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Investigation of Tannin content in *Diospyros mespiliformis* Extract using Various Extraction Solvents

Abstract

The present study was aimed to evaluate the tannin content accumulated in the unripe fruit, leaf and bark of *D. mespiliformis* (African Ebony) using acetone, methanol, aq. Methanol, hot and cold water extracts. A quantitative test was conducted on the extracts and a blue blackish precipitate was observed which indicates that the tannins were hydrolysable. The study revealed that the unripe fruits have the highest accumulation of tannin content but the extract weight was significantly higher in aq. methanol extract (15.94 g) and acetone extracts (13.52 g) from 100 g of dry samples. This was followed by the tannin extracted weight of the leaf in acetone and aq. Methanol extracts having 12.35 g and 11.55 g respectively while the acetone extracts from the bark was 12.33 g. Furthermore, the extracted weight of natural solvent (hot and cold water) was low hence, it is concluded that more tannin are extracted with aqueous methanol and acetone amongst others. Consequently, conservation of *D. mespiliformis* is highly important therefore, it is suggested that the leaves should be used instead of the fruits because of regeneration.

Keywords: *Diospyros mespiliformis;* Tannins; Unripe fruit; Leaves; Bark; Extracts

Research Article

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Introduction

Plants produce many bioactive compounds that are called secondary metabolites, and have a wide variation in chemical diversity, distribution and function [1]. Phytochemistry of plants has revealed the pharmacological effect and their uses in industries [2-4]. Secondary metabolites have long been known as a source of effective medical therapies such as anti-bacterial and anti-cancer as reported [5-7]. The classification is based on the precursor, basic structure and biosynthetic pathway [1]. These compounds are classified into four categories namely: alkaloids, phenols terpenoids, and non-protein amino acids [8]. Phenol group is characterized by the presence of an aromatic ring with one or two hydroxyl groups and consists of thousands of compounds some of which include anthocyanins, flavonoids, glycosides, pigments, lignin melanin, phenolic acids, phenylpropanoid, quinones, saponins, and tannins which are widely distributed in various plant species [9]. Tannins are polyphenolic compounds that have high molecular weight (over 1000 g) and can form a complex with protein. Structurally, tannins can be divided into two classes: condensed tannins and hydrolysable tannins [9,10]. Tannins are distributed in species throughout the plant kingdom and are commonly found in both angiosperms and gymnosperms.Tannins is produced by a chloroplast-derived organelle, the tannosome [11] which are physically located in the vacuoles or surface wax of plants, it contains gum, oil, latex, pigments or resin [12] and function as, excretory materials, minerals, pigments, and storage of reserves. Most common polyphenols are the condensed tannins found in virtually all families of plants, and comprising up to 50% of the dry weight of leaves. Tannins are located in the roots, wood, bark, leaves, and fruit of many plants [13-15]. In plants, tannins play a role of protecting plant from predation and also help in regulating plant growth [16]. Furthermore, it causes the astringency, colour, and flavour in tea [17]. Type of tannins in tropical woods is cathetic while the temperate woods are gallic in nature. There may be a loss in the bioavailability of still other tannins in plants due to birds, pests, and other pathogens. Likewise, the modification of tannins with time plays a significant role in determining harvesting times.

For instance tannins isolated from the stem bark of *Myracrodruon urundeuva* have been studied for their potential to affect 6-hydroxydopamine-induced toxicity [18]. Tannin can react with formaldehyde (condensation polymerization), because of the presence of phenol groups to form thermosetting products that can be used as an adhesive. In vitro, tannins showed antiviral antibacterial and antiparasitic effects [19-21] but no tannin-based drug has been developed for treating these or other diseases.

Diospyros Mespiliformis belongs to the family Ebenaceae and is extremely widespread in African countries such as Senegal east, Ethiopia, Kenya, south to Namibia, northern South Africa and Swaziland, and Nigeria [22,23]. Its commonly known as Ebony tree, Jackal berry or jackal bessie [24] and in Northern part of Nigeria it is commonly called Kanya (Hausa), Nelbi (Fulani) [23]. *D. mespiliformis* is a large deciduous tree found mostly in the savannahs, reaching about 10 meters in height [22]. Mature trees have dark gray fissured bark and adult tree reaches an average of 4 to 6 metres in height, though occasionally some trees reach can reach up to 25 metres. The foliage is dense and dark green with elliptical leaves, which are often eaten by grazing animals such as elephants and buffalo. The tree flowers in the rainy season; the flowers are imperfect, with genders on separate trees, and are cream-colored. In some parts of Africa, ripe fruits of *D. mespiliformis* are consumed as food because of its high nutrition

value while gum for binding loose pages and pasting papers on walls were extracted from the unripe fruits [25]. The fruits are sometimes preserved dried and ground into a flour, and are often used for brewing beer and brandy. The leaves, bark and root of *D. mespiliformis* have been reported to be medicinal containing some bioactive compounds including tannin, which are used as a styptic to staunch bleeding [25]. Also, the roots are consumed to purge parasites and are thought to be a remedy for leprosy. Industrially, the wood is highly useful because its almost impervious to termite damage, fine-grained and strong heart wood, and often used for making wood floors and furniture [26].

