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Original article

THE EFFICACY OF DATE FRUITS (*Phoenix dactylifera*) EXTRACTS ON SOME CLINICAL ISOLATES

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ABSTRACT

Date fruit (*Phoenix dactylifera*) are an excellent source of vitamin C and dietary fibers. They also supply vitamin A and B, different minerals and various amino acids. The antioxidants present in dates can also aid in lowering the risk of cancer and cardiovascular conditions, while ensuring a healthy immune system. In this study, the antibacterial activity of ethanol and methanol extract of *Phoenix dactylifera* fruits against selected clinical isolates; *Salmonella typhi, Shigella dysenteriae, Escherichia coli* and *Klebsiella pneumoniae* was examined. Date fruit were collected and extracted by maceration and the antibacterial activity was determined by agar well diffusion and tube dilution methods. Both extracts were found to be effective in inhibiting the growth of test bacteria in a concentration dependent manner, with the highest activity against *S. dysentariae* (19.30 $\pm$ 0.24mg/ml) and *E. coli* (10.00 $\pm$ 0.00mg/ml) at 150mg/ml while no activity was recorded at 40mg/ml. Methanol extract at all concentration has no activity against *K. pneumoniae*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results revealed that the ethanol and methanol extracts of *P. dactylifera* has bacteriostatic and bactericidal effect against *S. typhi*, *S. dysenteriae*, *E. coli* and *K. pneumoniae*. Further pharmacological investigation is recommended.

Key words: Antibacterial, effective, *Phoenix dactylifera*, MIC, MBC

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INTRODUCTION

The World Health organization (WHO) defines a medicinal plant as any plant in which some or all of its part can be used directly in the management of a disease [1]. From the earliest years of human history, many plant extracts whose formulations were derived by instinct, trial and error have been used to combat various human diseases [2]. Medicinal plants represent a consistent part of the natural biodiversity endowment of many countries in Africa [3]. Also, some of the pharmaceuticals currently available to physicians are derived from plants that have a long history of use as herbal remedies. An example is aspirin which contains the active metabolite salicyclic acid found in large amount in the bark of willow trees. Others include opium, quinine, digoxin etc. [3].

Many chemicals produced by plants have biological function [4]. Some of the compounds used in modern medicine are gotten from medicinal plants 80% having had a traditional use related to the current use of the active ingredient of the plant. The date natural products are profoundly sustaining and may have various potential medical advantages when used alone or in combination with therapeutic herbs [5]. In the past, dates were considered an integral part of the diet due to their high mineral and vitamin contents. Date seeds contain ingredients with nutritional value [6].

A variety of phytochemical compounds produced by plants include alkaloids, flavonoids, glycosides, polyphenols, vitamins, tannins, terpens and coumarin compounds. Medicinal plants are generating interest as a re-emerging health aid driven by the economic predicament of most developing countries as such the people resort to the use of traditional medicinal plant for healthcare [7].

The date palm (*P. dactylifera*) is a monocotyledonous woody perennial fruit species belonging to the Arecaceae family [8]. The beneficial health and nutritional values of date palm for human and animal consumption have been claimed for centuries. The fruit of the date palm contains tannin, which makes it an effective astringent. Dates have been used as a detersive and astringent in intestinal troubles, treatment for sore throats, colds, bronchial catarrh, fevers, gonorrhea, edema liver and abdominal troubles, and to counteract alcohol intoxication [8].

The sugar content of ripe dates is about 80% (this sugar includes sucrose and fructose); the remainder consists of protein, fiber, and trace elements including boron, cobalt, copper, fluorine, magnesium, manganese, selenium, and zinc. The caffeic acid glycoside 3-O-caffeoylshikimic acid (also known as dactylifric acid) and its isomers, are enzymic browning substrates also found in dates [9].

Due to the adverse effects of most antibiotics, increase in emergence of multidrug resistant strains of pathogens and the economic predicament of most developing countries, the search for new, cheap, effective and less toxic antimicrobial became necessary. This study was designed to investigate the antibacterial effect of the ethanol and methanol extract of *P. dactylifera* against some clinical bacterial isolates.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The sample of fresh *Phoenix dactylifera* fruits were obtained from Kure Market in Minna, Niger State, Nigeria. The fruit was identified at the Department of Biological Sciences, Federal University of Technology, Minna.

Extraction of Plant Material

The fruit of *P. dactylifera* was air dried for 21 days. The dried fruit was pulverized with mortar and pestle into fine powder, from which 100g of the fruit was weighed and soaked into 500 ml ethanol and 500 ml methanol separately in conical flask, plugged with cotton wool and kept on a rotary shaker at 220 rpm for 24 h. The supernatant was decanted and the solvent was slowly evaporated in an evaporating dish at room temperature to a constant weight and stored at 4°C in airtight bottles [10].

