Original Research Article

Screening of methanol extract for antimicrobial activity of *Hyphaene* thebaica L. fruit pulp from Sudanese folklore

Abstract

Aim: To evaluate the antimicrobial potential of the methanol extract of the fruit pulp of *Hyphaene thebaica* (Doum), a famous natural product in Sudan.

Methods: Agar-well diffusion test and minimum inhibition diffusion test (MIC).

Results: The investigation revealed that the extract has no antifungal effect against *Aspergillus niger* ATCC 6275 and *Candida albicans* ATCC 10231. While it shows remarkable antibacterial activity against *Staphylococcus aureus* ATCC BAA 1026 and *Pseudomonas aeruginosa* ATCC 10145. Whereas, weak antibacterial activity was recorded with *Bacillus cerius* ATCC 10876 and *Escherichia coli* ATCC 1637**.**

Conclusion: The fruit pulp of *Hyphaene thebaica* (Doum) have good antibacterial activity against some Gram-positive and Gram-negative bacteria. Accordingly, the consumption of this fruit would exert several beneficial effects by virtue of its antibacterial activity and further studies using different bacterial strains and clinical pathogens are recommended.

Keywords: Doum fruit, *Hyphaene thebaica*, antibacterial, antifungal, methanol extract

1.INTRODUCTION

The Western food culture that has spread to most parts of the world, led to excessive consumption of modern Western diet and fast food which are characterized by richness in saturated fatty acids, gluten, too much salt, high content of refined sugar and poor in natural bioactive ingredients has contributed to the spread of chronic diseases and immunodeficiency [1]. Fortunately, research efforts revealed that healthy food can provide various therapeutic benefits, promote health and prevent diseases, on this basis the term 'functional food' has been arisen, which defined as a diet that may have a particular health promotion or diseases prevention attribute, although this term was known in ancient medicine [2]. Accordingly, functional food is simply a diet rich in vegetables, whole grains, fruits, edible seeds, natural products and commensal

microorganisms. In the last decades, the world faced challenge of emerging and re-emerging infectious diseases and scientific communities constantly warn of the growing phenomenon of resistance to antimicrobial agents which has become a major source of morbidity and mortality worldwide [3]. Therefore, consuming "functional foods" may be of great benefit to tackle these health threats.

The fruit pulp of Doum-palm (*Hyphaene thebaica* L.), is well known of its therapeutic benefits since Pharaonic civilization (Covered vast areas of Egypt and Northern Sudan). Doum was mentioned in the papyrus and the tree appeared in many tomb paintings [4]. The local name "Doum" is well known in Sudan and Egypt. In Sudan, Doum-palm are grown in the Northern, central and western regions; The popular edible Doum pulps are sold in Sudan as dry fruits or crushed into powder, macerated in water and then consumed as a beverage and sometimes mixed with other fruits macerates, it is also prescribed for gastrointestinal disorders like diarrhea and other parts of the tree are also used in many different purposes [5]. Globally, Doum-palm is distributed along the Nile valley, sub-Saharan Africa, and Western India [6,7] (Figure 1).

Fig. 1. Doum-palm (*Hyphaene thebaica* L.). Source: [5]

In literature, studies showed that Doum fruit have good nutritional and pharmaceutical properties; it contains carbohydrates, fibers, essential minerals, vitamin B-complex substantial for good nutrition, in addition to good amounts of phenolic compounds which are important in regulate various physiological processes in human body [8, 9]. Studies on Doum fruits have revealed a wealth of curative agents; it was found that Doum fruit is a good source of potent antioxidants and anticancer compounds [10, 11], anti-inflammatory, anti-proliferative and anti-microbial activity [12].

Few scientific investigations were done concerning the biological activities of Sudanese Doum. Accordingly, the current study aimed to evaluate the antimicrobial capacity of Doum-palm fruit (*Hyphaene thebaica* L.), a famous natural product from Sudan, to be used as possible antimicrobial dietary supplement, especially in light of the growing phenomena of antibiotics-resistant pathogens in African countries.

