PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ANALYSIS OF HYPHAENETHEIBECA FRUIT EXTRACTS

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(Received 12th July 2024; revised 14th October 2024; accepted 22nd October 2024)

Abstract. Medicinal plants play vital roles in the control and prevention of various diseases such as cancers, diabetes, and cardiovascular diseases. These plants have been used as principal raw materials for production of various drugs due to their antimicrobial activities. The aim of this study is to carryout phytochemical screening and antibacterial analysis of Hyphane thebaica fruit extract. Extract the fruit using n-hexane, ethylacetate and ethanol. This study identifies the various chemical compounds present in the fruit extracted of hyphaenetheibeca found in Sokoto region and also evaluate it antibacterial activities agent (compound) for the possibility of using them in the development of new antibiotic to reduce cost of health care and mortality rate due to the resistance of bacteria to the already developed drugs. The fraction were tested for antibacterial activities using three bacterial strains (S.auriaus g+ve, E.coli g-ve and shagella g-ve, of the antibacterial screening that the fraction were to inhibition the growth of the bacteria with zone of inhibition that range 2-9mm standard method were adopted for both extract phytochemical and antibacterial studies. The result for the moisture content of the fruit extracted reveals that, the fruit extracted containing 94.14% of water. Cool extraction method was used to extract the fruit using three different solvent (n-hexane, ethylacetate and ethanol) the mass of the fraction obtained are 50g, 45g, and 40g with percentage yield of 5.8%, 2.2% and 2.32% for both n-hexane, ethylacetate and ethanol respectively.

Keywords: *hyphaenetheibeca, antibacterial agent, drug resistance, fruit extracts*

Introduction

Medicinal plants play vital roles in the control and prevention of various diseases such as cancers, diabetes, and cardiovascular diseases (Mohanta et al., 2003). These plants have been used as principal raw materials for production of various drugs due to their antimicrobial activities (Mohanta et al., 2003). Antimicrobial activity involves complex mechanisms such as prevention of cell membrane, cell wall formation and inhibition of nucleic acid and protein metabolism (Mohanta et al., 2003). Hyphaenethebaica is an example of such medicinal plants and belongs. To the mint family (Arecaceae). It is a desert palm fruit native to Nigeria, Senegal, Egypt, Tanzania, Kenya, Arabian Peninsula and West India (Srivastava et al., 2005). This fruit serves as source of minerals, (calcium, potassium, phosphorus, sodium, and magnesium), carbohydrate, B-complex vitamins, and fibres that are essential for good nutrition (Srivastava et al., 2005). The root is used in the treatment of bilharzia, while the fruit pulp is used in controlling hyperlipidaemia and hypertension. It's grows better in hot, arid and dry regions and can be found in the Northern part of Nigeria, such as Kano, Maiduguri. Pradhan and Tamang (2019) investigated the effect of Hyphaene thebaica, and found out that it lowered the carbohydrate metabolism, in vivo, reducing the

postprandial blood glucose. Hyphaenethebaica fruit is very cheap, and readily available therefore, it is expedient to investigate its, phytochemical constituent and inhibitory activity to some selected bacterial. This research project focuses on the phytochemical screening and antibacterial analysis of hyphaenethebaica fruit extract.