Phytochemical Analysis

Phytochemical analysis of the date fruit powder was carried out according to the method described by Agboola and Adejumo [11]

Bacterial Isolates

The bacterial isolates were obtained from the Department of Microbiology, Federal University of Technology, Minna, Niger State. They include *S. typhi*, *S. dysenteriae*, *E. coli*, *K. pneumoniae* which were maintained on nutrient broth and incubated at 33±2°C for 48h prior to further use.

Antibacterial Activity Test (Agar Well Diffusion Method)

The Agar well diffusion method was used to determine the antimicrobial efficacy of the extracts [12, 13] using concentrations of 40mg/ml, 80mg/ml, 100mg/ml and 150mg/ml respectively. With the aid of sterile cotton swab, an inoculum was taken from the standardized culture, adjusted to McFarland standard and streak on a sterile plate of molten Mueller Hinton agar. A sterile cork borer of 6 mm diameter was used to make five wells on each plate and 0.2ml of the supernatant of different extract concentration was dispensed into the well, pefloxacin (100mg/ml) was used as positive control. The inoculated plates were left for 1h to allow the extract diffuse into the agar. Each extract was analyzed in triplicate. The bacterial culture plates were then incubated at 37 ^{o}C for 24 h. The zone of inhibition was measured in mm at the end of incubation period.

Determination of the Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was estimated using the broth dilution method [14, 15]. The crude extract was re-constituted to make concentration 200 mg/ml. MIC was determined for the test isolates that showed significant sensitivity to the extract. One ml of the extract (200 mg/ml) was added to 1 ml of Mueller-Hinton broth to obtain a concentration of 100mg/ml which was subsequently transferred to a group of sterile tubes containing one ml of Mueller-Hinton medium in two-fold serial dilutions to make a tube containing descending concentrations of extracts (50, 25, 12.5, 6.25 and 3.123 mg/ml). Then, 1 ml from McFarland suspension of the test isolates was transferred using Eppendorf pipette and added to all tubes, separately. Control was equally set up by using Mueller-Hinton broth alone and test isolates without extract. The tubes were then incubated at 37^{o}C for 24 h. The MIC value was the tube with the lowest dilution with no detectable growth.

Minimum Bactericidal Concentration

The Minimum bactericidal concentration (MBC) was performed according to the method described by Doughari [17]. From the MIC tubes that showed no visible growth, 100 µl was poured on the surface of petri-dishes containing Mueller-Hinton agar. The inoculated Petri-dishes were then incubated for 24 h at 37°C and inspected for bacterial growth. The Petri-dishes that showed no visible growth were considered as the MBC for the plant extract.

RESULTS

Phytochemical Analysis

The phytochemical screening of methanol and ethanol crude extract showed that the crude methanol and ethanol extract contain alkaloids, saponins, tannins, phenols and flavonoids (Table 1).

Table1: Phytochemicals present in the crude Extract of *Phoenix dactylifera*

Phytochemical	Methanol Extract	Ethanol Extract
Alkaloids	+	+
Saponins	+++	+++
Tannins	++	++
Phenols	+	+
Flavonids	+	-

KEY: - = Absent, + = Present in trace concentration, +++ = Present in very high concentration

Antibacterial activity

Ethanol extracts of *P. dactylifera* showed varying antibacterial activity against the tested organisms (Table 2). *S. dysenteriae* was the most sensitive isolate to ethanol extract at concentration whilst *K. pneumoniae* was the least sensitive at all concentration. *E. coli* was the most sensitive isolate to methanol extracts of *P. dactylifera* (Table 3). The lowest concentration of ethanol and methanol extract of date fruit that could inhibit bacterial growth was 80 mg/ml. Based on the results, at 40 mg/ml concentration none of date extracts exhibited any antibacterial activities.

Table 2: Antibacterial activity of ethanol extract of *Phoenix dactylifera*

Test Organisms	Zone of inhibition (mm)				
	40mg/ml	80mg/ml	100mg/ml	150mg/ml	Pefloxacin (100mg/ml)
S. typhi	-	3.00 ± 0.00	4.20 ± 0.16	6.02 ± 0.02	30.00 ± 0.00
S. dysenteriae	-	10.00 ± 0.00	13.05 ± 0.04	19.30 ± 0.24	31.00 ± 0.00
E. coli	-	5.00 ± 0.00	4.00 ± 0.00	10.00 ± 0.00	29.00 ± 0.00
K. pneumonia	-	2.01 ± 0.01	4.00 ± 0.00	4.00 ± 0.00	31.00 ± 0.00

