O. M. Daniel



## Highlights

- Telfairia occidentalis leaf extracts are known to contain minerals and phytochemicals
- They also show free radical scavenging and reducing potentials
- Extraction temperature showed an effect on bioactive compounds of *T. occidentalis* leaf extracts
- Cold extracts of *T. occidentalis* leaves contain higher amounts of secondary metabolites and minerals, while the hot extracts contain higher antioxidant properties.

### RESEARCH ARTICLE

# Effect of extraction temperature on the phytochemicals, minerals and antioxidant properties of *Telfairia occidentalis* leaf extracts

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**Abstract:** *Telfairia occidentalis* leaves are known to possess many therapeutic potentials such as antidiabetic, antiinflammatory, anticholesterolemic etc. To maximize their use in phytomedicine, the conditions used in preparation methods are crucial. Therefore, this study estimated the effect of extraction temperature on the phytochemicals, minerals, and antioxidant properties of T. occidentalis leaves. The powdered samples of T. occidentalis leaves were extracted using water at 100°C and 25°C, respectively, followed by the phytochemical, mineral and antioxidant analyses of the plant extracts. The concentration of most constituent metabolites in the cold extract of *T. occidentalis* leaves was significantly (p < 0.05) higher than that of the hot extract. The highest mineral concentrations in hot and cold extracts of *T. occidentalis* leaves were sodium (2.22 mg/L) and potassium (2.77 mg/L), respectively. In the antioxidant study, the EC<sub>50</sub> values (131.20  $\pm$  1.70  $\mu g/ml$  and 105.08  $\pm$  0.54 µg/ml) of the hot extract of *T. occidentalis* leaves were significantly (p < 0.05) lower than those of the cold extract (228.17  $\pm$  23.03 µg/ml and 151.47  $\pm$  14.68 µg/ml). The study reveals that the extracts hold varying biological importance. The cold aqueous extract can be an excellent nutritional adjuvant for the supply of essential nutrients, whereas the hot aqueous extract can act as an important agent in ameliorating pathological conditions thrive on excessive free radical generation. Therefore, the different extraction procedures can be utilized based on the desired biological outcome.

**Keywords:** antioxidant; different temperature; minerals; phytochemicals; *Telfairia occidentalis*;

#### INTRODUCTION

Since ancient times, humans have depended on herbs and medicinal plants as a source of food and remedy mainly because plants produce a large number of bioactive compounds which can protect against free radical damage and hence prevent diseases (Swamy and Akhatar, 2019). In recent years, antioxidants and phytochemicals in fruits and vegetables have been employed as supplements to meet body needs (Oboh *et al.*, 2006; Boots *et al.*, 2008). The extraction of these bioactive compounds in plants

depends on many variables like temperature and solvent type because they can affect the efficiency of extraction as well as the antioxidant capacity (Onyebuchi and Kavaz, 2020). Therefore, there is a need to ascertain the extraction conditions that will be most efficient in obtaining the optimal bioactive components from plants.

Telfairia occidentalis is a leafy vegetable that belongs to the family Cucurbitaceae. It is commonly called 'fluted pumpkin' and is grown in tropical rain forests of West Africa and some other parts of the world, with the largest diversity in Southeastern Nigeria (Fasuyi and Nonyerem, 2007). Previous studies reported that T. occidentalis is rich in protein (21-37% CP), ash (14%), fat (13%), and fiber (13%) (Fasuyi and Nonyerem, 2007). It also contains micronutrients like iron, potassium, phosphorous, sodium, calcium, magnesium, thiamine, nicotinamide, making the leaves riboflavin, and potentially useful as a supplement (Kayode and Kayode, 2011). Furthermore, leaves of *T. occidentalis* are rich in phytochemicals and antioxidants like phenols, tannins, alkaloids, terpenoids, flavonoids, saponins, and ascorbic acid (Oboh and Akindahunsi, 2004; Kayode and Kayode, 2011; Ekpenyong et al., 2012). In addition, previous work also reported that dietary intake of T. occidentalis leaves could prevent garlic-induced hemolytic anemia in rats (Oboh and Akindahunsi, 2004). Also, studies showed that the aqueous extracts of T. occidentalis can reduce blood glucose levels and have antidiabetic effects in glucoseinduced hyperglycaemic streptozotocin (STZ) in mice (Aderibigbe et al., 1999). Furthermore, the leaf extracts of T. occidentalis is used to manage cholesterolemia, liver problems, and inflammatory conditions (Eseyin et al., 2010).

