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Research Article

Evaluation of Nutritional, Phytochemicals and Antioxidant Capacity of *Telfairia Occidentalis* F. and *Vernonia Amygdalina* Delile Leaves

Pass C. Chijindu[1], Onyeka B. Onyeukwu[2*], Udomah Ogheneoruese[3]

[1]*Department of Biological Sciences, University of Delta, Agbor, Delta, Nigeria*
[2]*Department of Chemical Sciences, University of Delta, Agbor, Delta, Nigeria*
[3]*Department of Biology, College of Education, Warri, Delta, Nigeria.*

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***CORRESPONDENCE**
Onyeukwu, O.B.
benjamin.onyeukwu@unidel.edu.ng
+234-9060500148

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ABSTRACT

This study assessed the nutritional composition, phytochemical constituents, and antioxidant activities of *Telfairia occidentalis* and *Vernonia amygdalina* leaves. Standard analytical methods were employed to evaluate the powdered and aqueous extracts of both plants. Findings indicated that *T. occidentalis* exhibited higher levels of moisture (11.43%), crude fiber (9.11%), protein (8.19%), and crude lipid (3.67%) compared to *V. amygdalina*. No significant difference was observed in total ash content between the two species. Qualitative phytochemical analysis identified the presence of saponins, cardiac glycosides, terpenoids, triterpenoids, flavonoids, and tannins in both plants, while anthraquinones and steroids were absent. Quantitatively, *V. amygdalina* contained higher concentrations of phenolics (25.17 ± 1.03 mg/g), flavonoids (25.86 ± 0.09 mg/g), saponins (7.61 ± 0.07 mg/g), and alkaloids (6.42 ± 0.07 mg/g). In contrast, T. occidentalis had greater amounts of tannins (12.47 ± 0.06 mg/g) and cardiac glycosides (2.46 ± 0.03 mg/g). The antioxidant capacity, measured by DPPH radical scavenging activity, revealed that both plants possess significant antioxidant properties. *V. amygdalina* demonstrated a higher scavenging ability with an IC_{50} of 10.37 ± 1.61 μg/mL, compared to *T. occidentalis* at 22.99 ± 0.61 μg/mL. However, both were less potent than standard ascorbic acid (0.49 ± 0.001 μg/mL). Total antioxidant capacity (TAC) analysis showed *V. amygdalina* had a significantly higher TAC (0.941 ± 0.001 mg/g ascorbic acid equivalent) than *T. occidentalis* (0.830 ± 0.002 mg/g). These results suggest that *T. occidentalis* and *V. amygdalina* leaves are valuable vegetable sources capable of meeting human nutritional needs and providing defense against oxidative stress-related diseases.

Keywords: *Vernonia amygdalina, Telfairia occidentalis, Antioxidant properties, Pumpkin leaf, Bitter leaf, DPPH*

INTRODUCTION

Fruits and vegetables are a broad category of plant foods that differ substantially in energy and nutrient content and are generally regarded healthy (Slavin and Lloyd, 2012). Fruits and vegetables are significant providers of nutrients such as vitamins and minerals in meals because they include phytochemicals that fight oxidative stress and act as antioxidants (Basu *et al.*, 2010). Many diseases stem from oxidative stress, which is caused by an imbalance in the relative amounts of vital components of oxidative metabolism (Fatima *et al.*, 2021). Oxidative stress occurs when a system can no longer effectively remove reactive oxygen species and functional metabolites (Goodarzi *et al.*, 2018). An organism's general health is determined by the complex equilibrium between pro- and antioxidants in the cellular environment (Fatima *et al.*, 2021). Antioxidants play a variety of biological roles, even at extremely low concentrations and they also help to strengthen the immune system and defend the body against diseases caused by the invasion of uncontrolled radicals (Pisoschi and Negulescu, 2011; Sunil, 2014). A number of ageing, autoimmune illnesses, cancer, heart disease, neurological disorders, and rheumatoid arthritis are among the persistent and degenerative ailments that are greatly caused by oxidative stress (Lien *et al.*, 2008; Goodarzi *et al.*, 2018).

