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THE *IN VITRO* ANTIBACTERIAL EVALUATIONS OF THE CRUDE PERICARP EXTRACT OF *HYPHAENE THEBAICA* (DOUMPALM) ON SOME PATHOGENIC BACTERIA ORGANISMS

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ABSTRACT

The Preliminary phytochemical and antibacterial evaluation of crude pericarp extract of *Hyphaene thebaica* (doumpalm) was ascertained. The crude pericarp powder of the plant was extracted for phytochemical screening by reflux method. The phytochemical analysis of the extract revealed the presence of tannins, steroids, saponins, carbohydrates, flavonoids, terpenes and terpinoids in low and moderate concentration. The crude pericarp extract exhibited activity on some laboratory isolates such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi*, *Escherichia coli* and *Shigella dysenteriae* and the rest were resistant. The effect of this extract on both gram positive and negative isolates indicates broad spectrum antibacterial activity. These organisms were sensitive to all the concentrations of the extract used except *salmonella typhi* that were resistant at 200 mg / ml. The minimum inhibitory concentration for Staphylococcus *aureus, Streptococcus pyogenes and Salmonella typhi* is 25 mg / ml and 50 mg / ml for *Escherichia coli* and *Shigella dysenteriae*. All the laboratory isolates has a minimum bactericidal concentration of 50 mg / ml. This finding concludes that *Hyphaene thebaica* have some important phytochemicals that can be used as a therapeutic agent in the inhibition of the multiplication of these pathogenic organisms.

Keywords: Phytochemistry, Antibacterial, Pathogenic bacteria, Crude pericarp extract, *Hyphaene thebaica*.

INTRODUCTION

In third world countries, treatment with synthetic antibiotics is not always possible due to their high cost and adulteration. Opportunistic infections therefore develop resistance in multiple forms to the synthetic antibiotics commonly prescribed. To overcome this problem people use preparation obtained from plants growing in their countries following folk tradition for medication [1].

Plants are therefore a source of large amount of drugs comprising of different groups such as antispasmodics, emetics, anti-cancer, antimicrobials etc. A large number of the plants are claimed to possess the antibiotic properties in the traditional medical system and are used extensively by the tribal people worldwide. Therefore, researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases based on their traditional claims of the plants given in traditional medical practice [2]. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from or molded on plant substances [3].

Plant-derived products contain a great diversity of phytochemicals such as steroids, phenolic acids, flavonoids, tannins, lignin and other small compounds [4]. These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities [5].

There are many plants that possesses potential antibacterial activity that are traditionally applied in the management of diseases caused bacterial organisms, these plants include *Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifer* and *Ziziphus mauritiana* ,Cassia occidentalis, *Moringa oleifera*, *Olea hochstetteri*, *Jatropha curcas* etc [6-8].

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Hyphaene thebaica, with common names doum palm and gingerbread tree, is a type of palm tree with edible oval fruit. It is native to the Nile valley in Egypt and Sudan and also found in West Africa and in riverine areas of Northwestern Kenya. The mesocarp is edible and may be eaten raw or ground into flour. The hard endosperm has been used in India as vegetable ivory and hand-crafted into buttons and beads [9].

Roots of doum palm are used for treatment of bilharzias while the fruit is often chewed to control hypertension and other ailments. The hard seed inside the fruit known as vegetable ivory is used to treat sore eyes in livestock using charcoal from the seed kernel as well as making buttons and small carvings and artificial pearls [10].

The aims and objective of this research is to ascertain the phytochemical content and antibacterial properties of the crude pericarp extract of *Hyphaene thebaica* on some laboratory isolates.

MATERIALS AND METHODS

Plant collection and Identification

Fresh pericarp of *Hyphaene thebaica* was bought in September 2012 from Gamboru market, Borno state, North eastern, Nigeria. The seeds were authenticated by a taxonomist at the Department of Biological Science, University of Maiduguri. Voucher specimen (No. 95) of this plant was kept in the toxicology laboratory, University of Maiduguri for reference.

Preparation of Aqueous *Hyphaene thebaica* pericarp extract

Fresh pericarp of *Hyphaene thebaica* collected were ground into fine powder and stored in a glass container. One hundred and fifty grammes of aqueous product is prepared by reflux method from three hundred and fifty grammes of initial powdered sample. The aqueous seed extract obtained was then concentrated, labeled and stored in a refrigerator at 4^{o}C.

Phytochemical Analysis of aqueous pericarp extract

The aqueous extract obtained from the pericarp of the plant was subjected to phytochemical test using standard method of Trease and Evans [11].

Test for tannins (Ferric chloride test) - Two millilitre of the aqueous solution of the extract was added to few drops of 10% Ferric chloride solution (light yellow). The occurrence of blackish blue colour shows the presence of gallic tannins and a green-blackish colour indicates presence of catechol tannins.