As a result of the various medicinal capacities ascribed to *T. occidentalis* leaves, it is popularly consumed in many households in Nigeria either as a soup or folk medicine preparation, and also used in managing various diseases like diabetes, anemia, and gastrointestinal disorder (Oboh *et al.*, 2006). Although this plant has many health benefits, no study has been undertaken to report how potential health benefits of *T. occidentalis* leaves can be maximized by

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using different extraction temperatures. Therefore, this study investigated the effect of extraction temperature on the phytochemicals, minerals, and antioxidant properties of *T. occidentalis* extracts.

#### MATERIALS AND METHODS

Fresh leaves of *T. occidentalis* were obtained from Tepatan area, Oyun-Sango, Ilorin, Kwara State, Nigeria and was authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria with the voucher number UILH/001/1493/2022. Chemicals and reagents used were of analytical grade.

### Preparation of extracts of Telfairia occidentalis

A modified method of Ramírez-Aristizabal *et al.* (2017) was used for the preparation of the plant extracts. The leaves of *T. occidentalis* were harvested, rinsed, and then spread out on a clean surface to dry at room temperature, after which they were further dried in an oven at 50°C and pulverized.

The hot extract was prepared by adding 1,000 ml of hot water (100°C) to the leaf powder that has been previously weighed (150 g). The infusion was left at room temperature for 10 minutes and was agitated manually at regular intervals, after which it was filtered and concentrated in a water bath at 50°C.

Preparation of the cold extract was done by weighing 150g of the leaf powder, and then infusing in 1,000 ml cold water (25°C) for 10 minutes. The mixture was agitated manually at regular intervals during the period of infusion after which it was filtered and concentrated in a water bath at 50°C.

# Determination of percentage yield of *Telfairia* occidentalis leaf extract

The percentage yield was calculated by the following formula:

$$\%$$
Yield =  $\frac{\text{weight of plant sample - weight of extract}}{\text{weight of plant sample}} \times 100$ 

# Screening of secondary metabolites in leaves of *Telfairia* occidentalis

The secondary metabolites present in the leaves of *T. occidentalis* were determined by the methods of Trease and Evans (1989), Sofowora (1993), Harborne (1998), Obadoni and Ochuko (2001), Olajire and Azeez (2001) and Jagadish *et al.* (2009).

### Mineral analysis of Telfairia occidentalis leaf extracts

Plant extracts were digested using Aqua Regia mixture, which was prepared by weighing 1g of the extract and adding 3 ml HCl: 1 ml HNO<sub>3</sub> mixture. This was left for 24 hours, after which a little deionized water was added to dilute the mixture and then filtered. The filtrate was made up to 100 ml mark in a volumetric flask using deionized water. The method described by Association of the Official Analytical Chemists (AOAC) (2005) using atomic absorption spectrophotometer (AAS) was used for mineral contents determination. The minerals determined were

potassium, sodium, calcium, iron, magnesium, copper, manganese, and zinc.

# Antioxidant analysis of *Telfairia occidentalis* leaf extracts

# 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity

The method of Meera *et al.* (2019) was adopted for the assay. Potassium persulphate (2.45Mm) and ABTS 7mM were mixed and dissolved in double distilled water while the solution was diluted with distilled water at 1:9 v/v ratios. Then a 190  $\mu$ l volume of the reagent was pipetted into a microtiter as well as with successive addition of  $10\mu$ l of the various concentrations of the extracts or standard (Ascorbic acid). The absorbance was measured at  $\kappa$ =735nm and the reagent blank reading was taken (A<sub>0</sub>) and after 6 minutes of the initial mixing. The antioxidant activity was calculated using the values before the start of the decrease of the absorbance and the last measurement value as well (A<sub>0</sub>-A<sub>6</sub>). The formula used is shown below:

$$\frac{\text{%ABTS Scavenging}}{\text{Activity}} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

# 2, 2-diphenyl-1-picrylhydrazine (DPPH) radical scavenging assay

The scavenging ability towards DPPH was estimated using method of Meléndezet al. (2014). DPPH reagent was prepared by dissolving 8mg of DPPH in Methanol (100ml) for a solution concentration of 80µL/mL. To determine the scavenging activity, 100µL DPPH reagent was mixed with 100µL of extract at different concentrations in a 96-well micro plate and was allowed incubating at room temperature for 30minutes. After incubation, the absorbance was measured at 514nm using the micro plate reader while 100% methanol was used as the control. The DPPH scavenging activity was measured using the formula below:

$$\begin{tabular}{ll} \begin{tabular}{ll} \beg$$

#### Ferric ion reducing antioxidant power (FRAP) assay

This FRAP solution was prepared as described by Benzie and Stain (1996). The FRAP working solution was prepared by mixing 10 volumes of acetate buffer (300 mM, pH 3.6) with 1 volume of TPTZ (40 mM dissolved with 40 mM HCl) and 1 volume of ferric chloride (20 mM in water).

