GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

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Elemental and phytochemical analysis of *Diospyros mespiliformis* leaves and stem bark in Nangere local government area of Yobe state, Nigeria

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GSC Advanced Research and Reviews, 2022, 11(03), 051–055

Publication history: Received on 24 April 2022; revised on 09 June 2022; accepted on 11 June 2022

Article DOI: https://doi.org/10.30574/gscarr.2022.11.3.0139

Abstract

Aqueous extract of *Diospyros mespiliformis* leaves and stem bark in Nangere Local Government area of Yobe State were analyzed for its elemental and photochemical constituents. The Elemental analysis which was carried out with Atomic Absorption Spectrophotometer (AAS) showed that the plant contains calcium [Ca], manganese [Mn], magnesium [Mg], lead [Pb], copper [Cu] and Zinc [Zn] in moderate quantity while cadmium [Cd] and cobalt [Co] were not detected. Qualitative method was used for the phytochemical analysis and the result revealed the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, phlobatannins, saponins, steroids, tannins and terpenoids in both the leaves and the stem bark extracts of the plant while glycosides are present in the leave extract but absent in the stem bark extract. The phytochemical and the elemental constituents of the plant may account for its varied enthnobotanical uses in traditional medicine in Nigeria.

Keywords: *Diospyros Mespiliformis*; Phytochemical Analysis; Elemental Analysis; Enthnobotanical

1. Introduction

The plant *Diospyros mespiliformis*, commonly known as "Kanya" in hausa and "Igidudu" in yoruba is a tall, evergreen tree 15-20 m high, with dense, rounded and buttressed stem belonging to the family *Ebenaceae*. It is called Jackal-berry or African ebony. It is found in Savannah and Northern low land forest [1]. The leaves are simple, alternate dark green with small hairs on the underside of older leaves. *D. mespiliformis* is dioecious, pollinated by bees and flowers in the rainy season while fruit ripening, which coincides with the dry season takes place 6-8 months after flower fertilization [2]. The fruit of this plant is a traditional food of high nutritive value in Africa [3]. *D. mespiliformis* is reportedly one of the most important genera of *Ebenaceae* which species have been used over the millennia in traditional medicinal systems [2].

The leaves, roots, stem bark and fruits contained antibiotic qualities and possess many medicinal uses. Roots and stem-bark are used to facilitate childbirth and are used for infections such as pneumonia, syphilis, leprosy, dermatomycoses, as an anthelmintic [4] while the leaves decoction is used as a remedy for fever, otitis and wound dressing agent [2]. The leaves is 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 [5]. Barks and roots are used as psycho-pharmacological drug and to treat tumor [6, 7].

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The aim of this study is to analyze some elements (Ca, Mn, Mg, Cu, Zn, Co, Pb and Cd) and phytochemicals (Alkaloids, Tannin, Saponins, Flavonoids and Glycosides) from the leaves and stem-bark aqueous extracts of *D. mespiliformis* in Nangere Local Government area of Yobe State. This is with the hope that the result obtained may provide additional information on both the nutritional and medicinal values of this plant. Selection of the medicinal plant for the present study was based on its ethno-medicinal usages. Since the plant of study is known to possess a number of medicinal uses of different purposes.

2. Material and methods

2.1. Sampling and Sample Preparation

2.1.1. Sampling

Sampling was done according to the method of Ayoola *et al*.,[8]. Fresh tender leaves and the stem bark of *D. mespiliformis* were collected in the month of April in the year 2019 from Nangere Local Government area of Yobe State. Each plant parts collected was washed with tap water and air-dried. The dried parts were chopped into pieces, milled into fine powder by pounding manually with a clean and sterile pestle and mortar for the extraction. The powdered samples were each collected into sterile cellophane bags and labelled to prevent mix up.

2.1.2. Sample Preparation

Representative samples were obtained from each sample by coning and quartering techniques as described by Crosby and Patel [9]. This method involves making a cone shape of the sample, flattened it and divides it into four equal parts; take the opposite two quarters and discard the other two quarters. This was repeated until the sample was reduced to the size required for final analysis and stored in an air tight container.