Hyphaenethebaicais commonly referred to as doum, and it is a type of palm tree with edible oval fruit which belongs to the mint family (Arecaceae). They have several vernacular names like doum palm, gingerbread palm, zembaba, mkoma, arkobkobai and kambash (Pradhan and Tamang, 2019). The doum palm is native to the northern half of Africa. It grows in the west from Mauritania and Senegal, and east to Egypt, Kenya and Tanzania. It tends to grow along the Nile River in Egypt and Sudan in the areas which contain groundwater. It is also native to the levant and the Arabian Peninsula (Israel, Sinai, Yemen and Saudi Arabia). It grows in wadis and at oases, but it is considered as drought-tolerant and sometimes grows on rocky hillsides. Also, it is very resistant to destruction by fire in scrub or a forest (Aboshora et al., 2014). The doum palm is a dioecious palm and grows up to 17 m (56 ft) high. The trunk, which can have a girth of up to 90 cm (35 in), the trunk divided into two branches, each branch divided again into two branches, and the ends of the branches contain tufts of large leaves (Ewansiha et al., 2017). The bark is smooth, dark gray and contains the scars of fallen leaves. The petioles are about 1 m long, sheathing the branch at the base and contain curved claws. The leaves are fan-shaped and measure about 120 by 180 cm (47 by 71 in). Male and female flowers are produced on separate trees (Ewansiha et al., 2017). The inflorescences are similar in general appearance, up to about 1.2 m (3 ft. 11 in) long, irregular in the branching and have two or three spikes in each branch (Ewansiha et al., 2017). Male flowers have a short-stalk, solitary in pits of the spadix, spathe-bracts encircling the spadix, pointed. Branches of female spadices become thicker in the fruiting stage. Woody fruits are produced in the female palm that continues on the tree for a long time. They are 6-10 × 6-8 cm, smooth, rectangular to cubical with rounded edges, shiny brown when ripe. Its fresh weight is about 120 g and dry weight is about 60 g and each one containing a single seed. The size of seeds is about 2-3.5 × 3 cm, the color is ivory, truncate at the base and the apex is obtuse (Bahl, 2010) (*Figure 1*).

Figure 1. *Hyphaenethebeca fruit.*

Galeotti et al. (2008) reported on the antioxidant, anticancer, antimicrobial, and anti-inflammatory activities of Hyphaenethebaica fruit due to the high amounts of phenol, and flavonoids. Hyphaenethebaica tree is one of the most useful plants in the world (Galeotti et al., 2008). Along the Nile, people used its fiber and leaflets to spin baskets. The fruits of doum palm contained antioxidants properties (Galeotti et al., 2008). Palms are used for firewood and charcoal. Leaves are probably the most important part of the

palm, providing the raw material used in basketry, making mats, brooms, coarse textiles, ropes, thatching and string (Khalif et al., 2005). Leaves may also be used as fuel. The fibers of roots obtained after soaking in water for 2-3 days, and flogging of the roots are used for making fishing nets. Due to the high amounts of fibers in the wood, it is difficult to cut them using an axe. Wood produced from the male palm is considered better than that of the female. It is often used for building, providing for support and rafters for houses, railway sleepers, planks, water ducts and wheels fence posts and raft construction. Dried bark is used to produce a black dye for leather wear (El-Olemy et al., 1994). Roots are used in the treatment of bilharzia, while fruit pulp is helped in the reduction control hypertension (El-Olemy et al., 1994). 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 (Rhen and Cidlowski, 2005). In Turkey and Kenya, the powder made from the outer covering of the fruit is added to water and milk and left to stand to make a mild alcoholic drink; in other countries, the terminal meristem is tapped for making palm wine. The thin dried brown rind is used in the manufacture of sweetmeats, cakes, and molasses. In Egypt, the fruit is sold in herbalist shops and is popular among children. Apart from the use of the fruit as food, juice is extracted from the young fruit and palm wine is prepared from the sap (Rhen and Cidlowski, 2005). Doum palm fruit in its powder form was applied in some food products as a source of fiber, stabilizer and minerals as well as for its potential healthy effect (Rhen and Cidlowski, 2005). Research on the fruit pulp of H. thebaica showed that it contains nutritional trace minerals, proteins and fatty acids, in particular the nutritionally essential linoleic acid (Salunkhe and Chavan, 1989). Also, aqueous doum palm extracts increased the viability and activity of some certain dairy starter cultures which used in the manufacture of some dairy products especially probiotics (Salunkhe and Chavan, 1989).

Materials and Methods

All solvents and chemicals used were of analytical grade and were obtained from sigma aldrich and BDH Chemicals England respectively. Nutrient Agar and Nutrient Broth were obtained from Titan Biotech L.T.D India.