Keys: - No activity. Values are means of triplicates ± SEM

Table 3: Antibacterial activity of methanol extract of *Phoenix dactylifera*

Test Organisms	Zone of inhibition (mm)				
	40mg/ml	80mg/ml	100mg/ml	150mg/ml	Pefloxacin (100mg/ml)
S. typhi	-	4.10 ± 0.08	6.00 ± 0.00	6.25 ±0.20	30.20 ± 0.08
S. dysenteriae	-	7.00 ± 0.00	6.93± 0.04	9.90 ± 0.08	31.00 ± 0.00
E. coli	-	6.05 ± 0.40	10.10 ± 0.08	10.00± 0.00	29.00 ± 0.00
K. pneumonia	-	-	-	-	31.00 ± 0.00

Keys: - No activity. Values are means of triplicates ± SEM

Minimum inhibitory concentration

Minimum inhibitory concentration was evaluated to test the bacteriostatic property date extracts. The ethanol and methanol extracts of *P. dactylifera* were effective in suppressing the bacterial growth with the MIC values between 9.37mg/ml - 18.75mg/ml for all test isolates, except for *K. pneumoniae* which show no sensitivity to methanol extract (Table 4).

Table 4: Minimum inhibitory concentration of *Phoenix dactylifera*

Organism	Concentration (mg/ml)	
	Ethanol	Methanol
S. typhi	9.37	9.37
S. dysenteriae	9.37	9.37
E. coli	18.75	9.37
K. pneumonia	18.75	-

Keys: - No activity

Minimum bactericidal concentration

Result of the minimum bactericidal concentration showed values between 18.75mg/ml - 37.5 mg/ml for ethanol extract and methanol extract values between 9.37mg/ml - 18.75mg/ml, both extracts of *P. dactylifera* has bactericidal effect against all test isolates (Table 5).

Table 5: Minimum bactericidal concentration of *Phoenix dactylifera*

Organism	Concentration (mg/ml)	
	Ethanol	Methanol
S. typhi	37.5	18.75
S. dysenteriae	18.75	9.37
E. coli	18.75	9.37
K. pneumonia	37.5	-

Keys: - No activity

DISSCUSION

The result for the phytochemical analysis of the methanol and ethanol of extract of *P. dactylifera* (table1) indicate the presence of alkaloids, saponins, tannins, phenols and flavonoids. Saponins and tannis were found in very high concentration than other phytochemicals which is in accordance with the work of Martín-Sánchez *et al*. [17]. Flavonoids and phenols in date fruit contribute to its therapeutic effects such as anti-oxidant, anti-inflammatory, antiproliferative, anti-viral, anti-fungal and anti-cancer activities [18, 19].

The antibacterial activity of ethanol and methanol extract of *P. dactylifera* on the four isolates showed varying degree of

activity, inhibiting the growth of the test organism at various concentrations. The inhibition zone for all test isolates was gradually increased with increase concentration of the extracts.

High antibacterial activity against all test isolates was observed at 150mg/ml, Ethanol extract had the highest activity against *S. dysenteriae* (19.30 ± 0.24mm) while methanol extract showed highest activity against *E. coli* (10.00± 0.00mm), this may be due to the presences of alkaloids, saponins, flavonoids and tannins in the fruit [20, 21]. The ethanol and methanol extract of fruit at all concentration showed lesser activity against *S. typhi* which may be due to the fact that it is an encapsulated bacteria and have been known to resist many antibiotics. This in accordance with the work of Abdullah and colleagues [22], who found that, methanol extract of ajwa date fruit at 200mg/ml concentration showed lesser activity against *S. typhi* compared to other isolates. Both extracts methods of extraction showed potential bactericidal activity.

Methanol extract showed the higher potential in killing the bacteria with the MBC value of 9.37 to 18.75mg/ml in all test isolates except for *K. pneumoniae* which show no sensitivity whilst the ethanol extract was 18.75 to 37.5 mg/ml. *S. dysenteriae* and *E. coli* were found to be the most sensitive bacteria to methanol extract with MIC and MBC of 9.37mg/ml. *E. coli* was found to be the most sensitive isolate to ethanol extract with MIC and MBC of 18.75mg/ml. On the other hand, *S. typhi* was the least sensitive isolate with the MBC of 37.5 mg/mL to ethanol extract. The MIC and MBC, showed that the ethanol and methanol extracts of date fruit has bacteriostatic and bactericidal property.

CONCLUSION

The present study revealed that the ethanol and methanol extracts of date palm fruit possess antibacterial activities against the tested isolates owing to its significant zone of inhibition, lesser MIC and MBC as well as its broad spectrum activity. The toxicity and *in vivo* therapeutic properties of date palm fruit should be investigated while further phytochemical and pharmacological studies are recommended.

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