Original Research Article

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

Abstract

Aim: To evaluate the antimicrobial potential of the methanolic 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 showed no antifungal effect against *Aspergillus niger* ATCC 6275 and *Candida albicans* ATCC 10231. While it exhibited remarkable antibacterial activity against *Staphylococcus aureus* ATCC BAA 1026 and *Pseudomonas aeruginosa* ATCC 10145. Whereas, weak antibacterial activity was recorded with *Bacillus cereus* ATCC 10876 and *Escherichia coli* ATCC 1637**.**

Conclusion: The fruit pulp of *Hyphaene thebaica* (Doum) has 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, methanolic 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 the 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 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 the 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 for 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-palms 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].

In literature, studies showed that Doum fruit has 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 regulating various physiological processes in the 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.

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 1). 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% methanolic (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 evaporated at 40 ^{o}C for disposal of the solvent and getting dry extract. During the experimental phase, the dry crude extract was reconstituted in 80% methanol (v/v), and 500 mg/ml was used as the tested extract.

Fig. 1. 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 aeruginosa* ATCC 10145 and *Escherichia coli* ATCC 1637. Fungal strains were *Aspergillus niger* ATCC 6275 and *Candida albicans* ATCC 10231.

2.3 Agar well diffusion method

Agar well diffusion method was used to evaluate the possible antimicrobial activity of Doum fruits methanolic extract [13]. Prior to the experimental phase, microorganisms were subcultured in a broth medium and incubated overnight for bacteria and for 48 hours for fungi to get microbes at the exponential phase. Two sets of sterile Petri-dishes containing either Nutrient agar and 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 swab. Then, three wells were made separately on each Petri-dish using a sterile cork borer (6 mm in diameter). Then, 100 µl from Doum methanolic extract (500 mg/ml) was dropped into two wells and 100 µl from referenced Chloramphenicol 2.5 mg/ml (for bacterial Petri-dishes) and referenced Clotrimazole 5 mg/ml (for fungal Petri-dishes) were dropped into the third well, Chloramphenicol was served as a referenced antibacterial drug and Clotrimazole was served as a referenced antifungal drug. The pre-experimental tests showed that the solvent (80% methanol) have no inhibitory effect on the tested microorganisms. Plates

seeded with bacteria and fungi were then incubated for 18-24 hours, 35 ^{o}C for bacteria and 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 millimeters (mm).

2.4 Determination of minimum inhibitory concentration (MIC)

Microorganisms that exhibited susceptibility to Doum methanolic extract at a concentration of 500 mg/ml (w/v) were subjected to minimum inhibitory concentration (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 MIC was considered as the lowest concentration which inhibited the growth of the respective microorganisms.

2.6 Statistical analysis

The results of this analysis are listed as mean ± SEM (Standard Error of Means). One-way Analysis of Variance (ANOVA) at ($p \leq 0.05$) was used to find out the significant differences among the tested microorganisms. SPSS software 14.0 (SPSS Inc., Chicago, USA) was utilized for statistical analysis.

3. RESULTS

The antibacterial potential of Doum fruit pulp was evaluated using well plate diffusion method, Table 1 shows the antimicrobial activity of methanolic extract of Doum pulp fruit. The presence of clear zones of inhibition produced by the methanolic extract on the tested microorganisms was considered positive result and recorded (Figure 2). 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 was observed. The highest inhibition zones were recorded with *Pseudomonas aeruginosa* ATCC 10145 (18.5±0.5 mm), followed by *Staphylococcus aureus* ATCC BAA 1026 (16.0±1.0 mm) and *Bacillus cereus* ATCC 10876, Those two organisms exhibited significant antibacterial activity at ($p \leq 0.05$). The least inhibition zone was recorded with *Escherichia coli* ATCC 1637. This result was remarkable when compared with the referenced antibiotic. Both of *Escherichia coli* and *Bacillus cereus* were non-significant at ($p \leq 0.05$).