Sample collection and treatment

The sample of Hyphaenethebaica fruit was bought in February, 2021 from Sokoto fish market opposite Dandima in Sokoto State, Nigeria. The fruit was washed with water to remove the dust and dried under shade for three days. The dried fruit of Hyphaenethebaica were ground using mortar and pestle then sieved to obtain fine powder. The powdered samples were stored in an air tight water free polyethene bag and used for the analysis.

Determination of moisture content

The fruit were taken in a pre-weighed empty dish (W_0) and their resulting weight was denoted by (W_1). The dish containing the fruit sample was transferred into an oven at 100 °C for 24 hours then removed, and the weight of the dish containing the dried sample was determined and denoted by (W_2). The percentage moisture content of the fruit was calculated using Eq. (1) below:

$$\%\ moisture = \frac{W_1 - W_2}{W_1 - W_0}\ X\ 100 \qquad \text{Eq. (1)}$$

Where; W_0=weight of the empty dish, W_1=weight of empty dish + sample, W_2=weight of the empty dish + dry sample.

Extraction of fruit of hyphaenetheibeca

The fruit extracted powder (50 g) was extracted by maceration in a 1000 cm^3 glass stoppered conical flask. Ethanol, ethyl acetate, n-hexane (200 cm^3) was first used to defat. The fruit extracted. The marc was allowed to dry and then extracted with ethanol. The extraction was done according to the order of increasing polarity of the solvents. In each case the bark was left in contact with the solvent for 24 hours with intermittent stirring to ensure maximum extraction. After 24 hours, the extracts were filtered using Whatman filter No.1 and the filtrates were transferred to evaporation dishes and concentrated using air circulated oven at temperature between 80-100 0C. The percentage yield of the extracts was calculated as follows (Eq. (2):

$$\%\ extract\ yield = \frac{weight\ of\ extract}{initial\ weight\ of\ sample}\ X\ 100 \qquad \text{Eq. (2)}$$

Qualitative phytochemical screening of extract

The tests for flavonoids, tannins, saponnins, steroids/triterpenoids, alkaloids, cardiac glycosides and anthraquinones were carried according to the methods described by Muhammad and Usman (2023); El-Beltagi and Mohamed (2013); Halilu et al. (2013); Hsu et al. (2006); Calixto (2000); Salunkhe and Chavan (1989) as well as Herborne (1973).

Bacteria strain

The test organisms were obtained from the Department of microbiology, Sokoto Sttate University. The microorganisms are standard laboratory strains of Staphylococcus aureus (gram +ve), Escherichia coli (gram –ve) and Shigella (gram –ve).

Antibacterial tests

The antibacterial test was conducted using the petri dish plate method described by Bhumi and Savithramma (2014). The Nutrient Agar plates prepared according to manufacturer's instruction were allowed to solidify for 15 minutes at room temperature and incubated without inoculum for 24 hours at 37 °C to ensure the sterility of the medium. The Nutrient Agar plates were flooded with 1 ml of the inoculum and the excess was removed using Pasteur pipette. Five wells (cups) of about 6 mm in diameter were cut on each Nutrient Agar plate using a sterile cork borer and the agar plugs were removed using sterile ampoule file. The extract solution (0.1 mL) was placed in each of the wells and were allowed to settle for two hours at room temperature and then incubated for 24 hours at 37 °C. The inhibition zone was observed and then recorded in millimeters using a transparent ruler. Standard antibiotic was used.

Minimum Inhibitory Concentration (MIC)

This was carried out as described by Usman and Osuji (2007). Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration where no visible turbidity would be observed in the test tubes. The MIC was determined for the micro-organisms were prepared using the broth dilution technique the stock solution (20 mg1ml) of the fruit extract was prepared by dissolving 0.2g of the fruit extract in 10 ml DMSO four concentration were prepared (0.25mlmL, 0.50mglmL, 1.00mglmL, and 2.00 mglmL) from the stock solution using serial dilution technique and later inoculated with 0.2ml, suspension of the test organism, After 24 hours incubation at 37°C, the tubes were observed for turbidity. The lowest concentration where no turbidity was observed was determine and noted as the Minimum Inhibitory Concentration (MIC).