2.MATERIALS AND METHODS

2.1 Plant material and extraction

Dry fruits of *Hyphaene thebaica* (Doum) were purchased from local markets in Khartoum, Sudan (Figure 2). After identification by plant taxonomists, wooden fruits were washed using distilled water and dried with towels. The husk of the fruit was crushed using mortar and pestle and then ground to a fine powder using a mechanical grinder. 100 grams of Doum fruit powder were macerated in 500 ml of 80% methanol (v/v) using a well-tighten dark bottle and kept at room temperature (25-35 ^{o}C) for up to 3 days. Then, the macerate was filtered twice using Whatman filter paper No.1, the filtered solution was kept in an incubator at 40 ^{o}C for up to 5 days to evaporate the

solvent and getting dry extract. At the experimental phase, the crude dry extract was reconstituted in 80%methanol (v/v) to make 500 mg/ml.

Fig. 2. Fruits of *Hyphaene thebaica* L. (Doum)

2.2 Microorganisms

Four referenced bacterial strains representing different Gram-positive and Gram-negative bacteria, and two referenced fungal strains were used in this study. The tested Gram-positive bacteria were *Staphylococcus aureus* ATCC BAA 1026 and *Bacillus cereus* ATCC 10876. While the tested Gram-negative bacteria were *Pseudomonas aerugenosa* ATCC 10145 and *Escherichia coli* ATCC 1637. Fungal strains were *Aspergillus niger* ATCC 6275 and *Candida albicans* ATCC 10231.

2.3 Agar well diffusion test

Agar well diffusion test was used to evaluate the possible antimicrobial activity of Doum fruits methanol extract [13]. Prior to the experimental phase, microorganisms were subcultured in broth medium and incubated overnight for bacteria or 48 hours for fungi to get microbes at exponential phase. Sterile Petri-dishes containing either

Nutrient agar or Sabouraud dextrose agar were prepared, by pouring 25 ml of the autoclaved medium on a sterile Petri-dish (90 mm in diameter) and left to solidify at room temperature. After that, microorganisms were spread over the agar medium using a sterile cotton swap. Then, three wells were digged separately on each Petri-dish using a sterile cork borer (6 mm in diameter). Then, 100 µl from Doum methanol extract (500 mg/ml) was dropped into two wells and 100 µl from referenced antibacterial drug (Chloramphenicol 2.5 mg/ml) or referenced antifungal drug (Clotrimazole 5 mg/ml) were dropped into the third well. Plates seeded with bacteria or fungi were then incubated for 18-24 hours, 35 ^{o}C for bacteria or for 48 hours, 35 ^{o}C for Fungi. After that, plates were inspected and The antimicrobial activities of the tested extracts were determined by measuring the clear zone of inhibition in millimeter (mm).

2.4 Determination of minimum inhibitory concentration (MIC)

All tested microorganisms exhibited susceptibility to Doum methanol extract at a concentration of 500 mg/ml (w/v) were subjected to minimum inhibitory concentration test (MIC) using agar-well diffusion method [14]. Two-fold serial dilution were prepared to get different concentrations 250, 125, 62.5, 31.25, and 15.62 mg/ml. 1 ml of each prepared inoculum of tested bacteria was spread over a sterile Nutrient agar Petri-dish using sterile wooden swap, followed by making five wells on each plate. Then, serial dilutions were transferred, one by one, to the respective wells. Plated were then incubated at 35^{o}C for 18 hours (for bacteria) for up to 48 hours (for fungi). The

as the lowest concentration which inhibited the growth of the respective microorganisms was considered as MIC.

2.6 Statistical analysis

3. RESULTS

Table 1 shows the antimicrobial activity of methanol extract of Doum pulp fruit. Presence of clear zones of inhibition produced by the methanolic extract on the test microorganisms were considered positive results and recorded (Figure 3). The yeast (*Candida albicans* ATCC 10231) and the fungi (*Aspergillus niger* ATCC 6275) showed no susceptibility against the tested Doum extract. While, varied degrees of bacterial susceptibility were observed. The highest inhibition zones were recorded with *Pseudomonas aerugenosa* ATCC 10145 (18.5±0.5 mm), followed by *Staphylococcus aureus* ATCC BAA 1026 (16.0±1.0 mm) and *Bacillus cereus* ATCC 10876. The least inhibition zone was recorded with *Escherichia coli* ATCC 1637. This result was remarkable when compared with the referenced antibiotic (Figure 4).

Table 2 displays the minimum inhibitory concentration (MIC) of methanol extract of Doum pulp fruit for bacteria which represented remarkable susceptibility. The MIC values were 62.5 mg/ml for *Staphylococcus aureus* ATCC BAA 1026 and 125 mg/ml for *Pseudomonas aeruginosa* ATCC 10145.