Test for saponins (Frothing Test) - Three millilitres (3ml) of the aqueous solution of the extract was mixed with 10ml of distilled water in a test-tube. The test-tube was stoppered and shaken vigorously for about 5 minutes, it was allowed to stand for 30 minutes and observed for honeycomb froth, which is indicative of the presence of saponins.

Test for alkaloids - One gram (1g) of the extract was dissolved in 5ml of 10% ammonia solution and extracted with fifteen millilitre of chloroform. The chloroform portion was evaporated to dryness and the resultant residue dissolved in 15 ml of dilute sulphuric acid. One quarter of the solution was used for the general alkaloid test while the remaining solution was used for specific tests.

Mayer's reagent (or Bertrand's Reagent) - Drops of Mayer's reagent was added to a portion of the acidic solution in a test tube and observed for an opalescence or yellowish precipitate indicative of the presence of alkaloids.

Dragendorff's reagent - Two millilitres (2ml) of acidic solution in the second test-tube was neutralized with 10% ammonia solution. Dragendorff's reagent was added and turbidity or precipitate was observed which was indicative of presence of alkaloids.

Tests for carbohydrate (Molisch's test) - Few drops of Molischs solution was added to 2ml of aqueous solution of the extract, thereafter a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking. The interface was observed for a purple colour, which is indicative of positive for carbohydrates.

Tests for carbohydrate (Barfoed's test) - One millilitre of aqueous solution of the extract and 1ml of Barfoed's reagent were added into a test-tube, heated in a water bath 60°C for about 2 minutes. Red precipitate shows the presence of monosaccharides.

Standard test for combined reducing sugars - One millilitre of the aqueous solution of the extract was hydrolyzed by boiling with 5 ml of dilute hydrochloric acid. This was neutralized with sodium hydroxide solution. The Fehling's test was repeated as indicated above and the tube was observed for brick-red precipitate that indicates the presence of combine reducing sugars.

Standard test for free reducing Sugar (Fehling's test) - Two millilitre of the aqueous solution of the extract in a test tube was added 5ml mixture of equal volumes of Fehling's solutions I and II and boiled in a water bath for about 2 minutes. The brick-red precipitate formed as result of reaction between aqueous pericarp extract of *Hyphaene thebaica* and Fehling solution I and II indicates the presence of reducing sugars.

Test for ketone - Two millilitre of aqueous solution of the extract was added a few crystals of resorcinol and an equal volume of concentrated hydrochloric acid, and then heated over a spirit lamp flame and observed for a rose colouration that shows presence of ketones.

Test for pentoses - Two millilitre of the aqueous solution of the extract was added an equal volume of concentrated hydrochloric acid containing little phloroglucinol. This is

heated over a spirit lamp flame and observed for red colouration, indicative of presence of pentoses.

Test for phlobatannins (Hydrochloric acid test) - Two millilitre of the aqueous solution of the extract was added dilute hydrochloric acid and observed for red precipitate that indicates presence of phlobatannins.

Test for cardiac glycosides - Two millilitre of the aqueous solution of the extract was added 3 drops of strong solution of lead acetate. This was mixed thoroughly and filtered. The filtrate was shaken with 5ml of chloroform in a separating funnel. The chloroform layer was evaporated to dryness in a small evaporating dish. The residue was dissolved in a glacial acetic acid containing a trace of ferric chloride; this was transferred to the surface of 2ml concentrated sulphuric acid in a test tube. The upper layer and interface of the two layers were observed for bluish-green and reddish-brown colouration respectively, which indicates the presence of cardiac glycosides.

Test for steroids (Liebermann-Burchard's test) - The amount of 0.5g of the extract was dissolve in 10ml anhydrous chloroform and filtered. The solution was divided into two equal portions for the following tests. The first portion of the solution above was mixed with one ml of acetic anhydride followed by the addition of 1ml of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green colouration indicative of steroids.

Test for steroids (Salkowski's test) - The second portion of solution above was mixed with concentrated sulphuric acid carefully so that the acid formed a lower layer and the interface was observed for a reddish-brown colour indicative of steroid ring.

Test for flavonoids (Shibita's reaction test) - One gram (1g) of the water extract was dissolved in methanol (50%, 1-2ml) by heating, then metal magnesium and 5-6 drops of concentrated hydrochloric acid were added. The solution when red is indicative of flavonols and orange for flavones.

Test for flavonoids (Pew's test) - To five millilitre (5ml) of the aqueous solution of the water extract was added 0.1g of metallic zinc and 8ml of concentrated sulphuric acid. The reaction mixture was observed for red colour indicative of flavonoids.

