



Antibacterial activity of *Phoenix dactylifera* L. (Date palm) Seeds Extract against *Escherichia coli*

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Abstract

This research determines antibacterial activity of *Phoenix dactylifera* against clinical isolate of *Escherichia coli*. Extraction, phytochemical screening and agar diffusion methods were employed to evaluate phytochemical profile, and antibacterial activity of *Phoenix dactylifera*. Alkaloids, cardiac glycosides and saponins are present in *Phoenix dactylifera* seeds. *Phoenix dactylifera* seeds showed activity against *E. coli* with zone of inhibition of 20.4mm. The Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *Phoenix dactylifera* seed extract are 1000µg/ml and 100µg/ml against *E. coli* respectively. *Phoenix dactylifera* seed could be very useful in the management of gastrointestinal infection due to *E. coli*.

Key words: Antibacterial, *Phoenix dactylifera*, phytochemical, Extraction, gastrointestinal, *Escherichia coli*

INTRODUCTION

Phoenix dactylifera (Date palm) is a flowering plant species belonging to the family *Arcaeae*. It is cultivated for edible sweet fruits and is integrated as part of Arabian diet as a good source of low cost food (Abdelhak *et al.*, 2005). It is a medium sized plant, 15-25m tall, growing with a single root system; the leaves are 4-6cm long. Date palm fruit is found useful in the treatment of inflammation, fever, paralysis, nervous disorders and memory disturbances (Al-Qarawi *et al.*, 2004). It is used as astringent in intestinal troubles, treatment of sore throats, colds, bronchial catarrh, fever, gonorrhoea, edema liver and abdominal troubles, and to counteract alcohol intoxication (Al-dalhan and Bhat, 2012).

Date palm seed powder is used in traditional medicines to treat toothache (Morton, 1987). Dates seed extract has ability to restore the normal function status of the poisoned liver, and was found protective against carbon tetrachloride hyper toxicity on the liver in rats (Al-Qarawi *et al.*, 2004). Date seeds have been studied as potential sources of edible oils and pharmaceuticals (Al-Shahib and Marshall, 2003). *E. coli* is a gram-negative, facultative anaerobic, rod-shaped bacterium, 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 (Al-Shahib

and Marshall, 2003). 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 (Al-Shahib and Marshall, 2003).

Dates provide essential nutrients and are claimed to have benefits in human health. Manickarasagan *et al.*, (2012) reported that dates by-products can be considered a good source of dietary fibre, total phenolics and an inexpensive source of natural antioxidants and could be used as a functional food ingredient. This study aimed to evaluate the antibacterial activity of *Phoenix dactylifera* ethanol seed extract on *Escherichia coli*.

MATERIALS AND METHODS

Sample collection and handling

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 using Voucher specimens with reference number 040616 deposited in the herbarium of Applied Science Department, Kaduna Polytechnic, Kaduna. The seed 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.

Ethanol 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.

Phytochemical screenng

The ethanolic extract of *Phoenix dactylifera* seed was phytochemically screened for the presence of alkaloids, tannins, steroids, saponins, and cardiac glycosides using the method reported by (Sofowora, 1993).

Clinical bacterial isolates

Clinical isolates of *Escherichia coli* cultures were obtained from Shehu Kangiwa Medical Centre (SKMC), Kaduna Polytechnic. These isolates were confirmed using Grams staining and biochemical tests (Cheesbrough, 2004).

Preparation of culture media

Nutrient agar and nutrient broth were prepared according to manufacturer's instructions and autoclaved at 121°C for 15minutes.

Preparation of over night broth culture

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

Preparation of 0.5 McFarland turbidity standards

A 1%v/v solution of sulphuric acid (H₂SO₄) was prepared by adding 1ml of concentrated H₂SO₄ to 99ml of water in a volumetric flask. The preparation was properly mixed. A 1%^{v/v} solution of barium chloride was prepared by dissolving 0.5g of dehydrated barium chloride (BaCl₂.2H₂O) in 50ml of distilled water in a volumetric flask. A 0.6ml of the barium chloride solution was added to 99.4ml of the H₂SO₄ solution and mixed properly. A small volume of the turbid standard solution was put into a screw cap bottle. The standard was kept at room temperature (28±2°C) prior to inocula standardization (Cheesbrough, 2000).

Standardization of inocula

The previously prepared overnight broth cultures of each bacterial isolate was adjusted to 0.5 McFarland standard. This was achieved by adding sterile saline solution to each broth culture till the turbidity matched standard (Hussein *et al.*, 1998).

Preparation of varied concentration of *Phoenix dactylifera* extracts

Two grams (2g) 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, using 1 milliliter sterile syringe, after calculating the required diluents volume using dilution formula (Ademuyiwa *et al.*, 1990).

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 in sterile Petri plates and allowed to solidify. Sterile wire loop was used to inoculate standardized bacterial inocula radially on the surface of the prepared nutrient agar. A sterile standard cork-borer (6mm) was used to bore wells on the surface of the agar. Using a sterile syringe, 0.1ml of the different extract were saperately dispensed in to the wells and allowed to stand for 20minutes at room temperature for proper diffusion of extract. The preparations were incubated at 37°C for 24 hours. Zones of inhibitions were measured in millimeters using meter rule.

