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Phytochemical Analysis of *Diospyros Mespiliformis (African Ebony)* Leaves and Bark

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ABSTRACT: As an antibacterial, antifungal, and anti-inflammatory agent, Diospyros mespiliformis, a member of the Ebeneceae family, is utilized in traditional medicine. This study attempts to search for phytochemicals in Diospyros mespiliformis's leaves and bark. Methanol was used to extract the crushed sample. Alkaloids, tannins, saponins, flavonoids, steroids, cardiac glycosides, phenolic compounds, coumarin, and carbohydrates were detected in the extracts of both the leaves and the the bark during an initial phytochemical screening; however, anthraquinone and terpenoids were not detected in the plant's leaves and bark respectively. The elemental analysis was also conducted on the plant where some trace elements were determined.

Keywords: Phytochemicals, methanolic extracts, elemental analysis

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INTRODUCTION

Plants have been used as medicine since the dawn of humankind. Traditional herbal medicine practitioners have highlighted the therapeutic usefulness of numerous indigenous plants in managing a variety of ailments (Mukhtar et al., 2022). It has been established that the reason why plant materials are used to treat illnesses is because plants contain chemical components. These substances are classified as phytochemicals, which are secondary metabolites. Plants create substances known as phytochemicals, usually to aid in their growth or to fend off intruders, predators, or diseases. Certain phytochemicals are poisons, whereas others are employed in traditional medicine (Molyneux et al., 2007). The ground sample of *D. mespiliformis* was examined for the presence of these phytochemicals such as tannins, alkaloids, flavonoids, cyanogenic glycosides, saponins, steroids, and carbohydrates. Phytochemicals are not specific in their use, therefore could exhibit several functions; bacterial, antifungal and antiviral (Deshi *et al.,* 2014).Various parts of theplants are used by traditional medicine practitioners inthe management and treatment of several disorders which include cancer, rheumatism, hypertension, fever, jaundice, inflammatory diseases among others (Ugwoke and Obasi, 2019).The World Health Organization (WHO) estimates that about 80% of the populationsliving in the developing countries rely almost exclusively on traditional medicine for their primary health care needs (WHO,2002).*Diospyros mespiliformis* Hochst (Ebenaceae) has a fantastic mutualism and symbiotic network with many living organisms, from human beings to small insects. There is a complex ecological system revolving around this tree. It is one of the savanna giants that can live for more than 200 years. It is a tall, upright tree that can reach a height of 25 m, with a trunk circumference of more than 5 m. It has a dense evergreen canopy. The bark is black to grey, with a rough texture. The fresh inner skin of the bark is reddish. Leaves are simple, alternate, leathery and dark green. The margin is smooth and new leaves in spring are red, especially in young plants. Flowers are cream-

coloured and bell-shaped (Abba et al, 2015). The leaves decoction is used as a remedy for fever, otitis and wound dressing agent (NRC, 2008). The leaves are also used for treatment of headache, arthritis and skin infections. The leaves and fruits are chewed or applied as infusion for treating gingivitis, toothache and for wound dressing to prevent infection(John, 2008). The aim of this research was to determine the phytochemical constituents on *Diospyros mespiliformis* Hochst (Ebenaceae).

MATERIALS AND METHODS

Collection and preparation

Fresh sample of the plant leaves and bark were collected randomly from different location in Katsina State. The samples were identified and authenticated at the Department of Biological Sciences, Umaru Musa Yaradua University, Katsina. The samples were washed severally with tap water and then with deionized water. They were air dried in the laboratory and crushed using wooden pestle and mortal. The crushed samples were sieved through 20-mesh sieve and the fine powder was stored in a polyethene bag for further use. The powdered sample was used for the analyses.