The micro plate FRAP assay was determined according to Jimenez – Alvarez *et al.* (2008); Firuzi *et al.* (2005) and Tsao *et al.* (2003) with some modifications. The extract at different concentrations was measured (20 $\mu$ l) and added to the 96-well micro plate followed by 280 $\mu$ l of the working FRAP solution. The mixtures were shaken, incubated at 37°C in the dark for 30 minutes, and then A<sub>593</sub> readings were recorded using a micro plate reader.

Determination of equivalent concentrations ( $EC_{so}$ ) of Telfairia occidentalis leaf extracts scavenging 50% of ABTS and DPPH radical

The equivalent concentrations (EC<sub>50</sub>) of *Telfairia* occidentalis leaf extracts scavenging 50% of ABTS and DPPH radical was obtained by the plot of concentrations (x-axis) against percentage inhibition (y-axis) (linear regression analysis) using Microsoft Excel 2016. The intercept (c) and gradient (m) were obtained from the line that fits best with the equation y = mx + c.

### Statistical analysis

All data were expressed as mean  $\pm$  standard error of mean (SEM). Statistical evaluation of data was performed by SPSS version 20.0 software (Statistical Package for Social Sciences, Inc., Chicago IL, U.S.A.) and GraphPad Prism version 6.0 (GraphPad software, San Diego, CA, U.S.A.) followed by Duncan's multiple range test for multiple comparison. Values were considered statistically significant at p< 0.05.

#### RESULTS AND DISCUSSION

#### Percentage yield of Telfairia occidentalis leaf extract

The extraction process is one of the important steps involved in the recovery of bioactive compounds from plants, and its efficacy is determined by many factors. Temperature is one of the variations that affect quantity and secondary metabolite composition of plant extracts (Pandey and Tripathi, 2014). In this study, the yield of the cold extract (30.66%) of *T. occidentalis* leaf was more than twice the yield of the hot extract (13%) (Table 1), this observation could be due to the difference in the temperature of extraction. The result obtained is in contrast to the observation made by Mohammad *et al.* (2013) where the yield of extraction of *Thymus vulgaris* leaf increased with increasing temperature.

**Table 1:** Percentage yield of *Telfairia occidentalis* leaf extracts under cold (TOC) and hot (TOH) conditions.

Samples	Percentage Yield (%)		
TOC	30.66		
ТОН	13.00		

# Chemical composition of *Telfairia occidentalis* leaf extract

Plants are known to produce diverse bioactive compounds in high concentrations which may be beneficial to the human body by protecting against free radical damage. The quality and quantity of these chemical constituents can be affected by heat treatment in the process of extraction (van het Hof et al., 2000a; 2000b). In this study, it was observed that both hot and cold aqueous infused extracts of T. occidentalis leaves contain saponins (167.53 mg/L, 166.72 mg/L), steroids (34.37 mg/L, 36.87 mg/L), glycosides (146.87mg/L, 155.63mg/L), flavonoids (66.38mg/L,73.70mg/L) and triterpenoids (16.23mg/L, 19.83mg/L) (Table 2 and 3) while phenol and tannins were present in the hot extract alone and reducing sugar was absent in both extracts. The concentrations of saponins (167.53 and 166.72mg/L) were higher in both extracts while triterpenoids (16.23 and 19.83mg/L) were the lowest. The concentration of constituent metabolites in the cold extract of *T. occidentalis* leaves was significantly (p < 0.05) higher than the hot extract except for saponins. This may be attributed to the higher yields obtained in the cold extract during the extraction.

Secondary plant metabolites are the numerous chemical compounds produced by plant cells through various metabolic pathways (Jones and Kossel, 1953). These secondary metabolites are shown to possess various biological effects, which provide the scientific basis for the use of herbs in traditional medicine in many ancient communities. They have been described of having various bioactivities such as antibiotic, antifungal and antiviral (Chiocchio *et al.*, 2021).

**Table 2:** Presence (+) or absence (-) of secondary metabolites observed in *Telfairia occidentalis* leaf extracts infused under cold and hot conditions.