Table 1. Antimicrobial activity of Doum fruit, methanol extract.

Tested compound	Zone of inhibition (mm)					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	Sa	Bc	Pa	Ec	Cand	Asp
Doum methanol extract (500mg/ml)	16.0±1.0	9.0±0.0	18.5±0.5	7.5±0.5	-ve	-ve
Chloramphenicol (2.5 mg/ml)	32.0	34.0	19.0	31.0	-	
Clotrimazole	-	-	-	-	38	32

(5 mg/ml)						

Sa= *Staphylococcus aureus* ATCC BAA 1026, Bc= *Bacillus cereus* ATCC 10876, Pa= *Pseudomonas aerugenosa* ATCC 10145, Ec= *Escherichia coli* ATCC 1637, Asp= *Aspergillus niger* ATCC 6275, Cand= *Candida albicans* ATCC 10231.

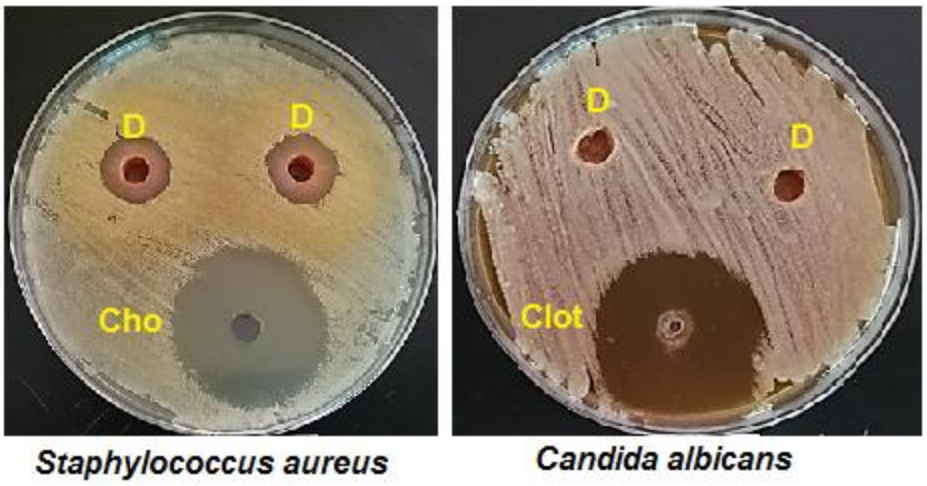

Fig.3. Representative photo of positive antibacterial activity and negative antifungal activity.
D=Doum methanol extract, Cho= Chloramphenicol, Clot= Clotrimazole

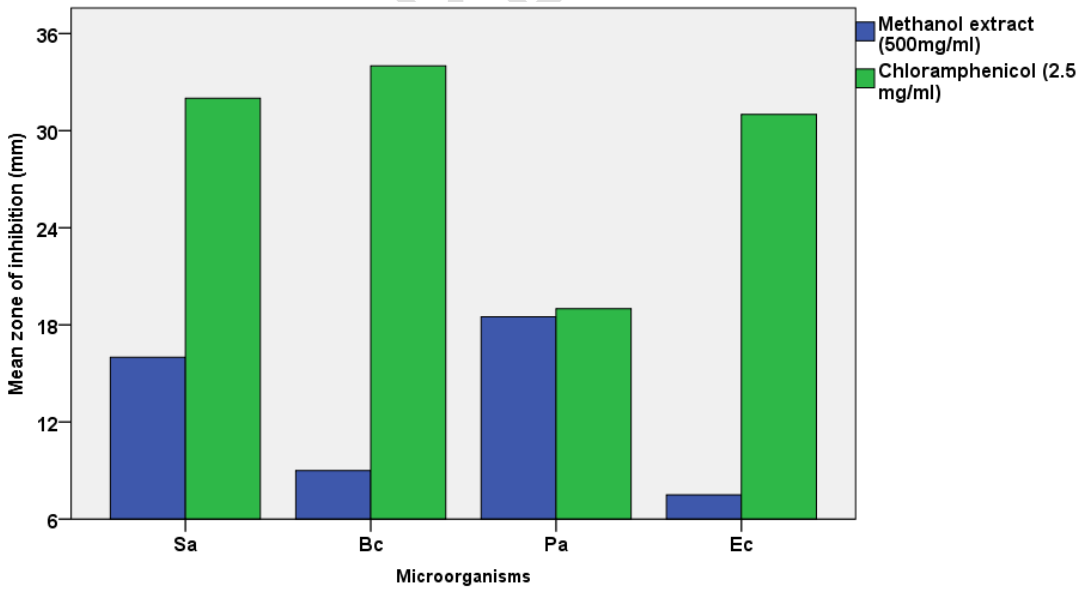

Fig.4. Antibacterial activity of Doum fruit, methanol extract.