Extraction of sample for phytochemical analysis

Diospyros mespiliformis leaves and bark were ground into powder and 100 g of it was extracted using a percolation method that involved 48 hours of immersion in 150 ml of methanol and 150 ml of water. After filtering each extract, a rotatory evaporator was used to evaporate it completely. The next test was conducted using the extracted result. After that, each extract was weighed, and the yield percentage was determined as follows:

$$\text{Percentage yield} = \frac{\text{mass of extract}}{\text{Mass sample taken}} \times 100$$

Qualitative analysis of phytochemicals

To determine whether secondary metabolites were present in the crude extracts, qualitative analysis was performed as previously described (Brain and Turner, 1975; Sofowora, 1993; Edeoga et al., 2005; Trease and Evan, 2000; Harbone 1973; Osuagwu et al., 2007; Mikail, 2010). The specific steps involved in the phytochemical screening process are as follows.

Test for alkaloids

1.0 ml of the methanolic sample extract was measured into a test tube, 5.0ml of 2% HCl was of the filtrate was treated by adding 5 drops of Wagner's reagent and shake. A reddish brown colouration added and placed on a steam bath for 10mins. It was filtered with the aid of whatman filter paper. 1.0ml was observed, indicating the presence of alkaloids.

Test for saponins

1.0ml of the methanolic sample extract was boiled with 5.0ml of distilled water in test tube for 5minutes in water bath. It was decanted while still hot. The filtrate was used for the following test;

Frothing Test

1.0ml of the filtrate was diluted with 4.0ml of distilled water and shaken vigorously for stable froth on standing. The stable froth was observed for 2minutes indicating the presence of saponins.

Test for flavonoids

1.0ml of the methanolic sample extract was measured into a test tube, 1.0ml of 10% lead acetate was added and shaken for 30seconds and kept to stand. Formation of yellow precipitate was taken as a positive result for flavonoid.

Test for tannins

1ml of methanolic sample extract was measured into a test tube and 1ml of 5% bromine water was added and shaken. The formation of greenish to red precipitate was recorded as evidence for the presence of tannins.

Test for Terpenoid

5ml of the methanolic sample extract was measured into a test tube, 2ml of chloroform was added, and 2ml of concentrated H_2SO_4 was added carefully by the side of the test tube to form a layer. Reddish brown colouration at the interface was formed, indicating presence of Terpenoid.

Test for phenol

1ml of the methanolic sample extract each was measure into a test tube, 1ml of 10% ferric chloride was added and shaken. The formation of a greenish brown colouration was taken as evidence for the phenolic.

Test for anthocyanins

2 ml of HCl was added to 2 ml of aqueous extract and

drop of ammonia was also added. The appearance of pink-red turns blue-violet indicates the presence of anthocyanins.

Test for Chalcones

2 ml of Ammonium hydroxide was added to 0.5 g extract sample. Appearance of reddish colour showed the presence of chalcones

Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of each sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins

Test for coumarins

3 ml of 10% NaOH was added to 2 ml of aqueous extract, formation of yellow colour indicates the presence of coumarins.

Cardiac glycoside test

To 0.5 gm of extract diluted to 5ml with distilled water and add 2 ml of glacial acetic acid and containing one drop of ferric chloride solution. This was underplayed with 1 ml of conc. sulphuric acid. Brown ring at the interface indicates the presence of a deoxy sugar characteristic of cardenolides

Steroid test

The extract was dissolved in 2 cm^3 of chloroform, 2 cm^3 of sulphuric acid was added carefully to form a layer. A reddish brown color at the interface indicates the presence of steroidal ring.

Elemental Determination

Air dried samples were investigated for elemental determination using Atomic Absorption Spectrophotometer (AAS) Buck scientific model AGV 210. 3g of each of the leaves and the stem bark powdered sample was weighed using a weighing balance and heated to ash for 2 hours, the ash was weighed and dissolved in 50cm3 of distilled water and was filtered into 100cm3 volumetric flask and 2 drops of 10% HNO3 acid was added and a warm water was added to make up to the mark for the elemental determination. Appropriate standard solutions were also prepared for element of interest. Calibration curve were constructed to obtained concentration of element.