Therefore, the demand for tannins in adhesive, food, dyes, medicine and tanning industries is on the increase on daily basis, as a result, there is a need to source for raw materials to enhance production. The juice of unripe fruits of *D. mespiliformis* has been found to have adhesive properties (informal information), and so far no scientific work has been carried out on any part of this plant to investigate the presence and quantity of tannins. Hence, the objectives of this study is to determine the tannin presence and quantity from unripe fruits, leaves and bark of *D. messpiliformis* using acetone, aq. methanol (70%), cold water, hot water, and Methanol solvent. Also, this study will recommend the vegetative part which can be harvested for tannin production that will not threaten or endanger the species.

Materials and Methods

Collection of plant material

The unripe fruits, leaves and barks of *Diospyros mespiliformis* were collected from Modibbo Adama, University of Technology (MAUTECH) Yola. After collection, the samples were taken to the herbarium for proper identification before taken to the laboratory for further extraction.

Extraction of the plant material

The unripe fruits, leaves and barks (Plate A & B) collected were washed under running tap water to remove dust, cut into pieces and was then spread on a clean table in the laboratory for air drying. The dried samples were ground with mortar and pestle to a particulate size of 1mm sieve screen and samples were stored.

Plate A: Leaf and Fruit of *Diospyros mespiliformis.*

Plate B: Stem bark of *Diospyros mespiliformis*

Extraction of tannin

The method of extraction of tannin followed the procedure reported by [27]. From all the grounded parts (unripe fruits, leaves and nark,), 100 g were measured from each and were placed in a volumetric of 1000 ml and hot distilled water 500ml was added. The flask was agitated in a mechanical shaker for 3 hours. The flask was allowed to stand for three (3) days after which the extract was filtered using Bucher's flask with Whatman No.1 filter paper. The same procedure was repeated for acetone, methanol, aq. Methanol (70% methanol and 30% distilled water) and cold water extractions. The filtrate in each case was concentrated to about 50 ml using water bath at temperature below 40°C for the organic solvents and above 40°C for water extraction. The concentrated extracts were allowed to stand for 72 hours. The extract was then obtained in gelled form and later in powdered form. The percentage by weight of sample extract was computed on the basis of the original air-dried samples.

Phytochemical test for tannins

The phytochemical test for the presence of tannins was conducted on the sample and a blue blackish precipitate was noticed which is an indication of the presence of Hydrolysable tannin.

Results and Discussion

The study has revealed the presence of tannin in the unripe fruit, leaf and bark of *D. messpiliformis* and the varying quantitative concentrations of tannin in these vegetative parts using hot water, methanol, aq. methanol (70%), acetone extraction and cold water extraction as presented in Table 1- 5. It was further revealed that among the parts, the unripe fruits had the highest tannins extracted weight across in all the solvent used. The results showed that the highest tannin weight extract was recorded for aqueous extract (15.94 g) from the fruits, followed by acetone extracts also from the unripe fruit (13.52 g) and aqueous extract from the bark (12.77 g) as shown in tables 3 and 4 respectively. Consequently, the study revealed that cold water has low tannin extractability because it had the least extracted weight amongst others (Table 5).

From 100 g of dry sample from the unripe fruit, leaf and bark extracted with hot water, it was revealed the highest extract weight 10. 11 g was recovered from the unripe fruits while the least extracted weight 7.52 g was recorded for the bark (Table 1).

Table 1: Hot water extraction.

Samples	Dry Sample Weight(g)	Extract Weight (g)
Unripe fruit	100	10.113
Leaf	100	8.689
Bark	100	7.552

It was further revealed that the highest tannin extract weight 12.01 g was recovered from the unripe fruits while the least extracted weight 10.72 g/100 g of dry samples was recorded for the bark in methanol extract (Table 2).

Table 2: Methanol extraction.

Samples	Dry Sample Weight(g)	Extract Weight(g)
Unripe fruit	100	12.01
Leaf	100	9.51
Bark	100	10.71

Table 3 showed that the highest tannin extract weight was recovered from the unripe fruits 15.94 g while the least tannin extracted weight 12.77 g was recorded for the bark when 100 g of dry sample were extracted in aq. methanol extraction. The aq. methanol extraction is very effective in extracting tannins from unripe fruit, leaf and bark of *D. messpiliformis*.