Determination of Minimum Inhibitory Concentration (MIC)

The tube dilution method was used as described by (Pelzer *et al.*, 1999). Standardized suspension of the test organisms were inoculated into series of test tubes containing sterile nutrient broth.

Varied concentrations of the extract were sequentially introduced into the inoculated test tubes.

The preparations were incubated at 37°C for 24 hours. Tubes without turbidity were recorded as MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration was determined by sub-culturing the minimum inhibitory concentration tubes that showed no growth on nutrient agar and incubating for 24hours at 37°C. The minimum bactericidal concentration was represented by the plate with the lowest concentration without growth (Roberts *et al.*, 2000).

RESULTS

Extraction of *Phoenix dactylifera* seeds yields 32.8% of extract (Table 1). The result of Gram's reaction presented in Table 2 confirms *E. coli* as Gram negative and is positive when tested for indole, motility and citrate utilization. The

phytochemical screening of *Phoenix dactylifera* seeds extract is presented in Table 3. Alkaloid, Cardiac glycosides and Saponins are present in ethanol seed extract of *Phoenix dactylifera* while Steroids and Tannins are absent.

Table 1: Percentage yield of *Phoenix dactylifera* seed extract

Extract	Initial weight of sample (g)	Weight of extract (g)	Yield (%)
Ethanolic seed Extract	25	8.2	32.8

Table 2: Gram reaction and biochemical characteristics of clinical bacterial isolates

Isolates	Gram reaction	Indole	Motility	Citrate
<i>E. coli</i>	-	+	+	+

Table 3: Phytochemical profiles of *Phoenix dactylifera* ethanol seed extract

Extract	Phytochemical profiles				
	Alkaloids	Cardiac glycosides	Saponins	Steroids	Tannins
<i>P. dactylifera</i> seed extract	+	+	+	-	-

KEY: + Means presence of phytochemical, - Means absent of phytochemical

Antibacterial activity of ethanolic extract of date seeds

The antibacterial activity of *Phoenix dactylifera* seed extract is presented in Table

4. The seed extract was found effective at 1000µg/ml as against *Escherichia coli* as it demonstrates higher zone of inhibition of 20.4mm.

Table 4: Zones of inhibition (mm) of ethanolic extract of date seeds against *E. coli*

Extract	Zones of inhibition (mm)			
	10µg/ml	100µg/ml	1000µg/ml	Ciprofloxacin (30µg/ml)
<i>Escherichia coli</i>	13mm	15mm	20.4mm	25mm

Table 4: MIC and MBC of *Phoenix dactylifera* seed extract against *Escherichia coli*

Test Organism	MIC (µg/ml)	MBC (µg/ml)
<i>Escherichia coli</i>	1000	1000

DISCUSSION AND CONCLUSION

This study investigates antibacterial effect of *Phoenix dactylifera* ethanolic seeds extract against *Escherichia coli*. 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 (Muktar and Tukur, 1999).

Dates have medicinal uses including anticancer, antihyperlipidemic, hepatoprotective activities and thereby serving as an essential healthy food in the human diet (Biglari *et al.*, 2009). The date fruit is used in folk medicine to treat the different infectious diseases probably because of their antibacterial ability, immuneomodulatory activity and antifungal property (Baliga *et al.*, 2011).

The medicinal use of extracts prepared from plant parts of the dates back to ancient times.

Furthermore, it has been proposed that its antioxidant constituents account for its beneficial therapeutic effects (Ljubuncic *et al.*, 2005). The antioxidant activity of phenolic compounds is a result of their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Frag, 2011).

The presence of phytochemicals such as Alkaloids, Cardiac glycoside and saponins in *Phoenix dactylifera* reveals the efficacy of seed extract against the bacterial isolate. (Habib and Ibrahim, 2009). Saponins are harmless in the body when taken orally (Fatope, 1994). The absence of tannins among the phytochemical components detected in this study is probably as a result of drying process of the *Phoenix dactylifera*.

Maillard and Berset, (1995) reported that in the process of drying, tannins are degraded by heat and maturation enzymes which result in release of phenolic compounds. *Escherichia coli* can be transmitted from person to product through unhygienic practice such as handling products with infected hands (Sawaya *et al.*, 2006). The extract has varying degree of antibacterial activities against *Escherichia coli*. The high concentration of the *Phoenix dactylifera* seed extract was found effective and thus useful in the control of the bacterial infection due to

Escherichia coli. The Minimum inhibitory Concentration (MIC) and Minimum bactericidal Concentration (MBC) will go a long way in providing therapeutic basis of *Phoenix dactylifera* seeds in treating bacterial infections due to *Escherichia coli* (Al-Farsi and Lee, 2008).

CONCLUSION

Phoenix dactylifera seeds extract has antibacterial effect against *Escherichia coli* due to abundance of phytochemical in the seeds.

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