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Antimicrobial Studies and Phytochemical Screening of Extracts of *Hyphaene thebaica* (Linn) Mart Fruits

[1]O.O. Dosumu, [1]F.O. Nwosu and [2]C.D. Nwogu
[1]Department of Chemistry, University of Ilorin, Ilorin, Nigeria
[2]Department of Chemistry, University of Ibadan, Ibadan

Abstract: *In vitro* antimicrobial properties of solvents extracts of *Hyphaene thebaica* (L.) Mart fruits showed that ethyl acetate extract was active against all the five pathogenic bacteria, *Staphylococcus aureus*, *Eschericia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* used in this study while methanol extract was active against *Pseudomonas aeruginosa* and *Klebsiella Pneumoniae*. Hexane extract showed little inhibition on the growth of *Bacillus subtilis* and out of the three fungi, *Aspegillus niger*, *Candida albicans* and *Penicillium sp*. used in this study, only *Penicillium sp*. growth was slightly affected by high concentration of methanol extract. The phytochemical screenings showed that tannins and flavonoids are present in the ethyl acetate fraction while saponin was found in methanol fraction.

Key words: *Hyphaene thebaica,* antimicrobial activity, phytochemical screening

INTRODUCTION

Hyphaene thebaica (L.) Mart belongs to the family Palmae and sub family Borassoideae. It is commonly called 'African doum palm' or 'ginger bread' palm. It is a small palm with the trunk normally branching into two-five branches exhibiting forking pattern. *Hyphaene thebaica* is a dioecious palm with the stem about 15 m high and 25 cm in diameter. It grows in the Sahel, in the hot dry savannah between 12-18 N from Senegal to Northern Nigeria, Chad, Zaire and North East Africa. It is called 'goriba' in Hausa.

The aqueous extract of *Hyphaene thebaica* is used in the treatment of bilharziasis, haematuria, bleeding especially after child birth and as haematinic agent[1,2]. It is also good as hypocholesterolenic agent[3], hypolipodemic and heamatinic suspensions[4]. The young leaves are readily eaten by livestock while old leaves are bitter and unpalatable.

The fruit is glubose-quandrangular, about 6×5 cm with a shinny orange-brown to deep chestnut skin (epicarp). The 'rind' (mesocarp) in some palm is unedible but of other it is very palatable, highly aromatic and sweet with a taste like ginger bread hence the English name. When eaten it serve as vermifuges and parasite expellant[2,5]. The presence of estrone in the kernel and pollen grains had been reported[6]. The chloroform extract of the fruits improve spermatic count of male rats at low concentration[7] but could decrease it at high concentration[8]. Previous work by Irobi and Adedayo showed that the aqueous extract of *Hyphaene thebaica* fruits has significant anti fungal properties on *Candida albicans*, *Microsporum gypseum*, *Trichlorophyton rubrum*, *Mucor sp.*, *Fusarium solani* and *Aspergillus niger* with the MIC range of 3.1 to 25%[9]. In another research on *H. thebaica,* it was observed that frog's heart and rat's intestine were stimulated by the aqueous extract of the fruits while dog's blood pressure was lowered[10].

Chemical investigation of the aqueous extract of the fruits revealed the presence of alkaloids, reducing sugars and glycosides[10]. This study aim to look at the effect of different solvent extracts of *Hyphaene thebaica* fruits on pathogenic bacteria and fungi. The phytochemical screening of the extracts preluding isolation and characterization of bio-active compounds of the fruits will also be done.

MATERIALS AND METHODS

Sample collection and extraction: The ripe *Hyphaene thebaica* fruits were collected from Gashua, Yobe state, North Eastern part of Nigeria in the month of August. The mesocarp was remove with knife and air dry at room temperature for 7 days. The dried samples were ground and subjected to hot solvent extraction using soxhlet. N-hexane, ethyl acetate and methanol were used respectively for the extraction. The extracts were vacuum dried and the thick dark brown crude extracts obtained were preserved for analysis.

Corresponding Author: O.O. Dosumu, Department of Chemistry, University of Ilorin, Ilorin, Nigeria

Phytochemical screening: Preliminary phytochemical screening of the extracts for saponin, glycosides, alkaloids, tannins and flavonoids using standard phytochemical screening methods[3] was done and the results shown in (Table 1).

The test for reducing sugars was done by dissolving a small portion of the extract in distilled water, filtered and warmed with Fehling solutions A and B. Brick red precipitate showed the presence of reducing sugars which was confirmed by the silver mirror result of Tollen's reagent test.

