

Preliminary Phytochemical Screening of *Diospyros Mespiliformis*

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Abstract

Ethno medical practice mostly revolves round phytochemistry. Phytochemical screening of medical plants would therefore help discover a wide range of more efficacious drugs safer and cheaper drugs. The various plant parts of *Diospyros mespiliformis* were collected from Basawa, Zaria. They were air dried, pulverised and serially extracted by cold extraction procedure starting with methanol. The methanol extract was then serially extracted with n-hexane, ethyl acetate and finally saturated butanol. The result revealed the presence of tannins, saponins, alkaloids, flavonoids, glycosides etc. in various extracts of the various plant parts. The work 'preliminary phytochemical analysis' of the root extract of the root, leaf and bark extracts of *Diospyros mespiliformis* has opened up a fresh line of research into discoveries of new drugs in many classes. It will be more fortunate if the new drugs thereafter discovered have better efficacy and less toxicity than the already existing ones in the classes.

Keywords: *Diospyros mespiliformis*; Phytochemical; Ethnomedical; Serial extraction

Introduction

Phytochemicals are non-nutritive plant chemicals produced by plants to protect them against environmental stressors like cold, heat, bacteria, fungi, etc. [1]. They have curative and prophylactic properties against a wide range of human and animal diseases [2]. There are many phytochemicals in fruits, leaves, bark and roots of plants. Each of these phytochemicals may cure or prevent a disease singly or in synergism with some others [3].

Diospyros mespiliformis is known in Hausa as *Kanya* and in Yoruba as *Igidudu*. It is commonly called Jackal-berry or African ebony. It is found in Savanna and Northern low land forest. It is an ever green tree of 12-15 m height but sometimes reaching up to 20 m or more in the rain forest [4]. The leaves are simple alternate dark green with small hairs on the underside of old leaves. The plant is diacious and flowers in the months of April and May. Mature fruits are large yellow berries [5]. The fruit of this plant is a traditional food of high nutritive value in Africa. The leaf extract is used against fever and syphilis. It is also used as an antihelmintic and as a wound dressing agent [5].

Since the claimed pharmacological activities of this plant should be kinked with one or more of its phytochemical constituents, there is the need to establish its phytochemical profile for the better prediction and easier confirmation of the medicinal value of this plant. This study was therefore necessary to establish the qualitative phytochemical profile of this plant.

Material and Methods

Plant material

The plant materials were collected from Basawa, Zaria, Kaduna state of Nigeria and scientifically identified at the botany laboratory, A. B. U. Zaria, Nigeria where a voucher number of 938 was issued. The parts collected are leaf, bark and root. These collected parts were air dried and pulverized using mortar and pestle into fine particles suitable for extraction.

Extraction

The extraction was serially carried out and starting with methanol. The methanol extract was then extracted with n-Hexane then with ethyl acetate and finally with saturated butanol.

500 g of each of the powdered plant parts was soaked in 2L of JHD methanol for 24hours, decanted and still another 2L of methanol poured and also left for 24hours and again decanted and still another 2L of methanol poured on each of the plant part and decanted for the 3rd time for exhaustive extraction. The decanted solution of each plant part was then filtered with Whatman filter paper size 1. The filtrate was evaporated in each case at 40°C using hot air oven, to get methanol extract which was then further extracted with subsequent solvent after the percentage yield was calculated

Yield

- Leaf - 25.2%
- Bark - 6.76%
- Root - 5.16%

10g of methanol extract of each plant part was dissolved in 200 ml of distilled water and 200 ml of n-Hexane in a separating funnel each and left for about 30 minutes. Then the mixture in each case was separated into n-hexane portion and water portion. The n-hexane portion was then evaporated to have n-hexane extract.

The water portion was then mixed again with ethyl acetate, 200 ml each and left also for about 30 minutes before separation into ethyl acetate and water portion. This was also repeated for more complete extraction. The methanol extract was extracted with n-hexane, ethyl acetate, and butanol and in order of increasing polarity.

Leaf methanol, root methanol, bark methanol, bark ethyl acetate, bark saturated butanol, root saturated butanol, root ethyl acetate, leaf saturated butanol, leaf ethyl acetate and leaf n-hexane extracts were dried at room temperature under the ceiling fan. 10 g of each of these extracts was individually dissolved in 100 ml of distilled water. These served as the stock for the phytochemical screening.

Screening procedure

Test for Flavonoids: To 3ml of the test extract was added 1ml of 10% NaOH (Sodium hydroxide) in a test tube. Development of a yellow colour indicated the presence of flavonoids [6,7].

