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Preliminary Phytochemical Screening and Antibacterial Activity of the Crude Methanol Leaf Extract of Diospyros Mespiliformis Hochst (EBENACEAE)

Abstract— Diospyros mespiliformis, is an evergreen tree found mostly in the savannas of Africa. The leaves have wide applications in traditional medicine which includes whooping cough, wound dressing, pneumonia, syphilis, arthritis and skin infections. The preliminary phytochemical screening showed the presence of cardiac glycosides, terpenoids, alkaloids, saponins, tannins, anthraquinones and flavonoids. The in vitro antibacterial study of crude methanol leaf extract (CME) was analysed using disc diffusion method (DDM), with two-gram positive (Staphylococcus aureus and Streptococcus pneumonia) and two-gram negative (Escherichia coli and Pseudomonas aeruginosa) bacteria. The standard antibiotic disc used was gentamicin (5μg/disc). The CME showed highest activity against Streptococcus pneumonia (12.5±1.12mm) at 150mg/ml and the lowest activity was seen against Staphylococcus aureus (11.5±1.11mm) at 200mg/ml as compared to 31.0±0.82mm and 41.0±0.82mm of the control (standard drug) respectively. Pseudomonas aeruginosa showed resistance against the CME at all concentrations including the standard drug.

Keywords— Diospyros Mespiliformis, Phytochemical, Antibacterial, Crude methanol leaf extract

I. INTRODUCTION

Diospyros mespiliformis is a tall, upright tree of 25m, with a circumference of more than 5m. It belongs to family Ebenaceae, and is related to the true ebony diospyros ebenum and edible persimmon diospyros kaki [4].

A. Taxonomy of Diospyros mespiliformis

a) Scientific classification

Kingdom Plantae

Division Magnoliophyta

Class Magnoliopsida

Order Eriocales

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Family Ebenaceae
Genus Diospyros

Specie Diospyros mespiliformis

b) Common and local names:

English	Ebony
Hausa	Kanya
Yoruba	Igi dudu
Igbo	Akawayi
Kibaku	Kwashina
Nupe	Buswachi
Fulani	Balchi
Kanuri	Bergen 2

B. Ethnomedical uses

Diospyros mespiliformis, have wide applications in traditional medicine which include the use of leaf decoction as a remedy for fever, whooping cough and for wounds [2; 8]. Bark and roots are used for treatment of ill health conditions such as malaria, pneumonia, syphilis,

leprosy and dermatomycoses, as an antihelmintic and to facilitate delivery [3]. In Nigeria the leaf is infused and taken as a mild laxative and as a vermifuge, for fever, dysentery and is applied to wounds as a haemostatic. The Hausas chew the leaf and fruit or apply an infusion for gingivitis and toothache [1], and locally in Dembo village, Zaria Kaduna State northern Nigeria, the plant is claimed to be useful in combination with the leaves of Annona senegalensis in the management of convulsive disorders (Personal Communication, 17th January 2011). The leaves are also used in traditional medicine for whooping cough, wound dressing, pneumonia, syphilis, arthritis and skin infections.

II. MATERIALS AND METHODS

a) Sample collection and identification

The fresh leaves were collected from polo ward MMC of Borno state in February, 2020 and was identified by Prof. S.S. Sanusi of the Department of Biological sciences, University of Maiduguri.

b) Sample preparations of plant material

The fresh plant sample was cleaned and air dried under shade at room temperature for several days. The air-dried leaves of the plant were pulverized using mortar and pestle then subjected for further analysis.

c) Extraction of plant material

1L of 95% methanol was poured into 5L round bottom flask; 250g of the sample was placed in the thimble and was inserted in the centre of the extractor. The Reflux was heated at 60°c. Liquid condensate drips in to the filter paper thimble in the centre, and the vapour rises through the vertical tube in to the condenser at the top which contain solid sample to be extracted. The extract seeps through the pores of the thimble and the siphon tube when it flows back down into the round bottom flask. It was allowed to continue for 3 hours, and then removed.

d) Phytochemical Screening

The methanol leaf extract of Diospyros mespiliformis was subjected to preliminary phytochemical screening to test presence or absence of alkaloids, saponins, flavonoids, tannins, anthraquinones cardiac glycosides and carbohydrates according to standard procedures [7].

e) Antibacterial

Gram positive (Staphylococcus aureus, Streptococcus pneumoniae) and gram negative (Escherichia coli, and pseudomonas aeruginosa) were used for this work. Respective organism/isolate was acceptically inside peptone water and allowed to reach a Mcfarland standard. It was poured unto the dried media. The media was freely allowed to dry of its content. Four different wells were made on the media containing the organisms. The wells were labelled according to the concentration of the extract (C,D,DD) and antibiotic control (gentamicin) served as control. 25 microliters of the concentrated extract were placed. The culture plate was incubated at 370c for 18hrs.The zones of inhabitation were measured to determine the activity of the extract against respective organisms.

