Evaluation of inhibitory effect of *Phoenix dactylifera* Ethanol Seeds Extract against *Escherichia coli and Staphylococcus aureus*

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ABSTRACT

This study evaluates inhibitory effect of Phoenix dactylifera against Escherichia coli and Staphylococcus aureus. Extraction phytochemical screening and agar diffusion methods were employed to evaluate phytochemical profile, and antibacterial activity of Phoenix dactylifera. Alkaloids, cardiac glycosides and sapponnins are contained in Phoenix dactylifera seeds. Phoenix dactylifera seeds have promising activity against E. coli with zone of inhibition of 20.4mm. The MIC and MBC of Phoenix dactylifera seed extract are 1000µg/ml and 100µg/ml against E. coli and S. aureus respectively. Phoenix dactylifera seed could be very useful in the management of gastrointestinal infections due to E. coli.

Keywords: Antibacterial effect, *Phoenix dactylifera, Escherichia coli, Staphylococcus aureus*

INTRODUCTION

Phoenix dactylifera (Date palm) is a flowering plant species belonging to the family Arcaeae. It is cultivated for edible sweet fruits as a good source of low cost food and is integrated as part of Arabian diet [1]. *Phoenix dactylifera* Date fruit has long been one of the most important fruit crops in the arid regions of the Arabian Peninsula, North Africa and the Middle East The spread of date cultivation later accompanied the expansion of Islam and reached Southern Spain and Pakistan outside the Arabian Peninsula. Date fruits are a main income source and staple food for local population in many countries in which they are cultivated, and have played significant roles in the economy, society, and environment of these countries [2].Date palm fruit is found useful in the treatment of inflammation, fever, paralysis, nervous disorders and memory disturbances[6]. It is used as astringent in intestinal troubles, treatment of sore throats, colds, bronchial catarrh, gonorrhea, edema liver, abdominal troubles, and to counteract alcohol intoxication. The fruits of date palm are a good source of phenolic compound and their associated antioxidant activity. Different cultivars of dates have different total phenolic and antioxidant activity[14]. *E. coli* is a gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus

Escherichia coli that is commonly found in the warm blooded organisms.*E. coli* is a common cause of intestinal infections. Symptoms of intestinal infection include diarrhea, abdominal pain, and fever. More severe cases can lead to bloody diarrhea, dehydration, or even kidney failure[4]. People with weakened immune systems, pregnant women, young children, and old adults are at increased risk for developing these complications. Most intestinal infections are caused by contaminated food or water. *Staphylococcus aureus* is a gram-positive non-motile coccal bacterium. It is frequently found in the nose, respiratory tract, and on the skin[1]. About 80% of individuals from developed countries use traditional medicines which have compounds derived from medicinal plants[10]. Despite the presence of various approaches to drug discovery, plants still remain the main reservoir of natural medicines. Interest in plants with antimicrobial properties has been revived because of antimicrobial resistance to some antibiotics. This resistance could be attributed to indiscriminate use of commercial drugs, self-medication, abuse or misuse of drugs[3]. Equally, certain antibiotics present undesirable side effects such as nausea, depression of bone marrow, thrombocytopenic purpura and agranulocytosis leading to the emergence of previously uncommon diseases. This has given scientists the impetus to search for newer and alternative microbial compounds from medicinal plants[3]. Besides, the high cost of conventional drugs, particularly in resource limited communities has led to the increased use of plants as an alternative for treatment of infectious diseases. Plant extracts with promising phytochemicals could have antimicrobial properties that are of great significance in treatments of infectious diseases. Their antimicrobial properties are due to compounds synthesized in the secondary metabolism of the plant. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of novel antibiotics. Multi-resistant bacteria are growing public health concern worldwide justifying investment in the search for alternative forms of treatment[5]. This study evaluates inhibitory effect of *Phoenix dactylifer*a ethanol seeds extract on *Escherichia coli* and *Staphylococcus aureus.*

MATERIALS AND METHODS

Sample Collection and Processing

Dried *Phoenix dactylifera* (Date) fruits were purchased in March, 2016 from Central Market, Kaduna. The sample of the *Phoenix dactylifera* fruits were identified and authenticated by Dr. Aminu Ado of the Department of Applied Science, Kaduna Polytechnic . Voucher specimens with reference number 040616 were deposited in the herbarium of the Department of Applied Science, Kaduna Polytechnic, Kaduna.

The seeds of the dried *Phoenix dactylifera* (Date) fruits were removed and ground using mechanical grinder until a powdery texture was achieved. The powdered sample was transferred into clean plastic container and

kept at room temperature (28 ± 2°C) prior to extraction.

Soxhlet Extraction of Phoenix dactylifera Seeds

Ethanolic solvent extraction of *Phoenix dactylifera* seed was carryout using soxhlet extractor. The extract was separated from the solvent using solvent recovery method. The recovered extract was stored at room temperature (28 ± 2°C).

Phytochemical Screening

The ethanolic extract of *Phoenix dactylifera* seed was phytochemically screened for the presence of alkaloids, tannins, steroids, sapponnins, and cardiac glycosides using the method reported by[16].

Clinical Bacterial Isolates

Clinical isolates of *Escherichia coli*and *Staphylococcus aureus* were obtained from Shehu Kangiwa Medical Centre (SKMC), Kaduna Polytechnic. The isolates were confirmed using Gram's staining and biochemical tests.

Bioassay

Preparation of Culture Media

Nutrient agar and nutrient broth were prepared according to the manufacturer's instructions. The media were sterilized by autoclaving at 121^0C for 15minutes.

Preparation of Over Night Broth Cultures

Two to three well grown colonies from each of the confirmed cultures were separately and aseptically introduced into the sterile nutrient broth in test tubes. These were incubated at 37^0C for 24hours.