Fluted pumpkin (*Telfairia occidentalis* Hook F.) is one of the most widely used and common herbs, cultivated primarily in west and central Africa, particularly in Ghana, Benin, Nigeria, and Cameron (Kayode and Kayode, 2011). The plant is also known by the common names Ugu (Igbo), Egusi-iroko (Yoruba), and Ikong-ubong (Efik and Ibibio) (Kayode and Kayode, 2011). Fluted pumpkin is a tropical vine plant belonging to the cucurbitaceae family (Odiaka and Odiaka, 2011). Nutrients including carbohydrates, proteins, vitamins, lipids, minerals, and fibre are found in fluted pumpkin (Telfairia occidentalis), as well as phytochemicals such phenol, oxalates, saponins, glycosides, flavonoids, alkaloids, and resins, some of which are crucial for the manufacturing of pharmaceuticals (Nwite et al., 2013; Imosemi, 2018; Njoku, 2020). Many therapeutic benefits of fluted pumpkin have been linked to the treatment and mitigation of many ailments (Orole et al., 2020).

Vernonia amygdalina is a medium-sized shrub cultivated in the tropics and is known by several common names among the Nigerian populace, including Onugbu (in Igbo) (Achuba, 2018). *V. amygdalina* grows widespread in Africa and is a member of the Asteraceae family. In several nations, particularly in Africa, the leaves are utilised in complementary and alternative treatment. The chemical components found in *V. amygdalina* leaves include coumarins, terpenes, flavonoids, polyphenolic chemicals and these compounds have a variety of potential uses, including as anti-inflammatory, anti-cancer, and antioxidant properties (Cho et al., 2020; Fawwaz et al., 2020).

According to reports (Pem and Jeewon, 2015, Rush et al., 2019, Orole et al., 2020 Bokelmann et al., 2022), eating enough green vegetables is crucial for preventing malnutrition and hunger, guaranteeing food security, and helping farmers make money. A major factor in the underutilization of these vegetables by many individuals is a lack of awareness regarding their nutritional and therapeutic benefits. In order to support and validate the use of *V. amygdalina* and *T. occidentalis* as nutraceuticals, this study evaluated the nutritional, phytochemical and antioxidant properties of *T. occidentalis* and *V. amygdalina* leaves.

MATERIALS AND METHODS

Chemicals

Ethanol, chloroform, Mayer's reagent, Dragendorff's reagents, Hager's reagents, Wagner's reagents, sodium hydroxide, sulphuric acid, tannin acid, ascorbic acid, quercetin, lead acetate, glacial acetic acid, trichloroacetic acid, methanol, phosphate buffer, Anthrone reagent, Folin C & D, Phenol (Sigma-Aldrich), hydrochloric acid, Barium hydroxide, (JHD Shantou) zinc sulphate, potassium ferricyanide, iron chloride (Loba Chemie Mumbia).

Collection and identification of plant samples

Fresh leaves of *Telfairia occidentalis* and *Vernonia amygdalina* were harvested from a Cottage farm in College of Education, Warri (Latitude 5° 3'5.11" N Longitude 5°40'44.11"E, and altitude 13.5-17.5 m.) and the leaves were carefully examined and identified by a Taxonomist at the Department of Botany, University of Lagos and they were deposited at Lagos University Herbarium (LUH) given the voucher numbers LUH 9013 and LUH 9014 respectively.

Preparation and extraction of plant samples

After giving the fresh leaves a thorough rinse and letting them air dry at room temperature, they were pulverized into a fine powder with an electric grinder and quantified. 1000g of the dry powdered plant materials were soaked in 5 liters of double distilled water to produce aqueous extracts, which were then stored at room temperature for 48 hours to ensure a thorough extraction. The extracts were filtered using cotton wool and Whatmann filter paper No. 42 (125mm) after 48 hours and the filtrate was concentrated using a rotary evaporator for 72 hours with a water bath temperature set to 40°C before freeze-drying. After that, the dried residue (raw extract) was kept in storage at 4°C. On each experiment day, aliquot amounts of the crude plant extract residue were weighed and dissolved in distilled water.

Proximate analysis

The proximate composition of the powdered sample was done in order to determine the moisture, ash, crude protein, crude lipids, crude fiber and nitrogen-free extracts (digestible carbohydrate) according to the official method of the Association of Official Analytical Chemists (AOAC, 2002).

Phytochemical Screening

The phytochemical screening of the sample was carried out according to the methods described by Evans & Trease (2002), Harborne (1998) and Sofowora (1993). The sample was screened for saponins, alkaloids reducing sugar, anthraquinones, cardiac glycosides, terpenoids, triterpenoids, steroids, phenolic compounds, Tannins and flavonoids and was quantitatively analyzed.

