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ANTIBACTERIAL POTENCY OF AQUEOUS AND METHANOLIC EXTRACTS OF *Diospyros mesiliformis* LEAF AND STEM BARK

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ABSTRACT
Diospyros mespiliformis"Jackal berry" (also known as African ebony and by its Afrikaan name Jackal bessie). In hausa its called kanya, igi dudu (black wood) in Yoruba and Onye-oji (Black fellow) in igbo. It is widely used locally in treating various ailments such as fever, whooping cough, wounds, malaria, pheumonia, syphilis, leprosy and host of other ailment without proper scientific validation. This investigation is aimed at validating the scientific use of Diospyros mespiliformis leaf and stem bark using two solvents, methanol and aqueous. Phytochemical screening of the crude extracts revealed the presence of bioactive compounds such as saponins, tannins, alkaloids flavonoids, steroids, terpenoids and glycoside. The extracts were tested against Escherichia coli, Salmonella typhi and Staphylococcus aureus respectively. The antibacterial activity of the stem bark extracts revealed higher activity against E. coli, S.typhi and S. aureus than the leaf extracts. The mean range of zones of inhibition of stem bark at 100mg/ml and 12.5mg/ml varies between 19.29± 3.82 and 5.71± 2.05 while that of leaf extract ranges from 17.71± 2.06 and 5.43±1.13 at 100mg/ml and 12.5 respectively.
Key words: Antibacterial, Phytochemicals, Diospyros mespiliformis, Minimum Inhibitory Concentration.

INTRODUCTION

Diospyros mespiliformis "Jackal berry" (also known as the African Ebony Kanya in Hausa, Igi dudu, Yoruba and Onye Oji, Igbo) is a large deciduous tree found mostly in Savannah of Africa. It is a tall tree that grows up to 25 meters in height. It has a dense evergreen canopy (Belemtougri *et al.,* 2006). *Diospyros mesipiliformis* has been used in Traditional medical systems including Ayurveda, Chinese and Africa (Abdulrauf *et al.,* 2000). Mature tree have dark grey fissured bark, an adult tree reaches an average of 4 to 6 meters in height, though occasionally trees reach 25 meters. 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 grows in the rainy season, the flowers are imperfect with genders on separate trees and are cream color. The female trees bears fruit in the dry season and these are eaten by many wild animals. They are oval shaped, yellow and about 20-30mm in diameter with purple fruits when they are ripen. The tree like marula is favoured by the Bantu cultivated lands in order to harvest the fruit. The plant is widely used in parts of Africa and a number of chemical constituents of therapeutic importance have been isolated (Burkill, 2005). *Diospyros mespiliformis* is widely used as a folkloric remedies for the treatment of fever, malaria whopping cough etc, it has a large range of medicinal uses (El-Kamali, 2011).

A traditional food plant in Africa, the fruit has potential to improve nutrition. *Diospyros mespiliformis* has a fantastic mutualism and symbiotic network with many living organism from human beings to small insects. There is a complex ecological system revolving around this tree. It is one of the savannah giants that can live for more than 200 years (Quideau *et al*., 2012). This study aim at screening the stem bark and leaf extract of *Diospyros mespiliformis* for the presence of bioactive compounds as well as antibacterial potential of methanol and aqueous extract against *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus*.

MATERIAL AND METHODS

Plant material

The plant *Diospyros mespiliformis* collected from Jibiya local government area of Katsina state, Nigeria in March 2018. It was authenticated at the Herbarium section of Department of Biological Science, Ahmadu Bello University, Zaria, with Voucher specimen number 02684 have been deposited for future references.

Processing of Plant Materials

The stem bark of *Diospyros mespiliformis* was washed with distilled water and shade dried. The dried material was powdered using mechanical method and resulting powder is sieved with sieve of 0.3mm aperture size and stored in airtight container (El- Kamali, 2011).

