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# In-Vitro Total Tocopherols, Phenol and Anti-oxidative Levels of Seeds' oil of *Telfairia occidentalis*

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***Abstract*** — The seeds of fluted pumpkins are usually eaten boiled or pulverized and taken as food additive locally. As a result of this manner of utilization by the locals, this study was conducted to ascertain the contents of the total tocopherol, phenolic and the antioxidative property present in the oil of the seed. The pods of the fluted pumpkin were gotten from a farm within the Benin metropolis, Nigeria. The seeds were ground in a blender and afterward exposed to soxhlet extraction. Other standard methods were employed in this study. The quantitative analysis of these parameters showed Total Tocopherols (76.0 mg/kg ± 3.11), Total phenolic (3.34 mg of garlic acid equivalent ± 0.15). Percentage inhibition of DPPH was recorded to be 47.95 % ± 3.47, Nitric oxide scavenging capacity was 56.47 % ± 2.37 and ABTS was 51.06 % ± 2.60 respectively. The findings revealed that the seed oil of *T. occidentalis* possessed beneficial antioxidant properties which could be harnessed in management of disease like diabetes and cancer among others.

***Keywords*—**Fluted pumpkin, total phenol, nitric acid, tocopherol, fluted pumpkin.

## I. INTRODUCTION

One of the most recognized basic need for life is food as a result of its provision of useful nutrients for both vitality and good health to all life's forms [1]. Generally speaking, its basic constituents are proteins, fats and oils, minerals in trace amounts, carbohydrates in various forms and different types of vitamins. They are all utilized by the body/system for sustenance of healthy lifestyle. Moreover, there just two sources for food, which are animals and plants [2].

Plants, in general, are far the most important wellspring of flavors for food and food products, fragrance for perfume industries and traditional medicine for practitioners. These ingredients could be oils, latex and gums. While fats and oils play vital roles in sourcing for nutrient in both human and animals. Although, from the ancient time, food securities have been a major problem confronting most developing countries [3].

*Telfairia occidentalis* (*T. occidentalis*) otherwise called fluted pumpkin, which has a place with the family cucuribitacea is a tropical vine grown in West Africa as a leaf Vegetable and for its consumable seeds. Its regular names incorporate "Ugwu"(in igbo), "ikong-ubong" (in Efik) and "Ebumwenkhen" (in Edo). It is indigenous to Southern Nigeria [4].

It grows in numerous nations in West Africa, however it is chiefly grown in Igbo land and Calabar land in Nigeria. *T. occidentalis* is known to force calming impact [5], erythropoietic worth [6], anticholesterolemic and insusceptible structure properties [7] and hypoglycemic impact [8, 9, 10, 11].

The medicinal properties and the level of efficacy of using the seeds of fluted pumpkin (*T. occidentalis*) seeds as described by the locals have triggered the seeds oil's phytochemical investigations. The aim of this research was to conduct screening of the antioxidant property of the seeds oil and determine the total tocopherol and phenolic content of the seeds oil of *T. occidentalis* using different standard methods.

## II. RELATED WORK

Shoots of *T. occidentalis* contain significant levels of potassium and iron, while seeds are made out of 27% rough proteins and 53% fats [12] The leaves contain a high measure of cancer prevention agents and hepato-protective and antimicrobial properties [13].

Despite the fact that pumpkin seeds are wealthy in oil stockpiling saves, it by and by has exceptionally low business esteem as an oilseed yet is possibly significant as a high protein oilseed for human and creature food [14].

Maybe the potential utilitarian advantages of pumpkin seeds could be improved comparably. Anyway a definitive achievement of using plant protein in food detailing rely to

 

a great extent on its practical properties and a portion of these useful properties incorporate water and oil ingestion limit, emulsification, protein solvency, mass thickness [15].

## III. METHODOLOGY

**Materials**:
The fluted pumpkin (*T. occidentalis*) was purchased from a nearby homestead at Egbaen Village, Ugbowo, Benin City, Edo State, Nigeria. It was distinguished in Plant Biology and Biotechnology Department of the Faculty of Life Science, University of Benin, Nigeria. The cases were part open and the seeds evacuated. The hard packaging of the seeds was evacuated and they were cut into smaller pieces and dried and afterward mixed into powdered structure. They were then put away at room temperature for sometime in the future.