Evaluation of antioxidant capacity

1, 1-diphenyl -2-picryl hydrazyl (DPPH) assay

With ascorbic acid serving as the standard, the antioxidant capacity was evaluated employing the method of Manzocco *et al.* (1998). To 2 ml of DPPH solution (0.3 mM), 0.2 ml of various aqueous leaf extract concentrations was added.

Following a half-hour dark incubation period, the absorbance at 517 nm was determined. To calculate the % inhibition of DPPH radical scavenging, use the following formula:

% inhibition of DPPH radical = $([A_0 - A_1]/ A_0) \times 100$

Where A_0 is the control absorbance (blank, without extract) and A_1 is the absorbance in the presence of the extract.

Total anitioxidant capacity

The procedure described by Prieto *et al.* (1999) was employed using ascorbic acid as a reference. In screw-capped tubes, 1.0 mL (0.20–1.00 mg/ml) of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added followed by 0.1 mL of aqueous leaf extract. After being sealed, the tubes spent 90 minutes at 95 °C in a thermal block. After cooling to room temperature, each tube's aqueous solution's absorbance was measured at 695 nm against a blank. Ascorbic acid equivalents (AAE) are used to express the total antioxidant capability.

Statistical analysis

The data are displayed as Mean ± Standard Error of Mean (SEM) (n = 3). For DPPH, analyses of variance (ANOVA) were conducted using the Least Significance Difference test in order to compare the outcomes while paired sample t-test was used to compare the outcome of the proximate composition, phytochemical analysis and total antioxidant. The threshold for statistical significance was set at $p < 0.05$. The linear regression plot of the extract's concentration against the percentage of inhibition and the bar charts created with Microsoft Excel were used to determine the IC_{50}.

RESULTS AND DISCUSSION

Results

Results of the proximate composition of *T. occidentalis* and *V. amygdalina* is presented in Table 1. *T. occidentalis* was significantly (p< 0.05) higher in moisture content (11.43%), crude lipid (3.67%), crude fibre (9.11%) and crude protein (8.19%) while *V. amygdalina* was significantly (p< 0.05) higher in carbohydrates composition (67.20%). There was no significant difference in the total ash composition of *T. occidentalis* (4.99%) and *V. amygdalina* (4.79%) at (p> 0.05).

Table 1. Proximate Composition (%) of powdered sample of T. occidentalis (Pumpkin leaf) and V. amygdalina (Bitter leaf)

Samples	Moisture content	Total ash	Crude lipid	Crude fibre	Crude protein	Carbohydrates
Telfairia occidentalis	11.430 ± 0.02^a	4.990 ± 0.01^a	3.670 ± 0.02^a	9.110 ± 0.02^a	8.190 ± 0.02^a	62.710 ± 0.01^a
Vernonia amygdalina	10.730 ± 0.02^b	4.790 ± 0.01^a	2.010 ± 0.01^b	8.040 ± 0.02^b	7.250 ± 0.03^b	67.200 ± 0.01^a

Results presented as Mean ± SEM. Values with different letters (a–b) have significant differences (p< 0.05).

Tables 2 and 3 show the qualitative and quantitative phytochemicals of *T. occidentalis* and *V. amygdalina*, respectively. Alkaloids, saponins, reducing sugars, cardiac glycosides, terpenoids, triterpenoids, phenolics, tannins and flavonoids were detected while anthraquinones and steroids were not detected in both leaves. *V. amygdalina* contained significantly (p< 0.05) higher saponins (7.61 mg/g), phenolic compounds (25.17 mg/g), and flavonoids (25.86 mg/g) compared to the saponins (3.99 mg/g), phenolic compounds (15.95 mg/g), and the flavonoids (17.95 mg/g) of T. occidentalis. The tannins (12.47 mg/g) and cardiac glycosides (2.46 mg/g) of *T. occidentalis* were significantly (p< 0.05) higher than the tannins (9.29 mg/g) and cardiac glycosides (0.55 mg/g) of *V. amygdalina*. The alkaloids (6.42 mg/g) concentration of *V. amygdalina* was not significantly different compared to the alkaloids (5.34 mg/g) concentration of *T. occidentalis* (P> 0.05).