Table 3: Aq. methanol extraction.

Samples	Dry Sample weight(g)	Extract weight(g)
Unripe fruit	100	15.94
Leaf	100	11.55
Bark	100	12.77

Acetone extracts also has high extractability rate. The results of study show that the highest tannin extract weight was recovered from the unripe fruits 13.52 g while the leaf had the least extracted weight 12.33 g (Table 4).

Table 4: Acetone extraction.

Samples	Dry Sample Weight(g)	Extract Weight (g)
Unripe fruit	100	13.52
Leaf	100	12.33
Bark	100	9.85

Table 5: Cold water Extraction.

Sample	Dry Sample Weight(g)	Extract Weight(g)
Unripe fruits	100	4.32
Leaf	100	4.11
Bark	100	3.92

As presented in Table 5, cold water extract had the least tannin extracted weight from the 100 g of dry sample from the unripe fruits, leaf and bark. However, highest tannin extract weight was recovered from the unripe fruits 4.32 g while the least extracted weight 4.11 g was recorded for the bark.

Plants generally produce mixture of compounds; therefore the extractability depends on the bonding nature (i.e. tightly or loosely) to the associated compounds such as proteins. The appearance of thick and brown coloured substance after evaporation in water bath indicates the presence of tannin is the sample. The high accumulation of tannins in the unripe fruits is a normal biological activity in plant. Tannins prevent immature harvesting of fruits by it unpalatable to wild animal, and it is active in photosynthesis and physiological process fruit formation and maturation however, tannins reduce as fruits ripen [16]. Hence, the tannin concentration is high in the unripe fruits. Additionally, the study showed that the highest tannin weight was from aqueous methanol and acetone extracts, this is in line with the reports of [28]; boiling aqueous methanol is the most effective solvent for condensed tannin and [29]; aqueous acetone is generally most effective in extracting both condensed and hydrolyzable tannins from plants. Nonetheless, the quantity of secondary metabolites discoverable in plants depend on the solubility of tannins which is influenced by the specific structure of the tannins, polarity, ecology, age of tree, climate and nutrients [30]. Extraction of bioactive compounds from plants requires a solvent capable of dissolving polar compounds especially tannins. Water is a good solvent even for tannins (hot water), but the best solvent is a mixture of organic solvents and water. The principle of extraction is that polar compounds dissolve in polar solvents and non-polar solvents compound in non-polar solvent. The extraction method used in this study was extracted maceration which enables the solvents to penetrate the cell wall and move into the cavity of the cell that contains the active substance so that the active substance is dissolved. Due to the difference between the solution concentrations of active substance in the cell, consequently, the solubility is proportional. The advantage of this extraction method is its simplicity [31]. Moreover, maceration is used since it process has a fairly high absorption effectiveness of the active substances contained in the leaves; fruits and bark of many plants. Medicinally, tannins possess anti-diarrhoeal, anti-microbial, anti-inflammatory, and anti-oxidant activities. Besides, it is used for leucorrhoea, gonorrhea, sore throat, rheumatic pains, skin infections, chronic cough, bronchitis, inflammation and as expectorant [32-34]. Tannins are parts of the plant materials that are used as animal feed [35] and a principal raw material in the leather industry [16]. Also, resins from tannins have been used to remove mercury and methyl-mercuty from solution [36]. Likewise, tannin is an element used a type of industrial particleboard adhesive developed jointly by the Tanzania Industrial Research and Development Organization and Forintek Labs Canada [37]. *Pinus radiata* tannins have been used for the manufacture of wood adhesives [38]. Withal, some tannin is used in foods and drinks [39]. Because they soluble in water and organic solvent [40,41].

Regardless of the importance of tannins extracted from *D. messpiliforis*, the continuous harvesting of the vegetative parts of *D. mespiliformis* particularly the fruits will pose threats to regeneration and conservation of this species therefore leading to its entire loss thus sustainable use is acutely salient. Although, it can also be regenerated through coppice and root suckers, however, this study has revealed that tannins are contained in other parts such as the leaf and bark particularly on the bark when aqueous and acetone extracts are used as solvents.

Summary and Conclusion

Tannin was extracted from the unripe fruits leaves and bark of *Diospyros mespiliformis* using various solvents (acetone, methanol, aq. Methanol, hot and cold water). A qualitative test was conducted on the extracts and a blue black precipitate was observed which actually indicates the presence of tannins. Aq. Methanol extracts had the highest tannin extractability followed by acetone extract. Aqueous Methanol shows the highest yield of tannin in all the samples, except in the bark extract where acetone recorded the highest yield. The plant under study therefore contains Hydrolysable tannin and the extract from the unripe fruits of the plant has the highest yield.

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Conflict of Interest

None.

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