Table 2. Minimum inhibitory concentration (MIC) of Doum fruit, methanol extract

Microorganism	MIC (mg/ml)
Staphylococcus aureus ATCC BAA 1026	62.5
Pseudomonas aeruginosa ATCC 10145	125

4.DISCUSSION

Overall, the methanol extract of Doum fruit was tested against two Gram-positive and two Gram-negative bacterial and two fungal strains. Only, one Gram-positive and one Gram-negative bacteria exhibited high antibacterial susceptibility, other bacteria showed weak results, and both fungal strains revealed no effect against tested extract. Accordingly, the methanol crude extract has a remarkable activity against some gram-positive and gram-negative bacteria. These findings are in agreement with several earlier studies. Aboshora et al [8] reported that methanol and ethanol extracts of Doum have recorded strong antibacterial activity against *Staphylococcus aureus* (Gram-positive) and *Salmonella typhi* (Gram-negative). Auwal et al [15] stated that the crude pericarp extract of Doum fruit exhibited broad spectrum antibacterial activity *Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi, Escherichia coli* and *Shigella dysenteriae*. Abd-ELmageed et al. [16] mentioned that the ethanol extract of Doum fruit represented good different degrees of antibacterial activity against *Escherichia coli, Salmonella typhimurium, Proteus mirabilis, Salmonella entrica , Shigella dysenteriae , Staphylococcus aureus, Bacillus cereus* and *Bacillus licheniformis.* On the other side, the current study disagrees with the results of Mohamed et al [17] which showed that methanol extract of Doum have antifungal potential in addition to its antibacterial activity. Hence, more future study on different extracts should be carried out. Moreover, in the current investigation,

MIC values showed that *Staphylococcus aureus* ATCC BAA1026 was the most susceptible bacteria towards Doum extract, because a lower MIC value indicates that less of the antibacterial agent is required in order to inhibit growth of the bacteria, whereas, well-diffusion test revealed that Pseudomonas aeruginosa recorded inhibition zone higher than *Staphylococcus aureus*. This could be attributed to the complicated structure of the outer membrane of *Pseudomonas aeruginosa* as a Gram-negative bacteria and the nature of the antimicrobial compound present in the crude extract of Doum, meaning that it is important to investigate and isolate the antibacterial molecule(s) from the crude. In general, the gram-negative bacteria are among the most resistant bacteria to antibiotics, although it has a thin layer membrane. Gram-negative bacteria are encompassed with a thin peptidoglycan cell wall, which itself is enclitic by an outer membrane consists of lipopolysaccharide, while Gram-positive bacteria lack that outer membrane but are surrounded by thicker layers of peptidoglycan [18]. Accordingly, to understand the mechanism of this bacterial susceptibility, isolation of the bioactive agents is recommended. Interestingly, the biosynthesis of silver nanoparticles (AgNps) from the aqueous extract of Doum fruit showed were found to inhibit 99% growth of both *Staphylococcus aureus* and *Escherichia coli* after 24 h of incubation [19]. Therefore, lots of researchers believe that natural products, functional foods and medicinal plants could be a future promising source for new antibacterial agents to tackle the growing threat of multi-drug resistant bacteria [20].

5.CONCLUSION

The widespread use of functional foods and natural products for health purposes has increased dramatically due to their great importance in disease prevention and health

promotion. The fruit pulp of Doum-palm (*Hyphaene thebaica* L.) is widely sold in various areas of Sudan, it is part of Sudanese folklore particularly in rural areas where consumed as a native snack food and beverage. This fruit showed a remarkable broad spectrum antibacterial activity. Therefore, Doum frtuit is recommended for further deep investigation, including fractionation, separation and purification of these bioactive antibacterial compounds and examine the extract against clinical bacterial isolates, for possible use in future. On the other side, such studies highlight the importance and possible curative effects when consume some functional foods during illness, particularly against infectious diseases.