Table 2. Phytochemicals Detected in aqueous extract of *T. occidentalis* (Pumpkin leaf) and *V. amygdalina* (Bitter leaf)

Phytochemicals	Test	Pumpkin Leaf	Bitter Leaf
Alkaloids	Mayer`s Test	+	++
	Wagner`s Test	+	+
Saponins	Frothing Test	++	+++
Reducing sugar	Fehling`s Test	+	+
Anthraquinones	Borntrager`s Test	-	-
Cardiac glycosides	Keller Killani`s Test	+	+++
Terpenoids	Liebermaan-Burchard	++	+++
Triterpenoids	Liebermaan-Burchard	++	+++
Steroids	Salkowski`s Test	-	-
Phenolic Compounds	Lead acetate Test	+	+++
Tannins	Ferric chloride Test	++	++
Flavonoids	Shinoda`s Test	+	+

+++ = abundant, ++ = moderately present, + = lightly present, - = absent

Table 3. Concentrations (mg/g) of Phytochemicals in aqueous extract of *T. occidentalis* and *V. amygdalina*

Samples	Saponins	Alkaloids	Cardiac glycosides	Steroids	Phenolic Compounds	Flavonoids	Tannins
Telfairia occidentalis	3.990 ± 0.02[a]	5.340 ± 0.02[a]	2.460 ± 0.03[a]	0.330 ± 0.02[a]	15.950 ± 1.02[a]	17.950 ± 1.07[a]	12.470 ± 0.06[a]
Vernonia amygdalina	7.610 ± 0.07[b]	6.420 ± 0.07[a]	0.550 ± 0.03[b]	0.740 ± 0.03[b]	25.170 ± 1.03[b]	25.860 ± 0.09[b]	9.290 ± 0.07[b]

Results presented as Mean ± SEM. Values with different letters (a–b) have significant differences ($p < 0.05$).

DPPH radical scavenging ability of aqueous extract of *T. occidentalis* and *V. amygdalina* leaves are shown in Figure 1. At 50% inhibitory concentration, *V. amygdalina* aqueous leaf extract was more effective at scavenging free radicals generated by stable DPPH's free radical (10.37 ± 1.61 µg/mL) than the *T. occidentalis* aqueous leaf extract (22.99 ± 0.61 µg/mL) but was significantly ($P < 0.05$) lower in scavenging DPPH's free radical when compared to the Ascorbic acid standard (0.49 ± 0.001 µg/mL). The ability of the aqueous extracts of *V. amygdalina* and *T. occidentalis* leaves to scavenge DPPH-radicals was thus demonstrated by the results. Results from the total antioxidants capacity of *T. occidentalis* and *V. amygdalina* (Figure 2) revealed a significant difference in the vegetables, with *V. amygdalina* having a significantly higher value (0.941± 0.001 mg/g AAE) than *T. occidentalis* (0.830 ± 0.002 mg/g AAE).

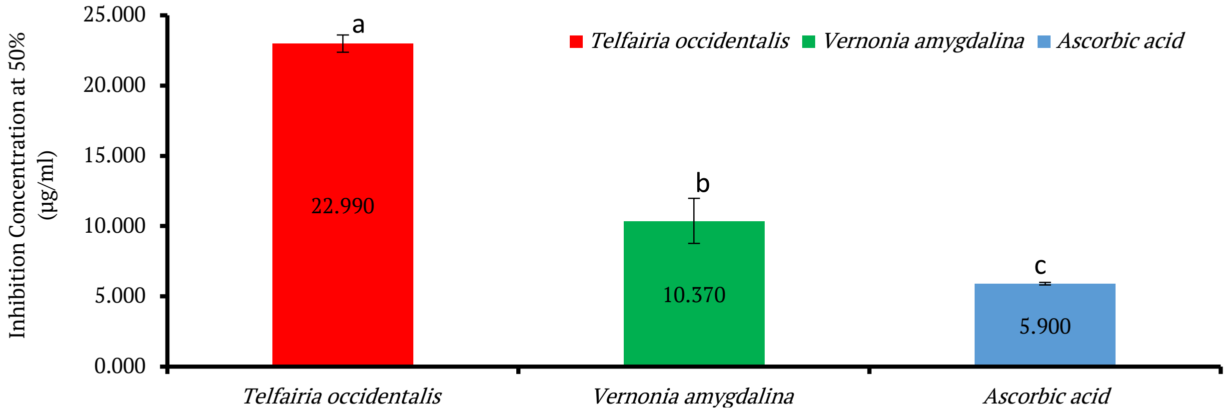

Figure 1. IC50 of *Telfairia occidentalis* and *Vernonia amygdalina* for DPPH radical.
Results presented as Mean ± SEM. Values with different letters (a–b) have significant differences ($P < 0.05$).