2.1.3. Sample digestion

The digestion process was carried out as described by Onwunka [10] as follows: 3.0 g of each of the powdered sample was weighed and pre-treated with 20 cm^3 nitric acid and allowed to stay overnight. A 10 cm^3 perchloric acid was added and heated gently, then vigorously until clear solutions were obtained. The solutions were allowed to cool and then transferred to 100 cm^3 volumetric flask and made up to mark with distilled water. The solutions were filtered and stored in plastic bottles for the analysis of Ca, Mn, Mg, Cu, Co, Zn, Pb and Cd using Atomic Absorption Spectrophotometer (AAS).

2.2. Phytochemical Determination

Qualitative phytochemical analysis of the leaves and stem bark extract of *D. mespiliformis* was carried out using standard qualitative procedures [11,12]. 15 g each of the powered plant leaves and stem bark were soaked in ethanol for 24 hours. The extracts were filtered through a whatman filter paper. The filtrates were then concentrated using a rotary evaporator with the water bath set at 40 ^0C [8]. Alkaloids, anthraquinones, carbohydrates, flavonoids, glycosides, phlobatannins, saponins, steroids, tannins and terpenoids tests were conducted in all the extracts and the results are shown in Table 1.

2.3. 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 50cm^3 of distilled water and was filtered into 100cm^3 volumetric flask and 2 drops of 10% HNO_3 acid was added and a warm water was added to make up to the mark for the elemental determination. Appropriate standard solution were also prepared for element of interest. Calibration curve were constructed to obtained concentration of element.

3. Results and discussion

Phytochemical screening conducted on aqueous extracts of leaves and stem bark of *D. mespiliformis* revealed that alkaloids, anthraquinones, carbohydrates, flavonoids, phlobatannins, saponins, steroids, tannins and terpenoids were present in both the leaves and the stem bark of the plant while glycosides are present in the leaves extract but absent in the stem bark extract as shown in Table 1.

It is evident that ethanolic extracts have significantly higher activity when compared to aqueous extracts, but due to some environmental issues, like non-destructive nature of water to both man and the environment, non-inflammability of water, operational simplicity, easy to obtain (low cost), etc, it was chosen to make the aqueous extract which has no negative impact on the result as all the bioactive compounds tested in the aqueous extracts of the leave and stem bark were present. However, in the stem bark aqueous extracts, glycosides were absent.

Phytochemical screening of the extract varies from one plant part to another as shown in the results. It could also vary from place to place due to geographical location, climatic conditions and soil condition of a particular area. This may explain why it could be possible to have differences in chemical composition of the same plant of study in other areas.

Table 1 Phytochemical analysis of aqueous extracts of leave and stem bark of *D. mespiliformis*

Phytochemicals	**Test**	**Inference**	**Leave Extract**	**Stem Bark Extract**
Alkaloids	Mayer's test	Appearance of yellow cream precipitate	+	+
Anthraquinones	Borntrager's Test	Ammoniacal layer turns pink or red	+	+
Carbohydrates	Molisch's Reagent	Formation of violet ring	+	+
Flavonoids	Alkaline Test	Intense yellow colouration	+	+
Glycosides	Liebermann-Burchard's Test	Brown ring	+	-
Phlobatannains	Hydrochloric acid test	Formation of red precipitate	+	+
Saponins	Frothing test	Produce foam or emulsion	+	+
Steroids	Liebermann-Burchard's Test	Brown ring at the junction, green at the upper layer	+	+
Tannins	Ferric chloride test	Formation of blue or green colour	+	+
Terpenoids	Liebermann-Burchard's Test	Brown ring at the junction, deep red at the upper layer	+	+