CONSENT AND ETHICAL APPROVAL
Not applicable

REFERENCES

1. Myles, I A (2014). Fast food fever: reviewing the impacts of the Western diet on immunity. Nutrition Journal, 13:61
2. Choudhary R, Tandon RV (2009). Consumption of functional food and our health concerns. Pakistan Journal of Physiology, 5(1): 76-83.
3. Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol. 2018;4(3):482-501. Published 2018 Jun 26. doi:10.3934/microbiol.2018.3.482
4. Ismail AAE, Mohamed SMM, El-Weshahy MHA (2016). Doum-palm in ancient Egypt. Journal of Association of Arab Universities for Tourism and Hospitality, 13(1):1-20.
5. Abdel-Raman NA (2019). Hyphaene thebaica (Doum): Distribution,Composition and Utilization. In: A. A. Mariod (ed.), Wild Fruits: Composition, Nutritional Value and Products, Springer Nature Switzerland AG 2019, p.427-435.
6. McKenna A (2012). Doum nut. Encyclopædia Britannica, https://www.britannica.com/topic/doum-nut [Accessed date: 17/1/2021].
7. Hsu B, Coupar IM, NgK. Antioxidant activity of hot water extracts from the fruit of the Doum palm, (Hyphaene thebaica). Food hem. 2006; 98(2): 317-328.
8. Aboshora W, Lianfu Z, Mohammed Dahir M, Gasmalla MAA, Musa A, Omer E , Thapa M (2014). Physicochemical, Nutritional and Functional Properties of the Epicarp, Flesh and Pitted Sample of Doum Fruit (Hyphaene Thebaica). Journal of Food and Nutrition Research, 2014, Vol. 2, No. 4, 180-186.

9. Aamer RA (2015). Physicohemical Properties of Doum (Hyphaene thebaica) Fruits and Utilization of its Flour in Formulating Some Functional Foods. Alexandria Journal of Food Science and Technology, Vol. 12, No. 2, pp. 29-39.
10. Hsu B, Coupar IM, Ng K (2006) Antioxidant activity of hot water extract from the fruit of the doum palm, hyphaene thebaica. Food Chem 98(2):317–328.
11. Faten, MAE. Antioxidant and anticancer activities of doum fruit extract (Hyphaene thebaica). Afr. J. Pure Appl. Chem. 2009; 3(10): 197-201.
12. El-Beltagi HS, Mohamed HI, Yousef HN, Fawzi EM (2018). Biological Activities of the Doum Palm (Hyphaene thebaica L.) Extract and Its Bioactive Components. In: Shalaby E, Azzam M (ed.): Antioxidants in Foods and Its Applications, IntechOpen, p.49-66.
13. Abdallah EM, Qureshi KA, Ali AMH, Elhassan GO (2017). Evaluation of some biological properties of Saussurea costus crude root extract, Biosci. Biotech. Res. Comm. 10(4): 601-611.
14. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M and Hatab SR (2018) Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. Frontiers in Microbiology, 9:1639. doi: 10.3389/fmicb.2018.01639
15. Auwal MS, Mairiga IA, Shuaibu A, Ibrahim A, Gulani IA, Wampana B, G. Lateefat I, Lawan FA, Sanda KA, Thaluvwa AB, Njobdi AN, Yagana KZ (2013). Preliminary phytochemical and in vitro antibacterial evaluation of the crude pericarp extract of Hyphaene thebaica (doumpalm). Journal of Medicinal plant and Herbal Therapy Research, 1:1-7.
16. Abd-ELmageed SM, Abushady HM, Amin AA (2019). Antibacterial and antioxidant activities of Physalis peruviana and Hyphaene thebaica extracts. African J. Biol. Sci., 15 (1): 73-86.
17. Mohamed AA, Khalil AA, El-Beltagi HES (2010). Antioxidant and antimicrobial properties of kaff maryam(Anastatica hierochuntica) and doum palm (Hyphaenethebaica). GRASASYACEITES, 61 (1), 67-75.
18. Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. Cold Spring Harb Perspect Biol. 2010;2(5):a000414. doi:10.1101/cshperspect.a000414
19. Bello AB, Khan SA, Khan JA, Syed FQ, Mirza MB, Shah L, Khan SB (2017). Anticancer, antibacterial and pollutant degradation potential of silver nanoparticles from Hyphaene thebaica, Biochemical and Biophysical Research Communications,490(3): 889-894.
20. Abdallah EM (2011). Plants: An alternative source for antimicrobials. J. Appl. Pharma. Sci., 1(6): 16-20.