Tannin Extraction and Analysis in Telfairia Occidentalis Seeds: A TLC and UV-Based Approach.

ABSTRACTS

In this study, we extracted tannins from Telfairia occidentalis seeds and identified them using thin layer chromatography (TLC) in conjunction with ultraviolet (UV) analysis. We employed the "spot matching" technique to obtain numerical values, tallied, and represented them. TLC revealed three distinct spots with Rf values of 0.37, 0.41, and 0.56. UV analysis further confirmed these compounds, depicting different colors: diosgenin showed dark green, glycyrrhizin appeared purple, and an unidentified tannin with an Rf value of 0.56 was black. Researchers can use this TLC-UV method to help them find and possibly separate bioactive compounds from Telfairia occidentalis seeds. These compounds could be used in the pharmaceutical industry and for other sustainable productions.

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.

Tannins, an important group of secondary metabolites, have been studied in various plants due to their diverse biological activities and potential applications. Although previous research has identified and characterized tannins in different species, the current literature on tannins in Telfairia occidentalis seeds remains scarce. A few studies, like Miteu and Ezeh (2022), Arukwe et al. (2012), and Owuna et al. (2019), have said that Telfairia occidentalis seeds contain tannins. However, not much is known about the exact types and properties of these tannins.

This study aims to contribute to the existing knowledge by identifying the specific tannin compounds present in Telfairia occidentalis seeds. The study was able to identify three different tannins by using the right extraction and identification methods. These are two known compounds (Diosgenin and Glycyrrhizin) and a new tannin that is black and has an Rf value of 0.56. These findings not only expand our understanding of the tannin composition in Telfairia occidentalis seeds but also uncover a previously unreported compound, highlighting the importance of further investigation into this plant species.

As a conclusion, while some earlier research has mentioned that Telfairia occidentalis seeds contain tannins, this study goes into more detail about the tannin composition by naming specific compounds, including a new tannin. The findings of this study may serve as a foundation for future research exploring the properties and potential applications of these compounds in various industries.