	Volatile oils	Anthraquinine	Balsams	Saponin glycosides	Steroids	Cardiac glycosides	Alkaloids	Glycosides	Saponins	Tannins
Leaf methanol extract	+	-	-	+	+	+	+	+	+	+
Root methanol extract	+	+	-	+	+	+	+	+	+	+
Bark methanol extract	+	+	-	+	+	+	+	+	+	+
Bark ethyl extract	+	+	-	+	+	+	+	+	+	+
Bark Butanol (saturated) extract	-	+	-	-	+	+	+	+	+	+
Root Butanol (saturated) extract	+	+	-	+	+	+	+	+	+	+
Root ethyl acetate extract	+	+	-	+	+	+	+	+	+	+
Leaf Butanol (saturated) extract	+	-	-	-	+	+	+	+	+	+
Leaf ethyl acetate extract	+	-	-	-	+	+	+	+	+	+
Leaf n-hexane extract	+	-	-	+	+	+	+	+	+	+
+ = presence of phytochemical substance -- = absence of phytochemical substance										

Table 1: Qualitative phytochemical analysis of Extracts of *Diospyros mespiliformis*

Test for Cardiac Glycosides (Keller- Mililani's test): To the extract was added 2 ml of 3.5% Ferric chloride solution and was allowed to stand for a minute. About 1 ml of conc. H₂SO₄ was carefully poured down the wall of the tube so as to form a lower layer. A reddish brown ring at the interface indicated the presence of cardiac glycosides.

Test for Steroids (SALKOWSKI): Dissolved in 3ml of chloroform was 0.5 g of the test extract. To this mixture was carefully added 2 ml of H₂SO₄ to form lower layer. A reddish-brown color at the interface indicated the presence of steroids.

Test for Tannins: Ferric chloride solution (5%) was added drop by drop to 3ml of the test extract in a test tube. Dark-green blue-black colors indicated presence of tannins [7,8].

Test for Saponins: To 5 ml of the test extract in a test tube was added 5 ml of distilled water and was agitated strongly. If the whole tube was filled with froth that lasted for several minutes indicated the presence of saponin [7].

Test for Glycosides: To 5 ml of the test extract in a test tube was added 2.5 ml of 50% H₂SO₄ The mixture was heated in boiling water for 15 minutes. It was then cooled and neutralized with 10% NaOH. Then 5 ml of Fehling's solution was added and the mixture was boiled. A brick-red precipitate indicated the presence of glycosides [7].

Test for Alkaloids: Stirred with 2 ml of each extract was 2 ml of 10% aqueous HCl. Treated with a few drops of Wagner's reagent was 1 ml and treated similarly with Mayer's reagent was 1ml. turbidity to precipitation with any of these reagents suggested the presence of alkaloids [7].

Test for Saponin Glycosides: To 2.5 ml of the extract was added 2.5 ml of Fehling's solution 'A' and "B". A bluish green precipitate showed the presence of saponin glycosides.

Test for Balsams: The test extract was mixed with equal volume of 90% ethanol. 2 drops of alcoholic ferric chloride solution was added to the mixture. A green color that may be produced indicated the presence of balsams [6].

Test for Anthraquinones: Half a gram of the test extract was shaken with 10ml of benzene and 5ml of 10% ammonia solution was added. The mixture was then shaken and the presence of a pink, red or violet

colour in the ammoniacal (lower) phase indicated the presence of anthraquinones.

Test for Volatile Oils: A milliliter of the solution was of the test extract was mixed with diluted HCl. A white precipitate that may be formed indicated the presence of volatile oils [9].

Results

In this study, preliminary phytochemical screening was conducted on various extract of *Diospyros mespiliformis* plant parts (leaf, root and bark). The results are presented in the table below (Table 1).

Discussion

Plants extracts contain different phytochemicals with various biological activities that can be of value in both medical and veterinary practice. Different plant extracts may therefore contain different phytochemicals each of which has its biological activity.

In this study, 10 extracts were phytochemically analysed. Flavonoids have been known to have antioxidant, antibacterial, antifungal; and antiviral activity [10,11]. Extracts such as leaf butanol and leaf ethyl acetate extracts may further be investigated for these activities since they contain flavonoids.

Tannins precipitate proteins of the wound, forming a protective layer on the wound, thus assisting in the arrest of bleeding and therefore promoting wound All the 10 extracts in this study contain tannin and could all be investigated for wound healing healings [12] activity. Tannins have also been reported to be used in treatment of diarrhea as an effective astringent medicine that does not only stop the flow of the disturbing substance in the stomach; rather controls the irritation in the small intestine [13].

Saponins have been linked with decreased cholesterolemia. They are also thought to aid in the absorption of calcium [14]. All the extracts analyzed in this study contain saponin as one of their phytochemicals. All these extracts are therefore candidate extracts for management or prevention of arteriosclerosis which is a condition precipitated by cholesterol.

Alkaloids like morphine and codeine have been reported to have analgesic properties. However, some of them like the morphine have addictive tendencies [15]. It may therefore be in place to investigate any of these extracts for discovery of alkaloids with analgesic properties and less or non-addictive tendencies.

Anthraquinones have been associated with anticancer, laxative and anti-arthritis properties [16]. Extracts positive of this secondary metabolite such as bark and root methanol extracts can further be investigated in these regards.

Conclusion

It is concluded that this preliminary phytochemical analysis of the root extract of the root, leaf and bark extracts of *Diospyros mespiliformis* has opened up a fresh line of research into discoveries of

new drugs in many classes. It will be more fortunate if the new drugs thereafter discovered have better efficacy and less toxicity than the already existing ones in the classes.

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