Minimum Bactericidal Concentration (MBC)

The minimal bactericidal concentration was determined from broth dilution test resulting from the MIC tubes as described previously by inoculating the content of each test tube on a nutrient agar plate. The plates were then incubated at 37°C for 24 hours. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration (Usman and Osuji, 2007).

Results and Discussion

Table 1 and *Table 2* give the details of the percentage moisture content and percentage yield and mass of the fractions obtained from Hyphaene thebaica fruit extract respectively. The fruit had a percentage moisture content of 94.14% and the mass of the faction of 2.93 g, 1.00 g and 0.93 g with percentage yield of 5.86%, 2.2%, and 2.32% for n-hexane, ethyl acetate and ethanol respectively. These indicate that n-hexane fraction contains more of the extract than others.

Table 1. *Percentage moisture content of Hyphaenethebaica bark (%).*

S/N	Weight of empty dish (W0)	Weight of empty dish + sample (W1)	Weight of empty dish + dry sample	Moisture content (%)
1	11.00	61.00	13.93	94.14

Table 2. *Mass of Fractions (g) and Percentage Yield of Hyphaenethebaica fruit extracts.*

Extract	Original mass (g)	Mass (g) of extract	Percentage yield (%)
N-hexane	50.0	2.93	5.86
Ethyl acetate	45.00	1.00	2.2
Ethanol	40.00	0.93	2.32

Qualitative phytochemical screening of hyphaenetheibeca fruit extract

Table 3 show the results of the qualitative phytochemical screening of the fractions obtained. Anthraquinones, and cardiac glycloside, were found present in all the factions, while flavonoids were only detected in ethyl acetate, and ethanol fractions. Alkaloids were only detected in n-hexane fraction and also Tannins were only detected in ethnolic fraction. No steroids were detected in the entire three fractions

Table 3. *Qualitative phytochemical screening of hyphaenethebaica bark extracts.*

Phytochemical	N-hexane	Ethyl acetate	Ethanol
Alkaloids			
Mayer's test	+	-	-

Wagner's test	+	-	-
Dragendorff's	+	-	-
Flavanoids			
Sodium hydroxide test	-	+	+
Iron (III) chloride test	-	+	+
Shinoda's test	-	+	+
Tannins			
Iron (III) chloride test	-	-	+
Lead acetate	-	-	+
Saponins			
Foam test	-	-	-
Anthraquinones			
Borntrager's test	+	+	+
Cardiac glycosides			
Keller-killiani's test	+	+	+
Steroids			
Salkowski's	-	-	-
Lieberman-Buchard's	-	-	-

Note: (+) means detected; (-) means non-detected.

Antibacterial activity of hyphaenetheibeca

Table 4(a) to *Table 4(c)* show the details of the results obtained from of the antibacterial activity test of the n-hexane, ethyl acetate and ethanol fractions of Hyphaenethebaica. The results deduced that all the three fractions has activity on bacterial strain with zone of inhibition ranging between 2-9 mm.

Table 4(a). *Antibacterial activity of the fraction of hyphaenethebaica*.

Concentration (mg/ml)	S. aurens	E. coli	Shagella
n-Hexane			
0.25	8.00	7.00	0.00
0.50	6.00	6.00	2.00
1.00	3.00	5.00	2.00
2.00	2.00	4.00	3.00
Ethyl acetate			
0.25	9.00	3.00	10.00
0.50	8.00	2.00	9.00
1.00	7.00	2.00	8.00
2.00	6.00	0.00	7.00
Ethanol			
0.25	2.00	6.00	5.00
0.50	2.00	5.00	4.00
1.00	0.00	4.00	3.00
2.00	0.00	0.00	2.00

Table 4(b). *Antibacterial activity of standard antibiotic (Amoxicillin).*

Antibiotic concentration	S. aureus	E. coli	Shigella
Amoxicillin (125 mg/ml)	12.00	10.00	10.00