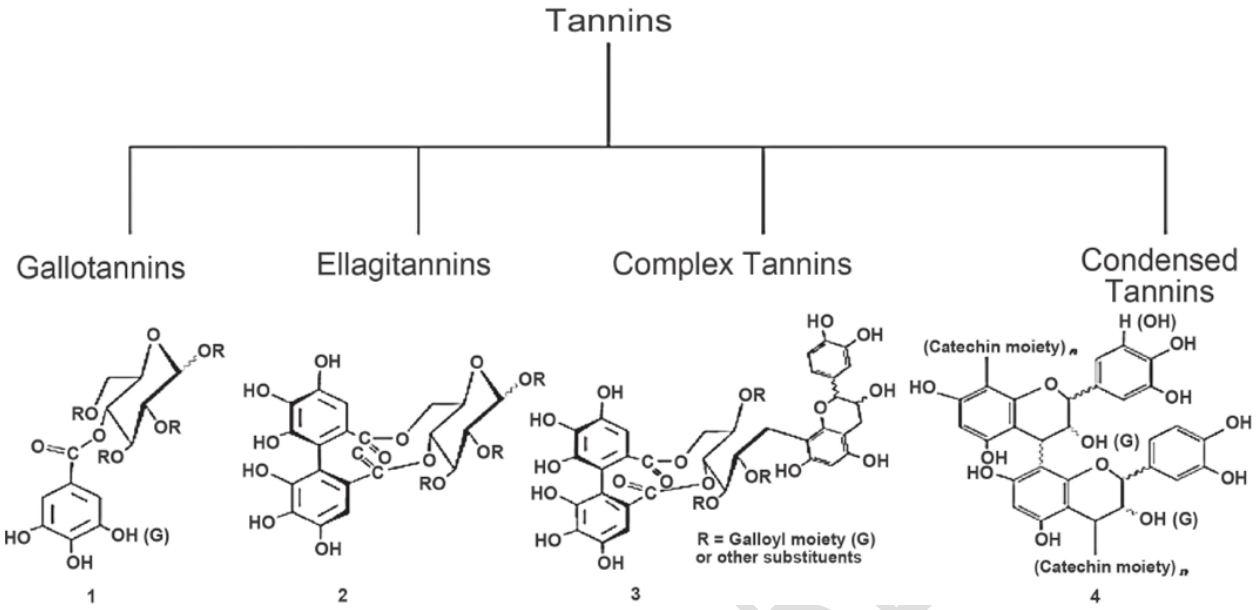

FIG 1. Classification of the tannins

2. Materials and Method

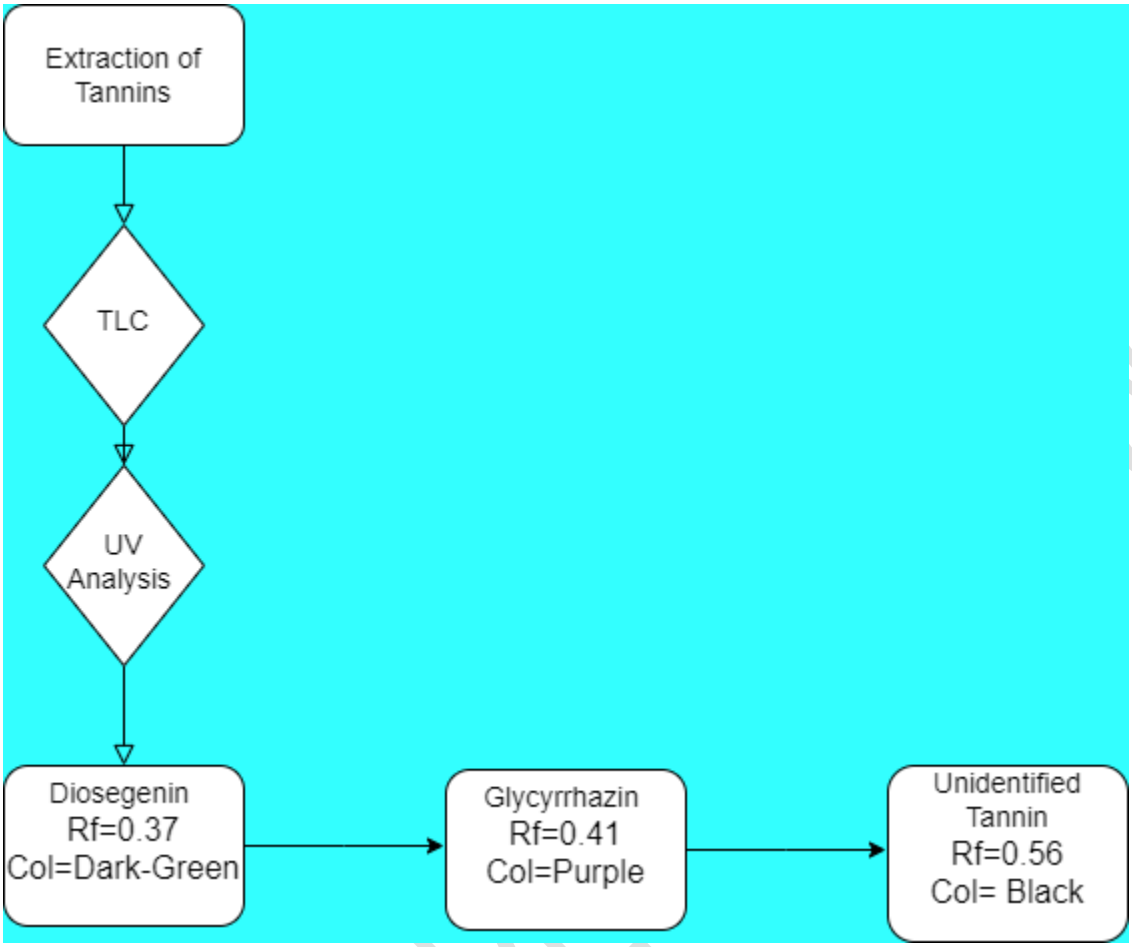

Fig 2. *Flowchart representing the extraction and identification process of tannins from Telfairia occidentalis seeds using Thin Layer Chromatography (TLC) and Ultraviolet (UV) analysis. The identified tannins, diosgenin and glycyrrhizin, are highlighted along with their Rf values and corresponding colours observed in the analysis. An unidentified tannin with an Rf value of 0.56 is also indicated.*

2.1 Material

The plant seed material (Telfairia occidentalis) was collected from Uga, Aguata Local Government Area, Anambra State, Nigeria. We thoroughly washed the seeds with distilled water to remove dirt, then air-dried them for two weeks before grinding them into a fine powder, 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:

A 2-gram sample was weighed and transferred into a flask. Next, we added 50 mL of 70% acetone to the flask and agitated the mixture to aid in the extraction of tannins.

We then transferred the mixture into a separatory funnel and used diethyl ether as the extracting solvent. We repeated this process five times until we achieved complete separation of diethyl ether and tannins.

We collected the lower layer of tannin extract and discarded the upper layer of diethyl ether. To remove any excess solvent, the collected tannin extract was evaporated using a water bath. We weighed the tannin extract after evaporation to determine its mass.

