Characterize and elucidate the structure of tannins present in the seed of *Telfairia occidentalis*

ABSTRACTS

In this study, the tannins were extracted and characterized from the seeds of *Telfairia occidentalis*. Thin-layer chromatography (TLC) identified the extracted tannins and ultraviolet analysis was used. Additionally, we obtained numerical values using "spot matching." The numbers obtained were tallied and represented. TLC showed three unique spots with Rf values of 0.37, 0.41 and 0.56. Ultraviolet analysis further confirmed these compounds, which depicted different colours: diosgenin showed Dark Green, and glycyrrhizin was Purple. The tannin, which was not shown in the reference materials, had an Rf of 0.56 and was black. Based on these analyses, scientists characterize these compounds for use in other sustainable productions and the pharmaceutical industry.

Keywords: *Telfairia Occidentalis* Seed; Tannins; Characterization; Diosgenin, Glycyrrhizin

1. Introduction

Telfairia occidentalis (T.O) (also referred to as fluted pumpkin) is a perennial arching climber native to tropical West Africa [1], which has served as a food and traditional medicine since time immemorial in many communities [2]. Its seeds are a source of proteins, lipids and other essential nutrients [3] and have long been used in soups, stews, [4] where they contribute to improving the nutritional status of the food. *Telfairia occidentalis* has extensive use in ethnomedicine, specifically in the treatment of anaemia in several ethnocratic communities in West Africa [5, 6]. Its seeds contain iron and vitamin B12, which prevent and treat anaemia. At the same time, the leaves have been reported to have a high iron concentration, hence their use in ethnomedicine and the treatment of anaemia [7]. It has also been traditionally adopted in promoting lactation among nursing mothers [8, 9]. A study confirmed the active galactagogue effect of T.O leaves, which showed that the aqueous extract of *Telfairia occidentalis* seed exhibited significant galactagogue activity [10]. Phytoestrogens in the plant might be responsible for the effect. Phytoestrogens are chemicals found in plants that mimic oestrogens in the body.

Telfairia occidentalis is nutritious and medicinal; as with other leafy vegetables, it has some antinutrients [11], including tannins, oxalates and phytic acid that can prevent the absorption of nutrients, such as calcium, iron and zinc in the digestive tract during digestion. However, this reduction is significantly reduced during processing, such as fermentation and cooking [9]; the plant is dioecious with separate male and female plants [3]. This feature is a functional advantage

for breeding. The way to use T. occidentalis is multifacet. The plants are non-toxic and nutrient-rich, accompanied by possibly healthful attributes due to the extraction of tannins; therefore, chemical characterization of these tannins from *Telfairia Occidentalis* will provide a novel contributory database on this plant for its medicinal potential and possibly its applications. Tannins are a type of polyphenols of plants. The term 'tannin' comes from the Greek verb 'to stretch' because of plants' astringent property and ability to bind to proteins [12,13]. Tannins can be one of several plant metabolites essential for the plants' defence and survival in the environment, especially against herbivores and other stressors [14,15]. Ingestion of toxins could poison an animal, so small mammals, large livestock, and plants maximize defence against harmful herbivores. For instance, in 'tannins effects on herbivores' research, the feeding tannins content lowers the digestibility and utilization of ingested plant matter, whereby tannins compete with other nutrients in ingested plant and interfere with their absorption due to the binding capacity with proteins and other plant nutrients [16]. High concentrations of tannins and other polyphenols antinutrients can bind proteins and other forms of nutrients, reducing their availability during digestion [17,18,19,20]. In ruminants, elevated tannins will decrease feed digestibility and microbial fermentation efficiencies, reducing nutrient utilization. Meanwhile, low levels of tannins in plant sources lead to better fermentation in herbivores' stomachs, more nutrients being used, and antibacterial action. For instance, in ruminants, tannins stimulate microbes' fermentation in the rumen, enhancing fibre digestion and nutrition utilization [23,24].

In horses, it can increase the digestion of forages and reduce the prevalence of enteroliths — mineral stones that can form in the digestive tract[25]. Tannins are therefore found in many legumes and field herbs at varying concentrations, some with relatively low concentrations [26] (e.g., white clover, Trifolium repens; red clover, T. pratense) and some with higher concentrations (e.g., birdsfoot trefoil, Lotus corniculatus; alfalfa, Medicago sativa) [27,28]. Because of this, the concentration of present tannins needs to be measured with every harvest to ensure the appropriate amount in the feed.