Metabolites	TOC	ТОН
Phenol	-	+
Saponin	+	+
Tannin	-	+
Alkaloids	+	+
Flavonoids	+	+
Steroids	+	+
Triterpenoids	+	+
Glycoside	+	+
Reducing Sugar	-	-

The array of important metabolites present in the hot and cold extracts of *T. occidentalis* in this study, such as phenol, saponins, steroids, glycosides, tannins, flavonoids and terpenoids (Table 2 and 3), confirmed previous studies (Usunobun and Egharebva, 2014; Orole *et al.*, 2020). The concentration of saponins was highest (167.53 mg/L

**Table 3:** Concentrations (mg/L) of selected secondary metabolites of hot and cold aqueous infused extracts of *Telfairia* occidentalis leaves.

Samples	Saponin	Steroids	Glycosides	Flavonoid	Terpenoid
TOH (mg/L)	$167.53 \pm 11.77^{b}$	$34.37 \pm 0.01^{\mathrm{a}}$	$146.87 \pm 0.02^{\rm a}$	$66.38\pm0.32^{\mathrm{a}}$	$16.23 \pm 0.06^{a}$
TOC (mg/L)	$166.72 \pm 3.51^{a}$	$36.87 \pm 0.01^{\text{b}}$	$155.63 \pm 1.10^{b}$	$73.70\pm0.01^{\text{b}}$	$19.83 \pm 0.07^{\rm b}$

Values are means of 3 determinations  $\pm$  SEM. Values with different superscripts down the column are significantly (p < 0.05) different. TOC= *T. occidentalis* leaf cold extract; TOH= *T. occidentalis* leaf hot extract

and 166.72 mg/L) amongst the secondary metabolites studied in the two extracts. Saponins have demonstrated properties numerous pharmacological anticancer, cholesterol-lowering, and antiviral (Zhao et al., 2008; Vinarova et al., 2015; Marrelli et al., 2016); while others have anti-inflammatory properties (Anthoni et al., 2006). Therefore, the high concentration of saponins in plants could provide greater health benefits when taken. Furthermore, plant-derived steroids possess several health benefits, such as antitumor, immunomodulatory, anti-inflammatory, antileishmanial, and antimicrobial activities (Chen et al., 2011). More still, Kamal et al. (2014) chronicled biological significance of plant-derived steroids extensively. Therefore, the significant amount of steroids in the plant extract implies its possibility to ameliorate the highlighted disease conditions. Equally, glycosides and triterpenoids possess several therapeutic attributes, such as antimicrobial, anti-inflammatory, chemotherapeutic, and vaccine adjuvants (Lacalle-Dubois and Wagner, 2017). Flavonoids are strong antioxidants and free radical scavengers which prevent oxidative cell damage. The hydroxyl groups of flavonoids have been reported to be responsible for the scavenging of the free radicals (Pourmorad et al., 2006; Omale and Okafor, 2008). Therefore, the free radical scavenging attributes of the plant extracts may be attributable to the significant amount of flavonoids in them. In addition, flavonoids have been reported to be potent as anti-inflammatory (Jang et al., 2020), anti-cancer (Tavsan and Kayali, 2019), antihypertensive (Hou et al., 2012) and hypolipidemic agents (Bae et al., 2014).

The mineral composition of the leaf extracts of T. occidentalis was also determined and the results are given in the Table 4. According to the results, the concentration of sodium (2.22 mg/L) was highest in the hot extract of T. occidentalis leaf while potassium (2.77mg/L) was highest in the cold extract. Iron concentration was the lowest in both the hot (0.01 mg/L) and the cold (0.03 mg/L) extracts. Furthermore, the cold extract of T. occidentalis leaf had significantly (p < 0.05) higher concentrations of calcium (0.39 mg/L), potassium (2.77 mg/L), copper (0.34 mg/L), zinc (0.34 mg/L) and manganese (0.33 mg/L) than the hot extract. Moreover, concentrations of iron, magnesium and sodium were not significantly (p > 0.05) different in the two extracts.

The importance of mineral elements in human nutrition cannot be over emphasized as they are essential components of enzyme systems which are required for metabolic processes and therefore deficiency of one can have great impact on metabolism and tissue structure thereby leading to various diseases (Soetan *et al.*, 2010). Presence of mineral elements as observed in the extracts of *T. occidentalis* leaves in this study, presents the plant as a good supplement in the diet.