Extraction of Plant Material

The powdered stem bark and leaf materials were subjected to successive solvent extraction using methanol and distilled water. 500 grams each of the powered plant materials were subjected to soxhlet extract 12-16 hours with 1000ml of the various solvents. The extracts obtained were later kept for evaporation to remove the excessive solvents. These extracts were stored in a cool dry place for the analysis of the presence of preliminary phytochemicals and in-vitro antibacterial activities.

Preliminary Phytochemical Analysis

All the plant extracts were screened for the presence of bioactive compounds namely alkaloids, glycosides, saponins, phytosterols, tannins, flavonoids and terpenoids by using standard methods (Cuilel, 2014).

Antibacterial activity test

The antibacterial activities of the methanol and aqueous extracts were determined using *E.coli, S.* Typhi and *S. aureus* obtained from Yusuf Dantsoho memorial hospital Kaduna, all the bacterial isolates were checked for purity and maintained in slant of nutrient agar. 10g of the extracts was weighed and dissolved in 10ml of DMSO to obtain a concentration of 100mg/ml. This was the initial concentration of the extracts used to determine the antimicrobial activities of the plant.

Standardization of Inoculum

Few colonies of the confirmed isolates were dispensed in normal saline to match the 0.5 McFarland standard for sensitivity test as described by NCCLS (1999).

Mueller Hinton agar was the growth medium used it was prepared according to the manufacturer's instruction, sterilized at 121^{o}C for 12 minutes, poured in to the sterile dishes and allowed to cool and solidity. The sterilized medium was then seeded with 0.1ml of the standard inoculums of the test organisms, the inoculums was spread evenly over the surface of the medium by the use of a sterile swab. By the use of standard cork borer of 6mm in diameter, a well was cut at the centre of each inoculated medium. 0.1ml of the solution of the extract at concentrations 100mg/ml 50mg/ml,25mg/ml and 12.5mg/ml were introduced into the well on the medium while 1mg/ml of the standard, profloxacin was used as positive control. The inoculated medium was incubated for 24hours at 37^{o}C, after which the plates were observed for the zones of inhibition of growth, the zone were measured with a transparent ruler and the results were recorded in millimeters (mm).

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of each extract was determined using the broth dilution method. Mueller Hinton broth was prepared; 10ml was dispensed into test tubes and was sterilized at 121^{o}C for 15minutes, the broth was allowed to cool. McFarland standard turbidity scale number 0.5 was prepared to give turbid solution. Normal saline was also prepared, 10ml was dispensed into sterile test tubes and the microbe was inoculated and incubated at 37^{o}C for six hours. Dilution of the test microbe was done using the normal saline until the turbidity matches that of the McFarland scale by visual comparison, at this point, the test tubes has a population density of 1.5x 10^{8} CFU/ml. Two fold serial dilution of the extracts were done in the sterile broth to obtain the concentration of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. Haven obtained the different concentration of the extracts in the sterile broth, 0.5ml of the test microbes in the normal saline was then inoculated at the different concentrations, incubation was done at 37^{o}C for 24hours after which the test tubes of the broth were observed for turbidity (Abba *et al.,* 2016; El-Mahmood *et al.,* 2018). The lowest concentration of the extracts in the broth which shows no turbidity was recorded as the minimum inhibitory concentration (Andrews, 2010).

Determination of Minimum Bacterial Concentration

The MBC was carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller Hinton was prepared, sterilized at 121^{o}C for 15 minutes, poured into sterile petri dishes and was allowed to solidity. The contents of MIC in the serial dilution were then sub- cultured on to the prepared medium; incubation was made at 37^{o}C for 24 hours after which the plates of the medium were observed for colony growth. MBC was the plates with the lowest concentration of extracts without colony growth (French, 2001).

RESULTS AND DISCUSSION

The phytochemical analysis of the crude extracts of methanol and aqueous stem bark and leaves extracts of *Diospyros mespiliformis* revealed the presence of tannins, saponins, glycosides, glycosides, alkaloids, flavounoids, steroids and terpenoids in all the extracts except anthraquione which was found to be absent in all except in methanol stem bark extract. The result agreed with the work of Yakubu and Mukar (2007). Flavonoids have been known to have antioxidant, antibacterial, antifungal and antiviral activity (Bors *et al.,* 1990). Alkaloids are known for their pharmacological activities such as anti-bacterial and anti-fungal activities (Shagal *et al.,* 2012). Saponins: are known for antibacterial activity against microorganisms, they also exhibit heamolytic activity upon injection into the blood stream as such it is advisable to administer it orally in medical application (Shagal *et al.,* 2012).