**Oil extraction:**
Dried *T. occidentalis* seeds were ground in a blender and afterward exposed to soxhlet procedure. Approximately 50 gm of ground seeds were put into a cellulose paper cone and extricated utilizing oil ether (b.p 40-60°C) in a soxhlet extractor for 8hours. The oil was then recouped by dissipating of the dissolvable utilizing rotating evaporator at 60 $^{o}$C [16].

**Total Tocopherol Determination:**
An AOCS standard strategy (Ce 8-89) (17) for tocopherol investigation was followed with minor alteration. An aliquot of dissolvable separated pumpkin seed oil was moved to a pre-weighed 1.5-mL HPLC auto-sampler vial, and any remaining dissolvable or dampness was dissipated in a vacuum stove (National Appliance Co., Portland, OR) for 6h at an encompassing temperature of 22 °C. After dissipation, the heaviness of the oil was controlled by gauging the vial once more, and afterward the oil was redissolved in 1 mL of HPLC grade hexane. Copy HPLC tests were set up thusly. A 1.5 mL aliquot of test was infused utilizing a business programmed injector for measurement. Test was eluted with 1% (v/v) isopropanol in HPLC grade hexane at a 0.650 mL/min stream rate with a Beckman The tocopherol content was communicated as parts per million fixation comparative with oil.

**Total phenolic content Determination:**
Total phenolic content of *T. occidentalis* seeds oil was assayed by the modified of Dewanto *et al* 2002, [18]. The different varied concentrations of 10µg, 20µg, 40µg, 60µg, 80µg, and 100 µg were analyzed with the addition of an aliquot of diluted extract added to 0.25mL of FolinCiocalteu reagent. The volumes were adjusted with distilled water to a final volume of 3mL and shaken thoroughly. The solutions were incubated and kept in the dark and later read at 760nm against prepared blank. The total phenol content of the oil parts were expressed as milligrams of gallic acid equivalents per gram of dry weight. The total sample was analyzed in three replicates

**1,1-diphenyl-2-picrylhydrazyl (DPPH) radical Determination:**
Antioxidant activity was determined by measuring the radical scavenging power of the fluted pumpkin seeds oil against DPPH. A volume of 50 µl of each sample oil were added to 2.95 mL DPPH solution (4.5 mg DPPH in 100 mL methanol), [19a,b]. After 30 minutes, the absorbance of each sample was measured at 517 nm using PerkinElemer Lambda 25 UV/VIS spectrometer. A triplicate set of tests were conducted for each sample extract. The percentage of scavenging activity of DPPH radical was measured using the following equation:

Percentage Radical Scavenging Activity (PRSA) =
$$( A_{control} - A_{sample} )/( A_{control} ) \times 100\ \%$$

Where;
$A_{control}$ is the absorbance of the control
$A_{sample}$ is the absorbance of the sample.

**Nitric oxide scavenging activity:**
The capability of *T. occidentalis* seed oil to rummage nitric oxide radical was resolved by the method as detailed by [20]. Precisely 2 mL of 10 M sodium nitroprusside was set up in 0.5 mL phosphate support saline (pH 7.4) and blended in with 0.5 mL of various weakenings of the seed oil (0.065–1.0 mg/mL) in a 96-wells plate. The subsequent blend was brooded at 25 0C for 2 hours, after which 0.5 mL was taken from the hatched blends and added to 1 mL sulfanilic corrosive reagent (0.33% in 20% icy acidic corrosive). The blend was additionally hatched at 25 0C for 5 min. From that point, 1 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was added to the blends and the subsequent arrangement was later hatched at 25 0C for 30 min.

The absorbance was perused at 540 nm and the IC50 was then assessed from alignment bend following estimation of rate nitric oxide radical searching limit of *T. occidentalis* seed oil utilizing the articulation by Eq. (1).

Rate restraint (1%) = ( Acontrol – Aextract )/( Acontrol ) ×100

Where;
$A_{control}$ is the absorbance of the control
$A_{extract}$ is the absorbance of the concentrate.