We calculated the percentage of tannins in the prepared sample using the following equation:

$$\% \text{ Tannins} = \frac{\text{Weight of dried Tannins}}{\text{Weight of Sample}} \times 100$$

This calculation provides a measure of the tannin content in the sample. We carried out all steps of the extraction process in a controlled environment to ensure accurate and precise extraction of tannins from the sample.

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:

We used a pencil to draw a horizontal line 2 cm above the base of the silica TLC plate. Using a capillary spotter, 10 µL of the analyte was collected and spotted on the pencil-drawn line, ensuring two different spots were made in distinct positions on the same plate.

We poured the mobile phase, a 10:3:1 ratio of acetic acid:water:HCl, into the chromatography chamber, making sure the depth was 1 cm below the pencil mark. We placed the glass plate containing the spotted analyte into the chamber, covered it with aluminum foil and a glass lid, and allowed it to develop for 6 hours.

After separation, we removed the silica gel plate from the chamber and marked the height of the solvent (solvent front) horizontally with a pencil. We then left the plate to dry.

We subsequently placed the glass plate in a beaker containing iodine crystals for 2 minutes to facilitate visualization of the separated components. We visualized the developed TLC plate under a UV transilluminator at 365 nm wavelengths, documenting the number of spots and their corresponding colors. We measured the distance each component (spot) moved using a ruler and a pair of dividers, and then calculated the retention factor (Rf) values.

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**

DISCUSSION

We identified the tannins extracted from Telfairia occidentalis seeds using thin-layer chromatography (TLC) and ultraviolet (UV) analysis. The TLC analysis, using an acetic acid: water: HCL (10:3:1) solvent system, revealed three distinct spots with Rf values of 0.37, 0.41, and 0.56, respectively. The UV analysis further corroborated these Rf values, revealing different colors for the tannins: dark green, purple, and black.

Diosgenin is responsible for the dark-green color of the tannin with an Rf value of 0.37, while glycyrrhizin is responsible for the purple color of the tannin with an Rf value of 0.41. However, the reference materials were unable to identify the tannin with an Rf value of 0.56 and a black color, indicating the need for further structural characterization using complementary techniques such as Fourier-transform infrared (FTIR) spectroscopy or nuclear magnetic resonance (NMR) spectroscopy.

A more effective approach for identifying unknown tannins would be to compare standard tannins with unknown tannins. Additionally, using different chromatographic methods, like gas chromatography-mass spectrometry (GC-MS) or high-performance liquid chromatography (HPLC), could give a fuller picture of the tannins in the sample. Optimizing the solvent system would also improve tannin detection and resolution in the given sample.

These identified tannins have potential applications in the pharmaceutical industry due to their known biological activities, such as antioxidant, antimicrobial, and anti-inflammatory properties. Additional biological assays, like cytotoxicity tests, could further evaluate the efficacy of these compounds and validate their therapeutic potential. Integrating complementary analyses, optimizing chromatographic conditions, and conducting biological assays in future studies will enable a more comprehensive understanding of the tannins in Telfairia occidentalis seeds and support their potential use in pharmaceuticals and other industries.

4.0 CONCLUSION

The extraction and identification of tannins from Telfairia occidentalis seeds revealed the presence of three distinct tannins with varying retention factor (Rf) values. Two of these tannins,

diosgenin and glycyrrhizin, were identified by UV analysis. However, a new tannin with a black color and an Rf value of 0.56 could not be found in the reference materials.

These findings contribute to understanding the tannin profile in Telfairia occidentalis seeds and emphasize the importance of further research to explore the potential applications and therapeutic properties of these compounds. The identification of a novel tannin compound may lead to new insights into the chemical composition of Telfairia occidentalis seeds and reveal unique biological activities that could be beneficial in various industries, such as pharmaceuticals or nutraceuticals.

The current study lays the groundwork for future investigations into the chemical composition, bioactivities, and potential applications of tannins in Telfairia occidentalis seeds. By examining the role of these tannins in the plant's physiology and their effects on human health, researchers can identify new avenues for the utilization of this plant in traditional medicine and modern healthcare practices. Furthermore, the discovery of novel compounds may inspire the development of innovative methodologies for extracting and purifying these compounds for use in various applications.

In conclusion, this study gives us useful information about the tannins found in Telfairia occidentalis seeds. It also shows how important it is to do more research to fully understand the properties and possible uses of these compounds. The discovery of a novel tannin compound warrants further investigation, which may ultimately contribute to the development of new natural products or therapeutic agents derived from Telfairia occidentalis seeds.

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