Tannins are compounds that have the ability to react with protein to form stable water insoluble components. They act as detoxifying agents and as such they have ability to precipitate the protein on the cell wall of bacteria thereby inhibiting the growth of bacteria (Shagal *et al.,* 2012). Tannins precipitate proteins of the wound , forming a protective layer on the wound, thus assisting in the arrest of bleeding and also used for wound, healings activity (Quideau *et al.,* 2012). It is also used in the treatment of diarrhea as an effective astringent medicine in the small intestine. The result of the zone of inhibition as shown in Tables 3 - 4 shows that the methanolic stem bark extract had zone of inhibition of between 13-16mm where as the aqueous stem bark extract had zone of inhibition of between 10-14mm when compared to the standard ciprofloxacin, this explains that the activity of *Diospyros mespiliformis* is comparable to the standard hence, the plant can be used to treat various diseases caused by these selected organisms. Similarly the result of the zone of inhibition of the leaf extracts (Table 4) revealed that the methanolic leaf extract had zone of inhibition between 12-13mm while the aqueous leaf extract had a value of 9-14mm. the standard drug had zone of inhibition between 15-19mm. The extracts of *Diospyros mespiliformis* can be substituted to the standard drugs.

The statistical analysis indicates that the difference between the zone of inhibition on stem bark differ significantly base on concentration ($p< 0.05$), it is also observed from the analysis that the zone of inhibition is significantly higher in the control and 100 mg/ml than in other level of concentration and it is found to be lower at 12.5mg/ml and 25mg/ml of concentration. The analysis also revealed that the difference between the zone of inhibition on leaf extracts differ significantly based on concentration ($p< 0.05$), from the analysis than the zone of inhibition is significantly higher in the control and 100mg/ml than in other level of concentration with lowest at 12.5mg/ml and 25mg/ml level of concentration.

The result of the minimum inhibitory concentration (Table 6-7) revealed that the methanolic stem bark extract had MIC range of between 6.22 to 12.5mg/ml whereas the aqueous stem bark extract had MIC of between 6.25 to 25mg/ml, the result shows that the stem bark extract had a high activity than the aqueous extract. Similarly, the MIC of the methanolic leaf extract and aqueous leaf extract as shown in table 5 showed MIC range of between 12.5 and 25 mg/ml while the aqueous leaf extract had MIC range of between 25mg/ml to 50mg/ml respectively. The result showed that the methanolic extract had better antibacterial activity than the aqueous extracts.

The minimum bactericidal concentration (MBC) is the lowest concentration that can kill the organism. The result of the MBC as shown in table 6 and 7 revealed that the methanolic stem bark extracts had MBC range of between 12.5 and 25mg/ml where as the aqueous extract showed MBC of 50mg.ml for all the organisms tested. The results of the methanolic leaf extract of *Diospyros mespiliformis* revealed an MBC of 25 and 50mg/ml while aqueous leaf extract showed a range of 50mg/ml to kill the tested organisms than the stem bark. It was deduced that stem bark extract have high antibacterial activity against the selected organisms than the leaf extract.