**2, 2'- azinobis (3-ethylbenzothiazoline-6-sulfonic corrosive) (ABTS) radical scavenging capacity:**
This test was done by the methodology of [21]. The $ABTS^{+}$ was created by responding 7 mM ABTS fluid arrangement with K2S2O8 (2.45 mM, last focus) in obscurity for 16 h and altering the pH to 7.0 with ethanol. Precisely 0.2 mL of the different dilutions of concentrates (0.065–1.0 mg/mL) was added to 2.0 mL $ABTS^{+}$ arrangement and the absorbance was estimated at 734 nm after 15 min.

 

## IV. RESULTS AND DISCUSSION

The results for the Total Phenol, Total Tocopherols and *In-Vitro* Antioxidative Properties of *Telfairia occidentalis* Seed Oil are presented below.

Table 1: Total phenol and total tocopherols content of *T. occidentalis* seed oil extract

| Parameter | Concentration |
|---|---|
| Total tocopherols (mg/kg) | 76.0 ± 3.1 |
| Total phenol (mg of garlic acid equivalent) | 3.34 ± 0.15 |

Note: Values are mean ± standard error of mean of triplicate determinations.

The result for the total phenol and tocopherol are as shown in the table above. From the result, it is indicative of a possible replacement effective enough compared to the available synthetic antioxidants sourced from other natural species of plants known to have antioxidants.

Table 2. *Invitro*1,1-diphenyl-2-picrylhydrazyl (DPPH), Nitric oxide and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging capacity of *T. occidentalis* seed oil extract

| Parameter | Concentration |
|---|---|
| DPPH (% inhibition) | 47.95 ± 3.47 |
| Nitric oxide (% composition) | 56.47 ± 2.37 |
| ABTS (% composition) | 51.06 ± 2.60 |

Note: Values are mean ± standard error of mean of triplicate determinations

The *In vitro* 1,1-diphenyl-2-picrylhydrazyl (DPPH), Nitric oxide and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging capacity of *T. occidentalis* seed oil extract are as stated in the table. From the results obtained, it revealed that the oil has a good oxidative stability. This showed that using this oil as against the regular vegetable oil van be of great environmental benefit as a result of the presence of levels of the anti-oxidants

**Discussion.**

As of late, there has been an expanding enthusiasm for considering phenolic mixes from seeds oils, since they speak to conceivably wellbeing advancing substances and have mechanical applications [22]. These normal mixes have demonstrated to have significant effect in the steadiness, tactile and dietary qualities of the item and may forestall disintegration through extinguishing of radical responses answerable to oxidation of lipid [23]. Wide ranges have been accounted for [24]. Antioxidants naturally present in plant sourced diets thus increase resistance in regards to damages induced by oxidation which may have a potential substantial health impact on humans [25]. Tocopherol homologues are phenolic cell reinforcements that happen normally in vegetable oils and give some assurance against oxidation by ending free radicals. The assurance of tocopherol homologues in the portion oils is significant attributable to their antioxidative impacts and their positive wholesome impacts in human digestion as natural cell reinforcements [26]. As shown in the results, the pumpkin seed oil had a significant level of tocopherol which would be relied upon to contribute great oxidative security of the oil during capacity and handling. The tocopherols content in the pumpkin seed oil was impressively higher than before detailed [27, 28].

Generally, amongst the most abundant antioxidants are hydroxylphenol compounds with different substitutions of rings and are basically characterized by the possession of low activation energies used for donation of hydrogen. From the results obtained, the seed oil of *T. occidentalis* has high nitric oxide content. The nitric oxide rummaging action of green tea have been concentrated by [29], who inferred that specific tannins had the capacity to show magnificent cell reinforcement movement. The outcomes from the cell reinforcement properties of the pumpkin seed oil showed the compound attributes of the oil, which may represent its detailed restorative advantages [30].

## V. CONCLUSION AND FUTURE SCOPE

As shown from the antioxidant property of the seed oil of *T. occidentalis* has the ability to produce various metabolites/compounds which may be useful for various purposes such as treating or managing some disease conditions or may also be used in producing antioxidant capsules and the seed oil can be viewed as a decent wellspring of characteristic cell reinforcements. However, it can be recommended as a substitute for pharmaceutical and industrial purposes.

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## AUTHORS' CONTRIBUTIONS

Each author participated in the research from the design to execution and the corresponding author was solely responsible for writing the manuscript.

## CONFLICT OF INTEREST

There are no conflicts of interest.

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