III. RESULTS AND DISCUSSION

A. Results

a) Plant extraction

The extractive for the CME of Diospyros mespiliformis from 250g plant material was found to be 4.25% as shown in the table below.

Table 1 $\label{table 1} \mbox{Yields of crude methanol leaf extract and fractions of } \\ \mbox{Diospyros mespiliformis}$

Sı	r. No.	Sample/Portion	Yield (g)	Colour	Percentage
	1	CME	58.85	Dark green	4.25 %

b) Phytochemical constituents of Diospyros mespiliformis

The preliminary phytochemical screening of methanol leaf extract of Diospyros mespiliformis revealed the presence of saponins, flavonoids, tannins, terpenol, carbohydrates, cardiac glycosides, cardenolide and



combined anthracene type of anthraquinones as shown in the table below.

TABLE 2

PHYTOCHEMICAL CONSTITUENTS OF CME METHANOL LEAF EXTRACT
OF DIOSPYROS MESPILIFORMIS

Alkaloids	-	
Saponins	+	
Flavonoids	+	
Tannins	+	
Carbohydrates	+	
Cardiac Glycosides	+	
Anthraquinones (combined anthracene)	-	
Anthraquinones (free)	-	

KEY: (+) = Present, (-) = Absent, CME = Crude Methanol leaf extract of Diospyros mespiliformis

Antibacterial Activity of Methanol Leaf Extract of Diospyros Mespiliformis

TABLE 3

SUSCEPTIBILITY TEST FOR CME ON THE TEST ORGANISM AT DIFFERENT CONCENTRATION MG/DISC AND THEIR ZONE OF INHIBITION (MM).

Concentration (mg/disc) of the CME and standard drug (μ g/disc) diameter zone of inhibition (mm) as mean \pm SEM

Test	Gentamici	Zones of inhibition(mm)		
organisms	n	100	150	200
Gram-positive ba				
Staphylococcu	41.0±0.82	0.00±0.0	11.7±0.9	11.5±1.1
s aureus		0	5	1
Streptococcus	31.0±0.82	11.7±1.2	12.5±1.1	0.00±0.0
pyogenes		5	2	0
Gram-negative b	acteria			
Escherichia	25.5±1.11	0.00±0.0	11.7±0.9	0.00 ± 0.0
coli		0	6	0
Pseudomonas	0.00±0.00	0.00±0.0	0.00±0.0	0.00±0.0
aeruginosa		0	0	0

KEY: (+) = Present, (-) = Absent, CME = Crude Methanol leaf extract of Diospyros mespiliformis

B. Discussion

Phytochemical screening of CME fractions showed the presence of cardiac glycosides, terpenoids, saponins, tannins, anthraquinones, and flavonoids. These classes of secondary metabolites such as alkaloids, saponins, tannins, anthraquinones, and flavonoids were reported to have curative activity against several pathogens and therefore could suggest the use of the plant traditionally for the treatment of various infections [5]. Staphylococcus aureus, Streptococcus pneumonia were active in the CME of Diospyros mespiliformis for the gram positive and gram negative Escherichia coli, was found active while pseudomonas aeruginosa was not reactive. It shows that Diospyros mespiliformis has the skin anti-infection and soft tissue infection because of the presence of Staphylococcus aureus and Streptococcus pneumonia. The CME of Diospyros mespiliformis shows the activity of E.coil which can serve as an anti-diarrheal, anti-urinary tract, and anti-pneumonia.

III. CONCLUSION

The result of this study indicated that the leaves of plants contain some major bioactive compound that inhibits the growth of microorganism thereby proving very effective as alternative source of antibiotics. This agrees with the report that the leaves are used to get rid of internal parasites, also act as anti-inflammatory, anti-infection, anti-diarrheal, anti-urinary tract, and anti-pneumonia [6]. The compounds identify in this study shows that they could be responsible for activity observed. Therefore, further studies should be carried out to identify the specific compounds and elucidation of the structure.

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