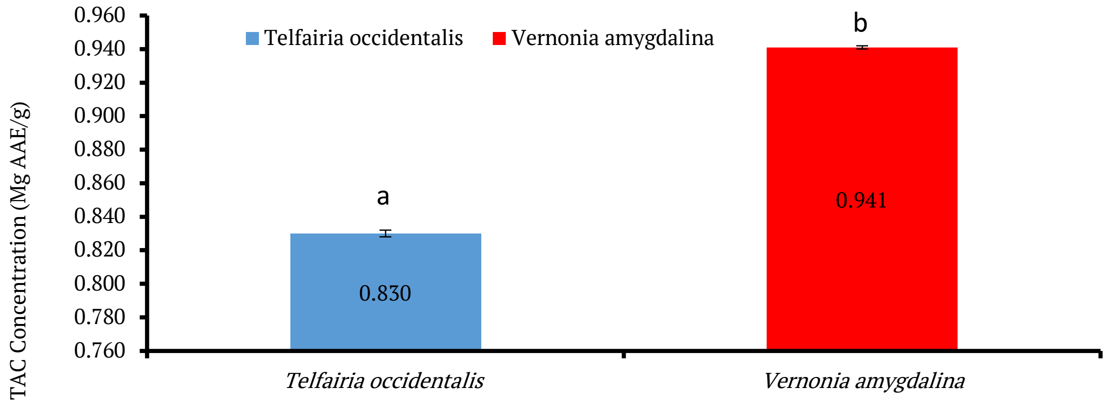

Figure 2. Total Antioxidant Capacity of *Telfairia occidentalis* and *Vernonia amygdalina*.
Results presented as Mean ± SEM. Values with different letters (a–b) have significant differences ($P < 0.05$).