Table 1: Phytochemical characteristics of *Diospyros mespiliformis*

Crude extract	Percentage yield of each extract (%)	
	Methanol	Aqueous
Stem bark	48.7	15.7
Leaf	10.63	6.60

Table 2: Phytochemical Characteristics of Stem Bark and leaf extracts of *Diospyros mespiliformis*

Bioactive compound	Leaf extract Methanol	Leaf extract Aqueous	Stem bark extract Methanol	Stem bark Aqueous
Tannins	+	+	+	+
Saponins	+	+	+	+
Alkaloids	+	+	+	+
Flavanoids	+	+	+	+
Glycosides	+	+	+	+
Anthraquinone	-	-	+	-
Steroids	+	+	+	+

Key: Present (+), Absent (-)

Table 3:Zone of inhibition of the stem bark extracts of *Diospyros mespiliformis*

	Zone inhibition (mm)		
Test organisms	Methanol extract stem bark	Aqueous extract Stem bark	Ciprofloxacin (5ug/ml)
E. coli	13	9	15
S. Typhi	12	11	16
S. aureus	13	14	19

Table 4: Zone of inhibition of the leaf extracts of *Diospyros mespiliformis*

	Zone inhibition in (mm)		
Test organisms	Methanol extract stem bark	Aqueous extract Stem bark	Ciprofloxacin (5ug/ml)
E.coli	16	12	18
S. Typhi	14	10	16
S. aureus	13	14	19

Table 5: Comparing difference in zone of inhibition of stem bark and leaf base on concentration level.

Concentration (mg/ml)	**Plant Part**	
	Stem bark	**Leaf**
	Mean ± S.D	**Mean ±S.D**
Control	19.29 ± 3.82^{c}	17.71 ± 2.06^{c}
100	15.71 ± 4.27^{c}	$15.00 \pm 6.16^{b,c}$
50	10.86 ± 4.26^{b}	12.29 ± 2.87^{b}
25	$7.57 \pm 2.15^{a,b}$	8.43 ± 1.90^{a}
12.5	5.71 ± 2.06^{a}	5.43 ± 1.13^{a}
F-value	18.603	15.391
P-value	0.000	0.000

Values are mean ± Standard deviation, Value with different superscript across a column have significant difference

Table 6: Minimum Inhibitory Concentration of stem bark extracts of *Diospyros mespiliformis*

Methanol stem bark extract (Aqueous)					
	Concentration (mg/ml)				
Test organisms	100	50	25	12.5	6.75
E. coli	- (-)	- (-)	- (-)	- (+)	+ (+)
S. Typhi	- (-)	- (-)	- (-)	- (+)	- (+)
S.aureus	- (-)	- (-)	- (-)	- (+)	+ (+)

Key: - no growth, + growth

Table 7: Minimum Inhibitory Concentration of leaf extracts of *Diospyros mespiliformis*

Methanol leaf extract (Aqueous)					
	Concentration (mg/ml)				
Test organisms	100	50	25	12.5	6.75
E. Coli	-	- (-)	- (+)	- (+)	+ (+)
S. Typhi	-	-	- (-)	+ (+)	+ (+)
S. aureus	-	- (-)	- (+)	- (+)	+ (+)

Key: - no growth, + growth

Table 8: Minimum Bacteriacidal Concentration of stem bark extracts of *Diospyros mespiliformis*

Methanol stem bark extract (Aqueous)					
Test organisms	Concentration (mg/ml)				
	100	50	25	12.5	6.75
E.coli	- (-)	+ (+)	- (-)	- (-)	- (-)
S.typhi	- (-)	+ (+)	- (-)	- (+)	- (-)
S.aureus	- (-)	+ (+)	+(-)	- (-)	- (-)

Key: - no growth
+ growth

Table 9: Minimum Bacteriacidal Concentration of leaf extracts of *Diospyros mespiliformis*

Methanol leaf extract (Aqueous)					
Test organisms	Concentration (mg/ml)				
	100	50	25	12.5	6.75
E. coli	-(-)	+ (+)	- (-)	- (-)	- (-)
S. Typhi	+(+)	+(-)	- (-)	- (-)	- (-)
S. aureus	-(+)	+ (+)	- (-)	- (-)	- (-)

CONCLUSION

The study showed that *Diospyros mespiliformis* leaf and stem bark could serve as potent antibacterial in ethnomedicinal sources. Treatment of typhoid, malaria, syphilis, leprosy etc. Phytochemical content of the plant were confirmed to contain tannins, saponins, flavonoids, steroids, glycosides, however no anthraquinone detected in the plant.

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