Microorganism and medium: Clinical isolates of human pathogenic bacteria and fungi namely *Staphylococcus aureus*, *Eschericia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Aspergillus niger*, *Candida albicans* and *Penicillium specie* were obtained from the microbiological laboratory unit of pharmaceutical microbiology department of University of Ibadan, Ibadan. The bacteria cultures were maintained on nutrient agar slants and fungi on sabourand dextrose agar slants. They were preserved at 4-5°C until needed for antimicrobial activity.

Nutrient broth, nutrient agar, Sabourand Dextrose Agar (SDA), tryptone soya agar (Oxoid Ltd, UK) were used in the assays. Methanol, ethyl acetate and n-hexane (Merck) were used to dissolve the extracts and as a negative control in the assay. For determination of the Minimum Inhibitory Concentrations (MIC) of the extracts, concentrations of 125, 100, 75, 50 and 25 mg mL^{-1} were made from the extracts. Gentamycin, 10 μg mL^{-1}, nystatin 10 μg mL^{-1} were I ncluded as standard reference drugs in the study.

Microorganisms assay: The cup agar broth diffusion procedure[12] was used. Overnight broth cultures of the organisms were used. The inoculums size was adjusted to 2.6×10^7 cfu mL^{-1} using Unicam gamma (1000) spectrometer at 540 nm. 15 mL of sterile molten agar was seeded with 0.5 mL of the bacteria isolates and sterile tryptone soya agar plate was similarly seeded with fungi. The plates were allowed to solidify and wells were made using a sterile 7 mm diameter cork borer. Different concentrations of the extracts (1 mL portions) were introduced into the wells with the aid of a Pasteur pipette and controls were set up containing solvent and gentamycin (10 μg mL^{-1}) for bacteria and nystatin for fungi. Diameters of zones of inhibition were determined after incubating plates at 37°C for 24 h (bacteria) and at 25°C for 72 h (fungi). Diameters of zones of inhibition>10 mm were considered active[12]. Antimicrobial studies were done in triplicates and diameters of zones of inhibition (mm) are expressed as the mean and standard errors on means. Student 'T' tests was used to test for probability at $p<0.5$.

Table 1: Phytochemical screening of the methanol and ethyl acetate extracts

Secondary metabolites	Ethyl acetate	Methanol
Alkaloids	-	-
Reducing sugar	+++	+++
Glycoside	++	+++
Saponin	-	+++
Tanins	++	-
Flavonoids	+++	+

Key:- Absent, + Low concentration, ++ Medium concentration, +++ High concentration

Thin Layer Chromatography (TLC) analysis: The dilute solutions of the extracts were spotted on pre-coated silica gel analytical thin layer chromatographic plate (0.02 mm) (Merck) and were developed in hexane/ethyl acetate (3:1) solvent system. The plates were viewed under UV lamp and in iodine vapour.

REUSLTS AND DISCUSSION

The result of the phytochemical studies in (Table 1) showed that the extract has no alkaloid but it is very rich in reducing sugar which is evident in it high concentration recorded in both the methanol and ethyl acetate extracts. This is buttress by the high concentration of glycoside in the methanol extract and medium concentration recorded in the ethyl acetate fraction. The methanol extract has high concentration of saponin while high flavonoid concentration was recorded in the ethyl acetate fraction. The use of some plants as medicinal plant for traditional treatment of diseases is due to the presence of flavonoids and saponin[13], hence the use of *H. thebaica* for the treatment of haematuria and schistosomiasis[2]. Flavonoids containing plants have been used as diuretic, laxative, emollient and poultice[14], hence, the use of *Hyphaene thebaica*, which is very rich in saponin and flavonoids in folk medicine, is not surprising. Medium concentration of tannin is found in ethyl acetate extract, it is not out of place then to tag the activity of this fraction to tannin. The presence of tannin in some medicinal plants have been found to be responsible for the antiviral and antibacterial activities exhibited by the plant[15-17], hence the local use of *H. thebaica* for the treatment of virus and bacteria induced diseases is supported[2,6].