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

The presence of some of these compounds has been demonstrated previously by other researchers. For example, the presence of alkaloids, saponins and tannins in the leaves and stem bark of *D. mespiliformis* has been demonstrated [13,14]. Similarly, the absence of glycosides in the stem bark of *D. mespiliformis* has also being demonstrated [13]. However, some of the results obtained are not in agreement with the previous findings. For example alkaloids were found to be absent in the stem bark of *D. mespiliformis* [15] which is contrary to other findings [13,14]. This might be due to climatic and environmental factors. Alkaloids, steroids, tannins and terpenoids were found present in both the leave and stem bark extracts of *D. mespiliformis*. Alkaloids are pharmacologically useful. They are the local anaesthetic, CNS stimulant (Cocaine, nicotine, coffiene, etc), analgesic e.g. Morphine and antimalarials e.g. guanine. Steroids (anabolic steroids) have been observed to promote nitrogen retention in Osteoporosis and in animals with wasting illness [16]. This also suggests that both the leaves and the stem bark extracts of the plant may possess some antibacterial potential most especially for antimalarial, antidiarrheal and antihemorrhagic potentials because of the presence of alkaloids and tannins in the leave and stem bark extracts [17,18]. Fruroquinolines and acridones have been reported to be compounds contained in plant alkaloids which are capable of curing malaria [19].

Phytochemicals constituents such as flavonoids, alkaloids and saponins present in the leaf and stem bark extracts were reported to possess biological activity against microbes [9, 13]. Flavonoids and tannins are proven to induce an important antimicrobial activity due to their possession of ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins and so forth [20]. Furthermore, the leaves and stem bark extracts of *D. mespiliformis* may have some antioxidant properties due to the presence of flavonoids and terpenoids in the extracts. Flavonoids have been known to have antioxidant, antibacterial, antifungal and antiviral activity [19]. Other studies confirmed that flavonoids and terpenoids produce antidiabetic activity, possibly by their antioxidant effects [19].

Elemental analysis of the extract presented in Table 2, showed that the plant contained major elements like Ca, Mn, Mg, Pb, Cu and Zn in moderate quantity while cadmium [Cd] and cobalt [Co] were not detected. The result of the elemental analysis of the aqueous extract of *D. mespiliformis* indicated the presence of macro and micro nutrients. Macro nutrients such as sodium, potassium and calcium regulate the fluid balance of the body and thereby influence cardiac output [21]. Calcium ions plays an important physiological and biochemical processes such as neuromuscular excitability blood coagulation, secretary processes etc [21]. Proper extracellular fluid and periosteal concentration of calcium and phosphate ions are required for bone mineralization [22]. Elements such as zinc and manganese are essential because they are important in several enzyme reactions as co-factors [22]. This study has provided some biochemical basis of the medical use of the extracts from *D. mespiliformis* in the treatment of infection, as a potential source of useful drugs and will help to develop new drug compositions.

Table 2 Elemental composition of leaves and stem bark extracts

Elements	Leave (Mg/Kg)	Stem Bark (Mg/Kg)
Ca	0.096 ± 0.02	0.190 ± 001
Cd	BDL	BDL
Mn	BDL	0.005 ± 0.03
Mg	1.450 ± 0.01	1.566 ± 0.01
Co	BDL	BDL
Pb	0.003 ± 0.01	0.001 ± 0.01
Cu	0.025 ± 0.02	0,021 ± 0.02
Zn	0.006 ± 0.01	0.004 ± 0.01

Mean ± SEM, BDL = Below Detectable Limit

4. Conclusion

The result of the elemental and phytochemical analysis reveals that the aqueous extracts of the leave and stem bark extract of *D*. *mespiliformis* plant contained elements such as calcium, manganese, magnesium, lead, copper, zinc and some primary bioactive constituents respectively. Therefore, phytochemical and the elemental constituents of the plant may account for its varied enthnobotanical uses in traditional medicine in Nigeria. With growing résistance of most pathogens to the activities of the common antibiotics, further work is required to isolate specific active constituents of the plant responsible for antimicrobial activity and antioxidant activity. Other related pharmacological studies such as in vivo investigation, drug formulation and clinical trials are highly recommended.

Compliance with ethical standards

Acknowledgments

The research team is profoundly grateful to Yobe State University, Damaturu Nigeria, for the elemental analysis of the plant extracts.

Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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