On the other hand, plant tannins have long been used in leather tanning. Quebracho, Chestnut and Mimosa trees provide tannins for leather tanning [29,30]. Moreover, its derived from the skins and seeds of grapes are an essential ingredient of winemaking, providing astringency, bitterness and colour [19,31]. In 2011, in an official European Commission report on the trade of vegetable tannins in the European Union (EU), 3.728 metric tons of vegetable tannins extracts, tannins, salts and derivatives were imported to the EU in 2011 [32]. Argentina (22.957 metric tons) was the leading supplier of quebracho tannins to the EU [33]. These imports highlight the continued importance of vegetable tannins in diverse applications, including leather tanning, wine production and others. Tannins from different sources can vary widely regarding their protein-binding properties and functionality in different applications. This is because different structures and compositions of plant tannins lead to differences in their ability to bind with proteins [34]. For instance, tannins derived from Quebracho, Chestnut trees [33,30] and other plants bind well to proteins, leading to the effectiveness of these tannins in leather production.

Similar differences in tannin-protein binding between grapes and other fruits can result in variations in wine's colour, astringency and bitterness [35]. Other factors other than plant source can also affect the binding of tannins with proteins, such as the ripeness of the plant material at the time of extraction of tannins.

An example is the more excellent protein-binding properties of tannins derived from unripe grapes than those from ripe grapes, producing more astringent wines [35]. In addition, metal ions and the pH of the solution can affect tannin-protein interactions. For instance, in wine, the pH and the presence of metal ions, such as iron (Fe) and copper (Cu), affects tannin-protein interactions [35]. The intricate nature of tannin-protein interactions highlights the importance of understanding the chemical composition and properties of tannins derived from different plant sources to exploit them fully for different applications.

This study aimed to characterize and elucidate the structure of tannins present in the seed of *Telfairia occidentalis*, commonly known as fluted pumpkin. Previous studies have focused on the characterization of flavonoids in *Telfairia occidentalis* [36], but to our knowledge, there needs to be more research on the characterization of tannins in this species. Therefore, this study aimed to fill this knowledge gap by identifying and elucidating the structure of tannins present in the seed of *Telfairia occidentalis*.

applications.

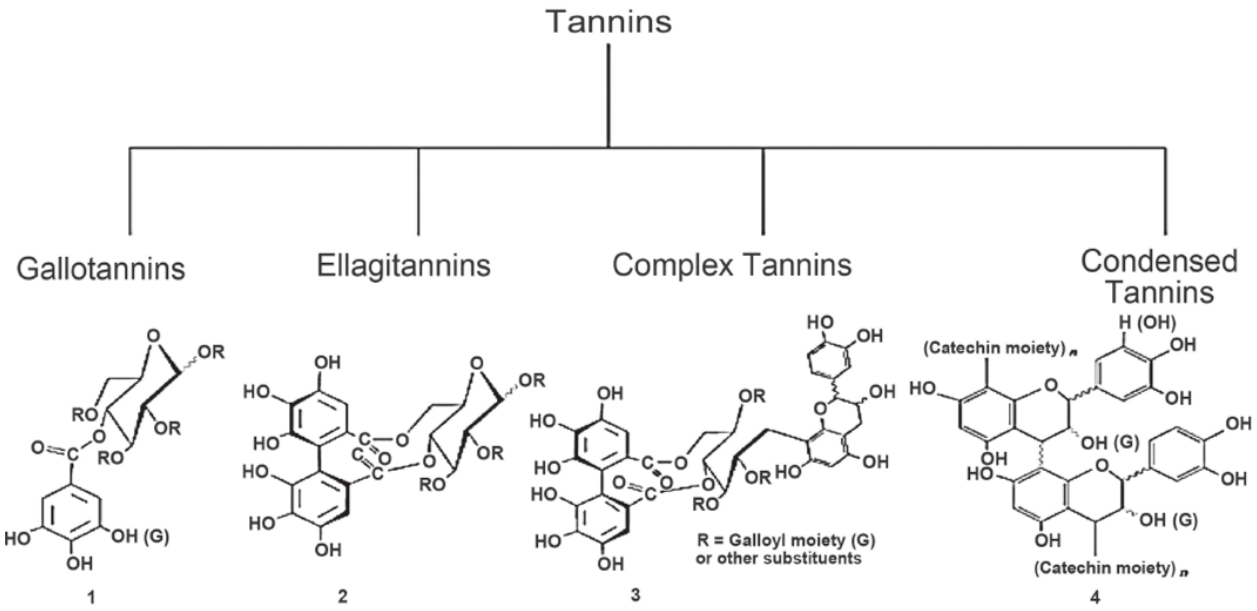

FIG 1. Classification of the tannins

2. Materials and Method

2.1 Sample Collections and Identification

The plant seed material (Telfairia occidentalis) was collected from Uga, Aguata Local Government Area, Anambra State, Nigeria. Dr. Orji, Biological Science Department, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, identified it. The plant seed was dried at room temperature, pulverized using an electric grinding machine, and stored in a well-covered plastic container for use.