Only organisms that showed significant antimicrobial activity were subjected to MIC test in order to determine the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, Table 2 displays the minimum inhibitory concentration (MIC) of methanolic 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. The lowest concentration of Doum methanolic extract needed to kill *Staphylococcus aureus* is 62.5 mg/ml. Whereas, the MIC of *Pseudomonas aeruginosa* was the higher than *Staphylococcus aureus*, and showed also the highest zone of inhibition with the well-diffusion method, meaning that the susceptibility of this bacterium is does-dependent.

Table 1. Antimicrobial activity of the methanolic extract of Doum fruit

Tested compound	Zone of inhibition (mm)					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	Sa	Bc	Pa	Ec	Cand	Asp
Doum methanolic 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 (5 mg/ml)	-	-	-	-	38	32

Sa= *Staphylococcus aureus* ATCC BAA 1026, Bc= *Bacillus cereus* ATCC 10876, Pa= *Pseudomonas aeruginosa* ATCC 10145, Ec= *Escherichia coli* ATCC 1637, Asp= *Aspergillus niger* ATCC 6275, Cand= *Candida albicans* ATCC 10231. * Significant ($p \leq 0.05$).

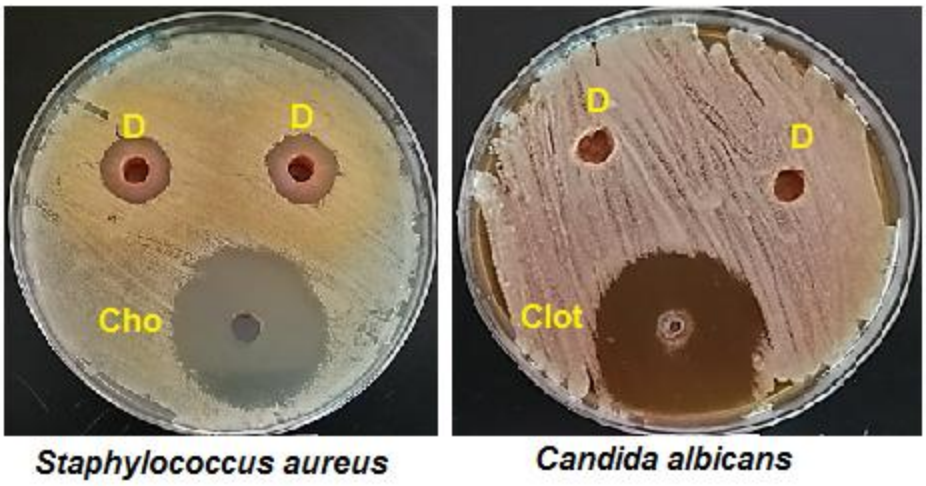

Fig.2. Representative photo of positive antibacterial activity and negative antifungal activity.
D=Doum methanolic extract, Cho= Chloramphenicol, Clot= Clotrimazole

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

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

4.DISCUSSION

There are growing scientific clues in the impact of geographical distributions and climate factors on the phytochemical constituents and biological activities of plants [15]. Information regarding the phytochemical properties of the Sudanese Doum is scant. However, it was reported that it is rich in polyphenols and flavonoids [16].

Overall, the methanolic 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 methanolic 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 methanolic and ethanol extracts of Doum have recorded strong antibacterial activity against *Staphylococcus aureus* (Gram-positive) and *Salmonella typhi* (Gram-negative). Auwal et al [17] 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. [18] 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 [19] which showed that methanolic extract of Doum has antifungal potential in addition to its antibacterial activity. Hence, more future studies 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 the growth of the bacteria, whereas, well diffusion method revealed that *Pseudomonas aeruginosa* recorded inhibition zone higher than *Staphylococcus aureus*. This could be attributed to the complicated structure of the outer membrane of the Gram-negative bacteria (*Pseudomonas aeruginosa*), 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 [20]. 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 [21]. 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 [22].

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 fruit 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 the future.

CONSENT AND ETHICAL APPROVAL

Not applicable

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