The result of the antibacterial studies in (Table 2) showed that ethyl acetate extract inhibited the growth of all the pathogenic bacteria used in this study. The effect is pronounced on *B. subtilis*, *P. aeruginosa* and *K. pneumoniae* particularly at high concentration and at this concentration, the effect was comparable to that of the reference drug, gentamycin. *S. aureus* and *E. coli* were

Table 2: *In vitro* antibacterial activity of *H. thebaica* fruits extracts

Plant Extracts	Extract Conc./ Control/Ref. (mg ml^{-1})	Diameters of zones of inhibition of bacteria (mm, p<0.05)				
		Gram positive		Gram negative		
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*K.pneumoniae*
Methanol						
	125	-	-	-	18 + 0.3	20 + 0.2
	100	-	-	-	16 + 0.5	18 + 0.4
	75	-	-	-	14 + 0.2	16 + 0.5
	50	-	-	-	12 + 0.1	14 + 0.1
	25	-	-	-	10 + 0.3	12 +0.1
	Methanol	-	-	-	-	-
	Gentamycin	34 + 0.2	38 + 0.5	36 + 0.1	40 + 0.5	24 + 0.1
Ethyl acetate						
	125	12 + 0.1	24 + 0.3	14 + 0.3	24 + 0.4	24 + 0.1
	100	10 + 0.3	20 + 0.2	12 + 0.1	20 + 0.4	20 + 0.4
	75	-	18 + 0.5	-	18 + 0.3	18 + 0.3
	50	-	16 + 0.4	-	16 + 0.1	16 + 0.5
	25	-	14 + 0.2	-	14 + 0.3	14 + 0.2
	Ethyl acetate	-	-	-	-	-
	Gentamycin	34 + 0.1	34 + 0.2	32 + 0.4	38 + 0.2	24 + 0.3
N-Hexane						
	125	-	14 + 0.1	-	-	-
	100	-	12 + 0.3	-	-	-
	75	-	10 + 0.3	-	-	-
	50	-	6 + 0.4	-	-	-
	25	-	-	-	-	-
	N-hexane	-	-	-	-	-
	Gentamycin	34 + 0.4	34 + 0.1	32 + 0.5	38 + 0.3	26 + 0.4

Table 3: *In vitro* antifungal activity of methanol extract of *H. thebaica* fruits

Concentration of extract (mg ml^{-1}) Control/Ref	Diameters of zones of inhibition of fungi (mm, p<0.5)		
	Aspergillus niger	*Candida albicans*	*Pencillium sp.*
125	-	-	18 + 0.2
100	-	-	16 + 0.1
75	-	-	12 + 0.4
50	-	-	-
25	-	-	-
Methanol	-	-	-
Nystatin	24 + 0.1	22 + 0.4	24 + 0.2

Table 4: R_f values of the TLC spots of hexane and ethyl acetate extracts

Extracts	R_f values
Hexane	0.97
Ethyl acetate	0.97, 0.78

only affected at high concentration and even to a little extent. The methanol extract has inhibition on the growth of *P. aeruginosa* and *K. pneumoniae*. Hexane extract had inhibition on *B. subtilis* growth only at 50 mg mL^{-1} and above with 14±0.1 mm for 125 mg mL^{-1} extract concentration.

The hexane and ethyl acetate extracts has no activity inhibition on fungi, it was only the methanol extract at high concentration that has. The methanol extract effect started at 75 mg mL^{-1} and the highest zone of inhibition, 18+0.2 mm recorded at 125 mg mL^{-1}. This observation was supported by Irobi and Adedayo's work which showed that polar solvent extract had high effect on the activities of wide range of fungal isolates[9]. The TLC of the hexane extract developed on hexane/ethyl acetate ratio 3:1 showed one spot with R_f value of 0.97 which was close to solvent front and could be the fat extracted from the mesocarp. The TLC of the ethyl acetate fraction showed two spots with R_f of 0.97 and 0.78. The compound with the 0.78 value could be tannin or flavonoid. The methanol extract of the sample which is much did not show any spot on TLC plate Shown in (Table 4). This suggests that the mesocarp contain mainly sugars which are soluble in polar solvent like methanol. This is visible in (Table 1) which showed high concentration of reducing sugars and glycosides. The biological activity which is tagged to this fruit for local treatment of diseases resides also in the methanol fraction but masked by high concentration of sugar. The activities of some plants have been traced to the sugars and glycosides present in them[18,19].

CONCLUSION

In conclusion, though the phytochemical studies of *Hyphaene thebaica* fruits do not show the presence of alkaloids, the presence of reducing sugar, glycosides, flavonoids, tannins and saponins were confirmed in the ethyl acetate and methanol fractions. Further work should be done on these fractions to isolate and characterize the organic bio-active compounds that are responsible for the antimicrobial activities recorded for the extract of this fruits.

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