Test for anthraquinones (Borntrager's reaction for free anthraquinones) - One gram (1g) of the powdered seed was placed in a dry test tube and 20ml of chloroform was added. This was heated in steam bath for five minutes. The extract was filtered while hot and allowed to cool. To the filtrate was added equal volume of 10% ammonia solution. This was shaken and the upper aqueous layer was observed for bright pink colouration, which is an indication of the presence of anthraquinones. Control test were done by adding 10ml of 10% ammonia solution in 5ml chloroform in a test tube.

Culture Media: Nutrient agar (Tab – Lemco powder 15.0g / l, Peptone, 10.0g / l, Sodium chloride, 5.0g / l, Agar, 15g / l) and Nutrient broth Tab – Lemco powder 15.0g / l, Peptone, 10.0 g / l, Sodium chloride, 5.0 g / l (Oxoid, England) of pH 7.3 were used for the investigation.

Microbial Cultures: Laboratory isolates of *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Corynebacterium pyogenes, Klebsiella pneumoniae, Salmonella typhi, Escherichia coli, Pseudomonas aeroginosa, Proteus mirabilis and Shigella dysenteriae* were obtained from the Department of Veterinary Medicine laboratory, University of Maiduguri, Nigeria. The isolates were cultured separately on nutrient agar plate for 24 h. Twenty five (25ml) millilitre of the culture media is poured into sterile Petri dish and allowed to solidify. A colony of each test organism was sub cultured on 10 ml nutrient broth and incubated at 37^{o}C for 12 hours. One millilitre of the sub - cultured organisms was inoculated on the agar plates.

Preparation of Inocula: The inoculum size of all bacterial isolates tested was standardized by the use of overnight broth cultures prepared by inoculating isolated colonies of test bacteria in 10ml of Nutrient broth which was incubated at 35°C for 24 hours. A loopful of overnight broth culture was diluted in 4ml of sterile physiological saline (0.8% W/V), such that its turbidity marched with that of 0.5 Mac Farland standard (Barium sulphate standard) considered to have a mean bacterial density of 3.3 x 10^6 CFU /ml. This was gauged by comparing the turbidity of the test suspension with the turbidity 1% (W/V) Barium sulphate solution against the background of a printed white paper [12].

Preparation of Antimicrobial Discs: Graded concentration of 200, 400, 600, 800 and 1000 mg / ml of the extract were measured and poured into different plates. About 1ml of sterile distilled water was added to each plate containing the extract and stirred. Filter paper discs (6mm) diameter were then placed in each plate and stirred so as to ensure the impregnation of the disc by the extract. Tetracycline (250 mg / ml) as the control drug was prepared and placed at the centre of each inoculated plate.

Incubation of Bacterial Isolates: The inoculated plates containing filter paper discs (6mm) impregnated with the extract and control drug were incubated at 37°C for 20 – 24 hrs.

Determination of Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration of the crude seed extract of *Moringa oleifera* was determined using the method of Greenwood, 1989 and Eloff, 1998 as described by Geidam *et al*. Serial dilution of the extract at the concentrations of 200, 100, 50, 25 and 12.5 mg/ml respectively was used to determine minimum inhibitory concentration and recorded as the least concentration of the extract that completely inhibited the growth of the organisms.

Minimum Bactericidal Concentration (MBC): Samples were taken from test tubes used in performing MIC assay and sub-cultured unto freshly prepared nutrient agar medium and later incubated at 37°C for 24hrs. The MBC was taken as the lowest concentration of the extract that inhibits bacterial growth on the agar plates [13].

RESULTS

The phytochemical analysis of the extract revealed the presence of tannins, steroids, saponins, carbohydrates, flavonoids, terpenes and terpinoids in low and moderate concentrations as shown below.

Antibacterial activity of *Hyphaene thebaica* aqueous pericarp extract

The crude pericarp extract exhibited activity on some laboratory isolates such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi*, *Escherichia coli* and *Shigella dysenteriae* and the rest were resistant as shown below. The effect of this extract on both gram positive and negative laboratory isolates indicates broad spectrum antibacterial activity. These organisms were sensitive to all the concentrations of the extract applied except *salmonella typhi* that were resistant to 200 mg / ml applied. The zone of inhibition range is 6 – 16 mm for the isolates to the extract and 17 - 24 for tetracycline that was used as a control drug.

Minimum inhibitory concentration of *Hyphaene thebaica* aqueous pericarp extract

The minimum inhibitory concentration for *Staphylococcus aureus*, *Streptococcus pyogenes and Salmonella typhi* is 25 mg / ml and 50 mg / ml for *Escherichia coli* and *Shigella dysenteriae* respectively shown on Table 3 below.

Minimum bactericidal concentration of *Hyphaene thebaica* aqueous pericarp extract

The sensitive laboratory bacterial isolates has a minimum bactericidal concentration of 50 mg / ml shown below.