# Antioxidant activities of *Telfairia occidentalis* leaf extract

One of the mechanisms by which cells in living systems are protected from damage is by scavenging of free radicals by

**Table 4:** Mineral constituents of hot and cold aqueous infused extracts of *Telfairia occidentalis* leaf.

Minerals	TOH (mg/L)	TOC (mg/L)
Ca	$0.26\pm0.01^{\mathrm{a}}$	$0.39 \pm 0.00^{\rm b}$
Cu	$0.19 \pm 0.01^{\rm a}$	$0.34 \pm 0.01^{\text{b}}$
Fe	$0.01\pm0.00^{\rm a}$	$0.03\pm0.00^{\rm a}$
K	$1.03\pm0.01^{\rm a}$	$2.77\pm0.00^{\rm b}$
Mg	$0.24 \pm 0.01^{\rm a}$	$0.24 \pm 0.01^{\text{a}}$
Mn	$0.18\pm0.00^{\rm a}$	$0.33\pm0.00^{\rm b}$
Na	$2.22\pm0.04^{\rm a}$	$2.27\pm0.00^{\rm a}$
Zn	$0.07 \pm 0.01^{a}$	$0.34\pm0.00^{\text{b}}$

Values are means of 3 determinations  $\pm$  SEM. Values with different superscripts across the row are significantly (p < 0.05) different. TOC= T. occidentalis leaf cold extract; TOH= T. occidentalis leaf hot extract.

antioxidants. The antioxidant activity of the leaf extracts of Telfairia occidentalis was tested using ABTS and DPPH radical scavenging methods, as well as FRAP method and the results are presented in Figures 1-3. The ABTS<sup>+</sup> and DPPH radicals can accept electrons and H• from available antioxidants (Braca et al., 2003; Ismail et al., 2004). In this study, the extracts showed ABTS radical scavenging activity in concentration dependent manner (Figure 1). The hot extract of *T. occidentalis* leaf had a significantly (p < 0.05) higher concentration of ABTS radical scavenging activity at 10, 50, 100, and 150µg/ml when compared to the cold extract and ascorbic acid. This result is similar to that obtained by Ramírez-Aristizabal et al. (2017), where they found that the antioxidant activity of tea increased in hot extracts than cold extracts when comparing various extracts of tea. Also, the results of the study carried out by Abdul Rahim et al. (2010) is almost similar to that obtained in this study, from their findings, they observed that the antioxidant activity of extracts of Plecranthus amboinicus increased with a rise in temperature.

The DPPH radical scavenging activity was not detected in all the extracts at  $10\mu g/ml$  but was detected at 50, 100 and  $150\mu g/ml$  in a concentration dependent manner (Figure 2). There was no significant (p > 0.05) difference observed in the DPPH radical scavenging percentage of the hot and cold extracts of *Telfairia occidentalis leaves* at  $100\mu g/ml$ . At  $50\mu g/ml$ , the cold extract of *T. occidentalis* leaves had a significantly (p < 0.05) higher scavenging activity than the hot extract, whereas the hot extract had a significantly (p < 0.05) higher activity than the cold extract at  $150\mu g/ml$  and was even comparable to the reference ascorbic acid.

The FRAP method measures the total antioxidant activity of a bioactive compound by its ability to reduce ferric ion (Fe<sup>3+</sup>) to ferrous ion (Fe<sup>2+</sup>) (Santos-Sachez *et al.*, 2019). In this study, the Ferric reducing antioxidant power of the hot and cold extracts of *T. occidentalis* leaves were not significantly (p > 0.05) different at 10, 50, 100 and 150µg/ml (Figure 3). However, there was significantly (p < 0.05) higher ferric reducing antioxidant power in ascorbic acid than the hot and cold extracts of *T. occidentalis* leaf at 100 and 150µg/ml.

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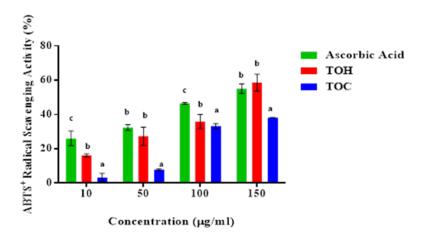
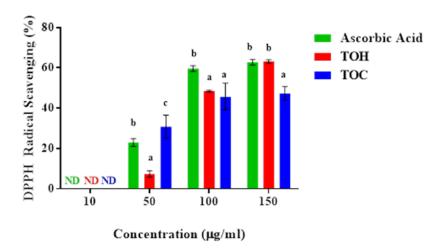
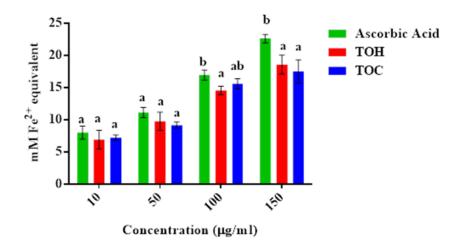