Discussion

Plants provide nutritional value and can be used medicinally. The proximate analysis revealed that *T. occidentalis* and *V. amygdalina* leaves contained moisture, ash, lipid, fiber, protein, and carbohydrates. The dried leaves of *T. occidentalis* and *V. amygdalina* have fairly low moisture contents, which would prevent the proliferation of spoilage bacteria and extend the leaves' shelf life. The moisture content reported in this study is lower than the moisture content of leaves reported by Auwal et al. (2023) and slightly higher than the moisture content of leaves reported by Omimakinde et al. (2018). Elevated levels of moisture have been shown to support the preservation of the protoplasmic content of cells (Gbadamosi et al. 2011). It also promotes the activity of hydrophilic enzymes and coenzymes that are necessary for leafy green vegetable metabolism (Ihenacho and Udebuani, 2009). In contrast, the results of this study indicate that *T. occidentalis* leaves have more moisture content, are more quickly digested after consumption and are more prone to bacterial degradation during storage than *V. amygdalina* leaves. The amount of ash on leaves is an indicator of the mineral content. The ash content reported in this study is lower than the 8.3% and 8.6% reported by Adeyeye and Omolayo, (2011) and Auwal *et al.* (2023) respectively. The low amount of crude fat reported in this study is consistent with the findings of Banerjee *et al.* (2012), who stated that vegetables have minimal fat content. It is well recognised that the fatty acids found in vegetables promote membrane fluidity and facilitate gaseous exchange between intracellular and extracellular osmosis (Lovejoy, 2002). The crude fibre value in this study agrees with the result of Iheanacho (2024) who reported 10.63% crude fibre of *T. occidentalis*. Olaoye *et al.* (2023) reported (2.32 % and 2.15 %) of crude fibre of some leaves. Eating foods high in dietary fibre has been linked to lower blood cholesterol levels and a lower risk of heart disease, hypertension, diabetes, colon cancer, and breast cancer, among other illnesses (Ebana *et al.*, 2019, China *et al.*, 2021). The result of this study is consistent with the 8.72% of crude protein reported by Spranghers et al. (2017). Protein-dense plant foods are those that have more than 12% of their calories from protein (Ali, 2020). This provides evidence of the low protein content of leafy greens. The leaves in this study have a high amount of carbohydrates. Previous authors have reported high content of carbohydrates in leaves (Omimakinde et al., 2018, Ali, 2020, Auwal et al., 2023, Olaoye et al., 2023). The high concentrations of carbohydrates in the leaves suggest that they provide a significant contribution to the food total energy content. Carbohydrates are primarily used by the human body as a fuel and energy source to support daily activities and physical exercises. Phytochemicals are evidence that these leaves contain bioactive compounds with potential medical use. The phytochemicals reported in this study support previous researches carried out by various authors (Elejere *et al.*, 2019, Okoye and Orakwue, 2019, Ali, 2020, Udosen and Osu, 2022) who have reported the presence and concentrations of phytochemicals such as alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, steroids, cardiac glycosides etc. Researches have shown that flavonoids may enhance the body's defense against several disorders such as injury, cancer, ageing, atherosclerosis, inflammation, and neurodegeneration by increasing the immune system's antioxidant levels (Pal *et al.*, 2012, Dilipkumar and Preeti, 2013). According to reports, phenols have antioxidant properties that guard against oxidative damage to cells by scavenging free radicals (Ugwu *et al.*, 2013). Because phenolics can scavenge or neutralise free radicals, they have anti-inflammatory properties and reduce the risk of heart disease (Omale and Okafor, 2008). There have been reports that digestive disorders can be treated with leaves that contain tannins (Akindahunsi and Salawu, 2005). The metabolism and growth of living things are significantly influenced by alkaloids (Edeoga *et al.*, 2006). It is a good chemical for plants since it keeps parasites and predators away. The antibacterial action of the alkaloid has been attributed to the presence of recognized antimicrobial agents (Usunobun and Okolie, 2016). The potential of natural compounds to function as antioxidants can usually not be determined by one asssay (Rahman *et al.*, 2015). Hence, we evaluated the antioxidant potency of aqueous leaf extract of *V. amygdalina* and *T. occidentalis* using two techniques in this study. In both technique used for the evaluation of antioxidant capacity, *V. amygdalina* was significantly higher than *T. occidentalis* although both vegetables recorded antioxidant ability, capable of scavenging free radicals. Enhancing the quality of life by preventing or delaying the development of chronic illnesses and potentially saving a significant amount of money on medical services are two major benefits of free radical scavenging and antioxidant capability (Alam *et al.*, 2013; Anigboro *et al.*, 2019). A number of researchers have investigated the antioxidant capacity of leaves and vegetables (Iyamah *et al.*, 2014; Agambi *et al.*, 2017; Anigboro *et al.*, 2019; Chijindu *et al.*, 2022; Hasibuan *et al.*, 2023; Onyeukwu *et al.*, 2023), indicating their potential as pharmacological and therapeutic ability to combat oxidative stress. The bioactive components of the plant's leaves may be responsible for the extract samples' demonstrated antioxidant properties in this investigation. Fruits and vegetables contain the highest concentrations of naturally occurring antioxidants, such as carotenoids, tocopherols, flavonoids, phenolic acids, vitamin C, and certain minerals (Abirami *et al.*, 2015; Kulczyński *et al.*, 2020). Compared to manufactured antioxidants, natural antioxidants have no negative health effects on humans (Azeem *et al*, 2021). A large class of phytochemical substances known as flavonoids and phenols are synthesized by plants during metabolic processes as secondary metabolites connected to physiological processes such as decreasing or eliminating oxidative radicals and regulating plant growth. The process underlying this antioxidant action is that reactive oxygen species produced are neutralized by phenols and flavonoids' functional hydroxyl groups (Kumar and Pandey, 2013.

CONCLUSION

This study revealed that *T. occidentalis* (pumpkin leaf) and *V. amygdalina* (bitter leaf) are rich in antioxidants and can be used to combat oxidative stress related diseases in which the participation of reactive oxygen species has been implicated. This research has revealed the importance of pumpkin and bitter leaf for human consumption, as traditional herbs and also as possible source for the formulation of drugs as both plants are good source of antioxidants.

AUTHORS' CONTRIBUTIONS

Conceptualization and supervision: PCC; Experimentation: UO and PCC; Data analysis: OBO; Writing-original draft preparation: UO and PCC; Writing-review and editing: OBO and PCC; Resources: OBO, PCC and UO. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST

No conflict of interest declared.

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