Table 1. Preliminary quantitative phytochemical analysis of the crude pericarp extract of *Hyphaene thebaica* (doumpalm)

Phytochemical constituents	Test	Inference
Tannins	1. Ferric chloride 2. Formaldehyde 3. Chlorogenic acid	+ + +
Saponins	1. Frothing	++
Alkaloids	1. Dragendorff's 2. Mayer's 3. Wagner's	- - -
Carbohydrates	1. Molisch's 2. Barfoed's 3. Combine reducing sugar 4. Free reducing sugar 5. Ketone's 6. Pentoses	+ - ++ ++ + ++
Phlobatannins	1. Hydrochloric acid	-
Cardiac glycosides	1. General test	++
Steroids	1. Lieberman's 2. Salkowski's	+ +
Flavonoids Terpenes / Terpinoids Anthraquinones	1. Shinoda's 2. Ferric Chloride 3. Lieberman- Buchard's 4. Salkowski's 5. Free Anthraquinones	++ + ++ ++ -

Key: -Absent, +low, ++ Moderate

Table 2. Antibacterial activity of the crude pericarp extract of *Hyphaene thebaica* (doumpalm)

Extract / Antibiotic	Amount of Extract &Tetracycline (mg / ml)	Zone of inhibition diameter(mm) Organisms									
		S aureus	*Strept. Pyogenes*	*B subtilis*	*Coryn. Pyogenes*	*K Pneumoniae*	*Salm typhi*	*E. coli*	*P. aeroginosa*	*Proteus mirabilis*	*Shigella dysenteriae*
Aqueous extract of *H. thebaica*	1000	11	10	R	R	R	16	10	R	R	12
	800	10	9	R	R	R	14	9	R	R	10
	600	10	8	R	R	R	12	8	R	R	7
	400	10	7	R	R	R	9	7	R	R	6
	200	9	**6**	R	R	R	R	6	R	R	6
Tetracycline	250	19	20	23	24	17	24	18	20	18	20

Key: R- Resistant

Table 3. Determination of Minimum inhibitory concentration (MIC) of *Hyphaene thebaica* crude seed extract

Organisms	Concentration of *Hyphaene thebaica* aqueous pericarp extract (mg/ml)				
	200	100	50	25	12.5
S aureus	-	-	-	-	+
Strept. Pyogenes	-	-	-	-	+
Salm. Typhi	-	-	-	-	+
E. coli	-	-	-	+	+
Shig. Dysenteriae	-	-	-	+	+

Key: + Growth observed, - Growth inhibited

Table 4. Determination of minimum bactericidal concentration (MBC) of *Hyphaene thebaica* crude seed extract

Organisms	Concentration of *Hyphaene thebaica* aqueous pericarp extract (mg/ml)				
	200	100	50	25	12.5
S aureus	-	-	-	+	+
Strept. Pyogenes	-	-	-	+	+
Salm. Typhi	-	-	-	+	+
E. coli	-	-	-	+	+
Shig. Dysenteriae	-	-	-	+	+

Key: + Growth observed - Growth inhibited.

Figure 1. Showing the picture of *Hyphaene thebaica* (doumpalm) pods at Gamboru Market Maiduguri, Borno State, Nigeria. The pericarp removed from this pods using Mortar and Pestle was used for the phytochemical content analysis and antibacterial activity screening

DISCUSSION AND CONCLUSION

In this paper, we analyzed the future of *Hyphaene thebaica* as a medicinal plant, particularly as a potential antimicrobial crude drug as well as a source for natural compounds that may act as new anti-infective agent on some microbial isolates. In the past few decades, the search for new anti-infective agents has occupied many research groups in the field of ethno pharmacology [14].

The activity of crude pericarp extract of this plant was tried *in vitro* on some bacterial isolates and revealed some level of activity on *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi*, *Escherichia coli* and *Shigella dysenteriae* indicating various zones of inhibition for *Streptococcus pyogenes* and *Shigella dysenteriae* with least zone of inhibition (6mm) and *Salmonella typhi* with highest zone of inhibition (16mm). The aqueous extracts of *Hyphaene thebaica* specie used by Amal *et al* [15] exhibited similar inhibitory effect on gram positive (*S. aureus, B. subtilis)* and gram negative (*P. aeruginosa, S. typhi*).

The variation in the antibacterial activity of the Amal *et al* [15] sample and the present sample may be as a result of variation in the amount of phytochemical contents in the two products which could result due to difference in the geographical location of where the pods were collected, extraction procedure, time of extraction, degree of processing, temperature of extraction, moisture content of the sample before extraction and its particle size [2]. Since the pericarp extract of this plant revealed the presence of flavonoids, glycosides, triterpenes and steroids as chemical compounds, this may explain some of their antimicrobial actions since antimicrobial actions of most of these phytochemical substances have been reported [16-19]. The crude pericarp extract of *Hyphaene thebaica* can therefore be used to treat conditions caused by the above species experimentally inhibited.

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