RESULT AND DISCUSSION

Phytochemical screening

The result of preliminary phytochemical screening of the leaves and bark of *D.mespiliformis* was shown in (Table 1). The result revealed that all the phytochemicals tested for were detected in all the samples except terpenoid which is absent in the bark of *D.mespiliformis* and anthraquinone which is absent in the leaves of *D.mespiliformis.* Alkaloids are well known for their wide pharmacological activities ranging from anti-bacterial and antifungal (Trease and Evans, 1989). However, the presence of alkaloids made it possible to ascertain their potential antibacterial activity on microorganisms. Saponins have different pharmacological effects due to the alkaloids in them. Because of the potential activity of Saponins as antibacterial agents, their presence serves as an indicator towards possible antibacterial activity. Moreso, since on injection into the blood stream, Saponins exhibit hemolytic activity. In other words, they should be administered orally in all their medicinal application (Trease and Evans, 1989**)**. The presence of tannins in the parts of the plants studied indicated the possible use of this plant in ethanobotanical medicine. Tannins are compounds that have the ability to react with protein to form stable water insoluble components. Since bacteria cell wall are made up of proteins, tannins, are seen as active detoxifying agents by precipitating the protein components and hence inhibiting their growth.Flavonoids have been referred to as nature's biological reaction transformer because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities (Senthilkumar, 2013).

Elemental analysis

The plant had moderate amounts of major elements like Ca, Mn, Mg, Pb, Cu, and Zn, according to an elemental analysis of the extract; however, no traces of cobalt (Co) or cadmium (Cd) were found. The presence of macro and micronutrients was revealed by the elemental analysis of the D. mespiliformis aqueous extract. Macronutrients like calcium, potassium, and sodium control the body's fluid balance, which affects cardiac output. Blood coagulation, secretary functions, neuromuscular excitability, and other physiological and biochemical processes are among the many functions that calcium ions play. Bone mineralization requires the right concentration of calcium and phosphate ions in the periosteal fluid and extracellular matrix (Neshwari and Sing, 2014). According to Robert et al. (2000), elements like manganese and

TABLE 1: The result, test and inference of preliminary phytochemical screening of the leaves and bark of *D.mespiliformis* (Brain and Turner, 1975; Sofowora, 1993; Edeoga et al., 2005; Trease and Evan, 2000; Harbone 1973; Osuagwu et al., 2007; Mikail, 2010)

Phytochemicals	Test	Inference	Result	
			Leaves	Bark
Alkaloids	Wagner's test	reddish brown colouration	+	+
Terpenoids	Salkowski test	Reddish brown colouration at the interface	+	-
Steroids	Salkowski test	Brown ring at the junction, green at the upper layer	+	+
Tannins	Bromine water test	formation of greenish to red precipitate	+	+
Cardiac glycosides	Killer-Killani Test	Brown ring at the interface	+	+
Anthraquinone	Borntrager's test	Ammoniacal layer turns pink or red	-	+
Flavannoids	lead acetate test	formation of yellow colouration	+	+
Plobatannins	Hydrochloric acid test	Formation of red precipitate	+	+
Sapponins	Frothing test	Formation of red precipitate	+	+
Coumarin	Sodium hydroxide test	formation of yellow colour	+	+
Chalcons	Ammonium hydroxide test	Ammonium hydroxide test	+	+
Anthocyanins	Ammonia test	Ammonia test	+	+
Phenols	Ferric chloride test	formation of a greenish brown colouration	+	+
Carbohydrades	Molisch's reagent	Formation of violet ring	+	+

Keys: + = Presence of phytochemical substance, --- = Absence of phytochemical substance

zinc are crucial because they function as co-factors in a number of enzyme reactions. This work will aid in the development of new drug compositions and has provided some biochemical support for the medical use of D. mespiliformis extracts in the treatment of infection.

Conclusion

Based on the preliminary phytochemical analysis, it has been discovered that D. *mespiliformis* leaves and bark contain various secondary metabolites such as Alkaloids, phenols, tannins, saponins, steroids, glycosides, flavonoids and other significant compounds. These compounds play a crucial role in the plant's natural defense mechanism against pests and herbivores while also providing therapeutic potential to many medicinal plants. The findings of this analysis provide valuable insights into the potential applications of D. *mespiliformis* in various fields such as medicine and agriculture. As further research is conducted, we may discover even more potential benefits of the secondary metabolites found in this plant.

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