Table 4(c). *Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the fraction of hyphaenethebaica fruit.*

Test	MIC (mg/ml)	MBC (mg/ml)

Ethanol		
S. aureus	0.50	1.00
E. coli	0.25	0.25
Shigella	0.25	1.00
n-hexane		
S. aureus	1.00	2.00
E. coli	0.50	1.00
Shigella	0.25	1.00
Ethylacetate		
S. aureus	0.25	1.00
E. coli	1.00	2.00
Shigella	0.50	1.00

Base on the results obtained from the extraction of hyphaenetheibeca fruit. *Table 1* shows that the percentage moisture content is 94.14% and table 3.2 revealed that n-hexane, ethyl acetate and ethanol had a mass fraction of 2.93, 1.00 and 0.93 with percentage yield 5.86%, 2.2% and 2.32% respectively. Polar compound can only be extracted by polar solvent, example ethanol (Vandenbeldt et l., 1992). While non-polar compound can only be extracted by non polar solvent example haxane. The results of the extraction shows that hyphaenetheibeca consist of more non polar compound than polar ones. The phytochemical screening of the hyphaenetheibeca fruit extract revealed the presence of anthraquinones cardiac glycoside. In all the three fractions and tannins, while saponins and steroid were not detected (as shown in *Table 3*). while flovonids is detected in Ethyl acetate and ethanol fraction only and alkaloid is present only in n-hexane fraction and the saponins are present in ethanol only while none was detected in steroid and tannins in all of the three fractions (Vandenbeldt et l., 1992). These classes of secondary metabolites are known to be responsible for the antibacterial activities demonstrated by plant extracts (Vandenbeldt et l., 1992). Alkaloids, saponins have been reported to exhibit antimicrobial properties (Vandenbeldt et l., 1992). The chloroform extract exhibited good antibacterial activity on all the test organisms with zones of inhibition ranging between 2-9 mm (*Table 4*(*a*). The n-hexane fraction showed a high level of activity on gram positive bacteria than gram negative bacteria, but all the organisms were found to be susceptible.

The demonstrated activity shown by the fractions may be due to the presence of saponins and alkaloids (as shown in *Table 3*) that are known to have some antibacterial activity as reported by Zhao and Yu (2016). This results when compared with Amoxicillin (Standard Antibiotic as in *Table 4*(*b*), the zone of inhibition produced by the antibiotic against the test organisms was found to be appreciable in relation to those activities produced by the extracts for S-aureus, E-coli and Shigella under study. However, according to Bhumi and Savithramma (2014), diameter of zones of inhibition ≥10mm is considered active. Also from these results, it can be deduced that the n-hexane fraction is bacteriocidal on S-aureus, E-coli, and Shigella at concentration of 2.0mg/mL, 1.0mg/mL, and 1.0mg/mL respectively while ethyl acetate fractions shows concentration of 1.0mg/mL, 2.0mg/mL and 1.0mg/mL for S-aureus, E-coli, and Shigella respectively. Also ethanol fractions are bactericidal on all the organisms at concentration of 1.0 mg/mL, 0.25mg/mL and 1.00mg/mL for S-aureus, E-coli, and Shigella. In view of the above results, all the extracts showed considerable activity against S. aureus, a gram positive bacterium known to play a significant role in invasive skin diseases and also on E-coli (gram-ve) and Shigella (gram-ve) (Zhao and Yu, 2016).

Conclusion

Base on the discussion it can be conclude that anthraquinones, cardiac glycoside extract produced more of the phytochemicals in all the three fraction. Similarly the plants contain alkaloide, cardiacglycoside, anthraquinones, flavonoids and tannins but not saponin and steroid. This suggest the production for the use of the plant for the development of new drugs. The fruit shows strong antibacterial activity against Staphyloccus (gram +ve), thus if properly hamessed it can contribute through the reduce of cost of health care and mortality rate due to resistance of bacterial to the already manufactured drugs.

Acknowledgement

This research is self-funded.

Conflict of interest

The authors confirm that there is no conflict of interest involve with any parties in this research study.

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