Preparation of 0.5 McFarland Standard

A 1%$^{v}/_{v}$ solution of sulphuric acid (H_2SO_4) and 1% $^{v}/_{v}$ solution of barium chloride ($BaCl_2.2H_2O$) were used to prepare 0.5 McFarland standard. The standard was kept at room temperature (28 ± 2^0C) prior to inocula standardization[7].

Standardization of Inocula

The previously prepared overnight broth cultures of each bacterial isolate was adjusted to the 0.5 McFarland standard. This was achieved by adding sterile saline solution to each broth culture till the turbidity matched the standard[9].

Preparation of Varied Concentration of Phoenix dactylifera Extract

A Two (2g) grams of the ethanolic extract of *Phoenix dactylifera* was weighed using the analytical weighing balance and was dissolved in 20ml of sterile distilled water. It was mixed thoroughly until dissolved solution was obtained. This formed the stock solution. The stock solution was used to prepare 10, 100 and 1000µg/ml by diluting with sterile distilled water[2].

Antibacterial Activity of Phoenix dactylifera Seed Extract

The agar well diffusion method was used to determine the antibacterial activity of *P.dactylifera* seed extract. The sterile nutrient agar was poured into sterile Petri plates and

allowed to solidify. A sterile standard cork-borer (6mm) was used to cut wells on the surface of the agar. Sterile wire loop was used to inoculate standard bacterial inocula radially on the nutrient agar pour. A 0.1ml of the different concentrations of the extract was separately put into dug wells using sterile 1ml syringe. The preparations were incubated at 37^0C for 24 hours. Zones of inhibition were measured in millimeters.

Determination of Minimum Inhibitory Concentration (MIC)

The tube dilution method was used as described by[12]. Standardized suspension of each clinical bacterial isolate was inoculated into separate series of test tubes containing nutrient broth. Varied concentrations of the extract were sequentially introduced into the inoculated test tubes. Cotton wool and aluminum foil were used to cover the test tubes. The preparations were incubated at 37^0C for 24hours. Tubes without turbidity were recorded as MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration of the plate extract was determined by sub-culturing the test tubes from minimum inhibitory concentration tubes that showed no growth on nutrient agar and incubating for 24hours at 37^0C. The minimum bactericidal concentration was represented by the plate with the lowest concentration without growth[13].

RESULTS

Extraction of Phoenix dactylifera Seeds.

The result of *phoenix dactylifera* seed extraction is shown in Table 4.1. A total of 11.8% yield is obtainable from the seed.

Table 1: Yield (%) of *Phoenix dactylifera* Seeds Extract

Extract	Initial weight of sample (g)	Weight of extract (g)	Yield (%)
Ethanol seed Extract	200	8.2	11.8

Table 2: Gram's Reaction and Biochemical Characteristics of Clinical Bacterial Isolates

Isolates	Gram's reaction	Indole	Motility	Citrate
E. coli	-	+	+	+
S. aureus	+	-	-	-

Table 3: Phytochemical Profiles of *Phoenix dactylifera* Ethanol Seed Extract

	PHYTOCHEMICAL PROFILES				
Extract	**Alkaloids**	**Cardiac glycosides**	**Sapponnins**	**Steroids**	**Tannins**
P. dactylifera seed extract	+	+	+	-	-

KEY: + means presence of phytochemical **;** **-** means absent of phytochemical. Antibacterial Activity of Ethanolic Extract of *Phoenix dactylifera* Seeds

Table 4: Zones of Inhibition (mm) of Ethanolic Extract of Date seeds against *E. coli* and *S. aureus*

	Zones of Inhibition (mm)			
Extract	**10µg/ml**	**100µg/ml**	**1000µg/ml**	**Ciprofloxacin**
E. coli	20.4	15	13	25
S. aureus	15	12	10	30

MIC AND MBC of *Phoenix dactylifera* Ethanol Seed extract against *E. coli* and *S. aureus*

Table 5: MIC and MBC of *Phoenix dactylifera* Seed Extract Against *Escherichia coli* and *Staphylococcus aureus.*

Test Organism	**MIC (µg/ml)**	**MBC (µg/ml)**
E. coli	1000	1000
S. aureus	100	100

DISCUSSIONS

This study investigates antibacterial effect of *Phoenix dactylifera* ethanolic seeds extract against *Escherichia coli* and *Staphylococcus aureus.* Many plants in different location have been recognized as a source of cure for ailments in their region of existence. The plant parts mostly used include seed, back, leaves[11].The presence of vast array of phytochemicals in *Phoenix dactylifera* ethanolic seeds reveals the efficacy of the seeds against the bacterial isolates. Several seeds which are rich in tannins have shown possession of antibacterial activities against number of organisms[9]. Sapponnins are hemolytic on red blood cells and harmless when taken orally and they have in the body[8].

Escherichia coli and *Staphylococcus aureus* is within a normal flora of the skin of man and can be transmitted from person to product through unhygienic practice such as handling products with infected hands[15]. The extract has varying degree of antibacterial activities against *Escherichia coli* and *Staphylococcus aureus*. The high concentration of the *Phoenix dactylifera* seeds extract was found effective and thus useful in the control of the bacterial infection due to *Escherichia coli* The MIC and MBC will go a long way in providing therapeutic basis of *Phoenix dactylifera* seeds in treating bacterial Infections due to *Escherichia coli*[4].

CONCLUSIONS

Phoenix dactylifera seeds extract has promising antibacterial effect against *Escherichia coli* due to abundant of phytochemicals in the seeds. Further studies should be carried out to establish the antibacterial effect of leaf and stem of *Phoenix dactylifera plants*. Government should invest in further research on other uses and application of *Phoenix dactylifera* to fully harness and maximize the potentials of the seeds.

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