2.2 Equipment and Reagents used for Extraction

2.2.1 Equipment Used

Test tube

Conical flask

Separatory funnel

Beaker

Weighing balance

Filter paper

Spatula

Water bath

Busen burner

Sample container

2.2.2 Reagents Used

Acetone

Diethyl ether

2.2.3 Extraction of Tannins:

1. Two grams of the prepared sample are weighed and placed into a flask, where 50 millilitres of 70% acetone are added. The mixture is then shaken to facilitate the extraction of tannins.
2. The mixture is poured into a separatory funnel, where diethyl ether is used as the extracting solvent. This process is repeated five times until complete separation of diethyl ether and tannins is achieved.
3. The tannins extract, which is the lower layer, is collected, and the upper layer of diethyl ether is discarded.
4. The collected tannins extract is evaporated using a water bath, a heating process that removes excess solvent. After evaporation, the tannin extract is weighed to determine its mass.

The percentage of tannins in the prepared sample is then calculated using the ratio of the tannins extract weight to the initial sample weight, multiplied by 100%. This calculation provides a measure of the tannin content in the sample. The entire extraction process is performed in a controlled environment to ensure the accurate and precise extraction of tannins from the sample.

Mathematically:

% Tannins = Weight of dried Tannins ÷ Weight of sample × 100/1

2.3 Characterization of Tannins in the Seed of *Telfairia Occidentalis* using TLC Method

2.4 Equipment and Reagents Used

Ruler

Pencil

Aluminum

Silica TLC

Capillary spotter

Beaker

Glass plate

UV transilluminator

HCL

Acetic acid

Water

Iodine crystal

Procedure:

1. A horizontal line (2cm above the base of the silica TLC plate) was drawn with a pencil
2. 10ml of analyte was collected using a capillary spotter and spotted on the line drawn on the plate (two different spots were made in different positions on the same plate)
3. The mobile phase (Acetic acid: water: HCL in the ratio of 10:3:1) was poured into the chromatography chamber, making sure it was 1cm below the pencil mark
4. The glass plate with the spotted analyte was placed into the chamber, which was then covered with aluminium foil and a glass cover and allowed to stand for 6 hours.
5. After separation, the silica glass plate was removed, and a pencil was used to mark the height reached by the solvent (solvent front horizontally, and the plate was allowed to dry)

6. The glass plate was placed in a beaker containing iodine crystal for 2 minutes
7. The different spots of the analyte components were visualized in the UV transilluminator at 365nm wavelengths, and the number of spots and their corresponding colours were recorded. Then, the distance moved by each of the components (spots) was measured and recorded using a meter rule and a pair of dividers

Rf (Retention factor) values were calculated using the formula below:

R_f = Distance travelled by the analyte ÷ Distance travelled by the solvent = Solvent front

Finally, the corresponding isolates separated were identified by comparing their R_f values with the standard factor values of standard known compounds.

3.0 RESULTS AND DISCUSSIONS

Table 1. Organoleptic Description of Seed of *Telfairia* Occidentalis

Parameters	Inference
Color	Milk color
Texture	Powdery and soft
Odor	Unobjectionable

Table 2. Percentage yield of Tannins in the seed of *Telfairia* Occidentalis

Number of Determination in %

Ist	2nd	3rd	Mean value %	Sample
5.8	5.65	5.85	5.77	Tannins

Table 3. Characterization of compounds with reference to Rf Value and UV Analysis

Isolates	**Solvent Front (cm)**	**Sample Front (cm)**	**R_F Values**	**R_F × 100**	**UV Color**	**Compound Identified**	**Samples**
A	**8.0**	**2.97**	**0.37**	**37.00**	**Dark-green**	**Diosegenin**	**Tannins**
B	**8.0**	**3.3**	**0.41**	**41.00**	**Purple**	**Glycyrrhazin**	**Tannins**
C	**8.0**	**4.5**	**0.56**	**56.00**	**Black**	**Unknown**	**Tannins**

The tannins extracted from Telfaria occidentalis seeds were characterized using thin-layer chromatography (TLC) and ultraviolet (UV) analysis. The TLC analysis, using an acetic acid: water: HCL (10:3:1) solvent system, produced three distinct spots with Rf values of 0.37, 0.41, and 0.56, respectively. These Rf values were further corroborated with UV analysis, which revealed different colours for the tannins: Dark-green, Purple, and Black. The Dark-green colour observed for the tannin with an Rf value of 0.37 was attributed to the presence of Diosgenin. In contrast, the purple colour corresponding to the tannin with an Rf value of 0.41 was attributed to Glycyrrhizin. However, the reference materials could not identify the tannin with an Rf value of 0.56 and a Black colour.

4.0 CONCLUSION

The extraction and characterization of tannins from Telfairia occidentalis seeds revealed the presence of three distinct tannins with varying Rf values. Diosgenin and Glycyrrhizin were identified by UV analysis, while a novel tannin with a Black colour and an Rf value of 0.56 could not be identified in the reference materials. This study provides insight into the tannin profile of Telfairia occidentalis seeds, suggesting that further research is needed to explore the potential therapeutic properties of these tannins and identify and characterize the novel tannin compound. The findings from this study lay the groundwork for future research into the chemical composition and potential applications of tannins in Telfairia occidentalis seeds.

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