Figure 1: 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) concentration of the hot and cold extracts of *Telfairia occidentalis leaf*. Values are means of 3 determinations  $\pm$  SEM. Values with different superscripts in bars are significantly (p < 0.05) different. TOC= *T. occidentalis* leaf cold extract; TOH= *T. occidentalis* leaf hot extract.



**Figure 2:** DPPH radical scavenging activity of the hot and cold extracts of *Telfairia occidentalis leaves*. Values are means of 3 determinations  $\pm$  SEM. Values with different superscripts in bars are significantly (p < 0.05) different. ND: Not detected. TOC= *T. occidentalis* leaf cold extract; TOH= *T. occidentalis* leaf hot extract.



**Figure 3:** Ferric reducing antioxidant power (FRAP) of the hot and cold extracts of *Telfairia occidentalis leaves*. Values are means of 3 determinations  $\pm$  SEM. Values with different superscripts in bars are significantly (p < 0.05) different. TOC= *T. occidentalis* leaf cold extract; TOH= *T. occidentalis* leaf hot extract.

The ABTS and DPPH scavenging capacity of the extracts were also evaluated by calculating their EC $_{50}$  values, which correlates to the concentration of the extract that is needed to effectively scavenge 50% of the free radicals available in the reaction mixture. The hot extract of *T. occidentalis* leaves showed ABTS and DPPH scavenging EC $_{50}$  values (131.20  $\pm$  1.70µg/ml and 105.08  $\pm$  0.54µg/ml respectively) that was significantly (p < 0.05) lower than the cold extract (228.17  $\pm$  23.03µg/ml and151.47  $\pm$  14.68µg/ml respectively), and was comparable to those of the reference ascorbic acid for both ABTS and DPPH (Table 3). This presents the hot extract with a better scavenging activity because a low EC $_{50}$  value indicates high free radical scavenging activity and vice versa

Although it has been reported that thermal treatment can cause a depletion of antioxidants like vitamin C in some plants (Salihin *et al.*, 2004), application of heat can as well increase the bioavailability of some components in plants (van het Hof *et al.*, 2000a; 2000b). Therefore, the results of this study which clearly indicates that the extracts obtained using hot water were more effective towards recovering optimal amount of antioxidant components from *T. occidentalis* leaves may be due to the fact that increase in temperature of extraction increased the bioavailability of some of the components of the hot extracts.

**Table 3**: Equivalent concentrations of hot and cold extracts of *T. occidentalis* leaf scavenging 50% of ABTS and DPPH radical ( $EC_{50}$ ).

SAMPLES	EC50 (μg/ml)		
	ABTS	DPPH	
Ascorbic acid	$123\pm4.94^{\rm a}$	$103.82 \pm 1.261^{a}$	
ТОН	$131.20\pm1.70^{\mathrm{a}}$	$105.08 \pm 0.54^{\rm a}$	
TOC	$228.17 \pm 23.03^{\rm b}$	$151.47 \pm 14.68^{b}$	

Values are means of 3 determinations  $\pm$  SEM. Values with different superscripts down the column are significantly (p < 0.05) different. TOC= T. occidentalis leaf cold extract; TOH= T. occidentalis leaf hot extract

It is clearly indicated from the results of this study that the extracts obtained using hot water are more effective towards recovering optimal amounts of antioxidant components from *T. occidentalis* leaves.

#### **CONCLUSION**

The results obtained in this study revealed that the cold aqueous extract of *T. occidentalis* leaves contains a higher amount of secondary metabolites and minerals than the hot aqueous extract. However, the hot aqueous extract of *T. occidentalis* leaves possesses higher antioxidant properties than those of the cold aqueous extract. Therefore, the cold aqueous extract can be an excellent nutritional adjuvant for essential nutrient supply, whereas the hot aqueous extract can act as an important agent in ameliorating pathologic conditions that thrive on free radical generation. Therefore, a different plant extraction procedure can be utilized based on the desired biological outcome. Further studies are required to fractionate the extracts, characterize their effects, and identify their bioactive constituents.

### DECLARATION OF CONFLICT OF INTEREST

The authors declare no competing interests.

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