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Phytochemical content, antioxidant properties and antibacterial activities of date (*Phoenix dactylifera L.*) seed extracts

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ABSTRACT

This study evaluated the antioxidant and antibacterial properties of date (*Phoenix dactylifera L.*) seed extracts against some bacterial pathogens of food-borne diseases. The phytochemical constituents and antioxidant properties of the date seed were determined using a standard chemical method. In vitro antibacterial activities of the crude extracts of date seeds against the pathogens were determined using the agar well diffusion method. The phytochemical screenings of the extracts revealed the presence of oxalate, phytate, flavonoid, saponin, tannin and cardiac glycoside. The screening of the date seed extracts for antioxidant compounds revealed varied concentrations of total antioxidant capacity, ferric reducing antioxidant property and flavonoid. Methanol and aqueous date seed extracts exhibited appreciable antibacterial activity against *E. coli, K. pneumoniae, S. typhimurium, B. subtilis* and *S. flexneri* whereas, n-hexane extract had a mild effect on all test organisms. At 200 and 100 mg/mL of the crude extracts, all the test isolates were inhibited. Varied Minimum inhibitory and Minimum Bactericidal Concentrations of different date seeds extracts showed potential bacteriostatic and bactericidal action against the test pathogens. It can be deduced that the important bioactive compounds in date seeds may be responsible for the observed antibacterial activity against the causative agents of food-borne illnesses.

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1.Introduction

Spoilage of foods by microorganisms is a global challenge facing developing and even developed countries; these biological agents widely cause deterioration of all classes of foods which in most cases has resulted in food waste and economic losses.

The yearly losses of global foods have been estimated to reach up to 40% attributed to microbial activities (1,2). Once access has been gained by microorganisms to foods, they proliferate and produce toxic metabolites that either cause food spoilage within a short period of

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time by utilizing the available nutrients or food poisoning a condition of distress to consumers (3).

Food-borne illness or diseases are pervasive food safety problems that are significant to public health concern arising from the consumption of foods contaminated with live microbial pathogens and/or their poisonous metabolites (3,4). These spoilage microorganisms and microbial pathogens have developed mechanisms by which they survive in the presence of chemical preservatives and certain unfavorable conditions such as modified atmosphere packaging, low temperature, vacuum packaging and pasteurization (5). Thus, the risk associated with the use of synthetic or chemical additives as food preservatives on human health is considered to be a major concern among consumers and this has brought down their uses where necessary (6,7).

In recent times, an increasing tendency towards the application of natural substances of plant origin as food preservatives instead of chemically synthesized ones has been recommended to inhibit the growth of microbial pathogens and spoilage microorganisms and also to prolong the keeping quality of foods (8,9). Artificial food preservatives of plant origins have been frequently used to inhibit the growth of food spoilage microorganisms because of the existence of antimicrobial substances in different parts. The phytochemical and antioxidant compounds such as carotenoids, phenolic and its derivatives, and carotenoids previously extracted from the leaves, roots, flowers, stems, fruits and seeds are gaining importance both as potential nutraceuticals and food preservatives (3,10). Consequently, numerous studies targeting the antimicrobial properties of some plants against microorganisms already implicated in food spoilage or poisoning have been conducted. Among many plants previously investigated, (*Phoenix dactylifera* L.) seeds have not been extensively studied for their antimicrobial and antioxidant properties. Despite this interest, scarily information is currently available on the antioxidant, phytochemical and antibacterial activities of date (*Phoenix dactylifera L.*) seeds. With this goal, this study aimed at evaluating the antibacterial and antioxidant activities of date seeds (*P. dactylifera* L.) extracts against some bacterial pathogens.

2.Materials and Methods

2.1. Source of date fruit

Fresh date seeds (*P. dactvlifera* L.) were purchased from the hawkers at the South gate of the Federal University of Technology, Akure (FUTA), and Nigeria in sterile polyethylene bags and transferred to FUTA Microbiology laboratory for chemical and microbial analysis.

2.2. Source of bacterial isolates

Food and typed bacterial isolates from food sources namely: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Bacillus subtilis* and *Salmonella typhimurium*, were obtained from the Culture bank of the Department of Microbiology, FUTA. The identities of the isolates were authenticated using standard conventional methods (11).

2.3. Preparation of date seeds

Date seeds were processed into powdery form according to the methods of Chandrasekaran and Bahkali (12). The seeds used were collected and the seeds were separated from the fruits, washed with distilled water, air dried and ground into fine powder.

The weights of the powdered form of seeds were recorded.

2.4. Extraction of the phytochemical constituents of date seeds

The phytochemical constituents of date seeds were extracted as described by Olukunle and Adenola (13). The finely grounded dried date seeds were divided evenly into three portions and separately distributed into 3 sterile plastic containers respectively. Each portion was homogenized with 1 L sterile distilled water, ethanol and n- hexane respectively and kept for 3 days. Large particles were initially removed from the homogenate by sterile muslin cloth and then filtered using Whatman No. 1 filter paper. The solvents were removed from the filtrate and concentrated in a rotary evaporator. The extraction efficiency was quantified by determining and comparing the weight of each of the yield of the extracts.

2.5. Antibiotic sensitivity pattern of bacterial isolates

The antibiotic susceptibility pattern of the isolates was determined using agar disc diffusion method according to the prescription of the Clinical and Laboratory Standards Institute (CLSI). *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Bacillus subtilis* and *Salmonella typhimurium* were grown in sterile nutrient broth for 24 h at 37°C, after which they were adjusted to 0.5 McFarland Standard. With the aid of a sterile swab stick previously immersed into bacterial suspension, the surface of Mueller Hinton agar was streaked. After that, commercially antibiotics (Ciprofloxacin 5 µg, Gentamycin 10 µg, Streptomycin 10 µg, Amoxacillin 25 µg, Pefloxacin 5 µg, Septrin 25 µg, Ampiclox 10 µg, Rocephin 30 µg, Erythromycin 15 µg and Zinnacef 30 µg) discs were placed on the surface of inoculated plates and incubated at 37°C for 18 h. After incubation, the zones of inhibition were measured (mm) (14).

2.6. In-vitro antibacterial activity of date seeds extracts

Antibacterial activity of date seed extracts against the aforementioned bacterial pathogens was evaluated using agar-well diffusion with different concentrations of the extracts with commercial antibiotics and sterile distilled water serving as positive and negative controls respectively. Mueller Hinton agar streaked with bacterial suspension and with wells filled with the extracts were incubated for 24 h at 37°C. The antibacterial activity was interpreted from the size of the diameter of the zone of inhibition measured to the nearest (mm) (15).

2.7. Minimum inhibitory and bactericidal concentration of date seeds extracts

Minimum inhibitory and bactericidal concentration of date seed extracts against the bacterial pathogens were determined in accordance with the guidelines of the Clinical and Laboratory Standards Institute. The varying concentrations of the Dimethyl sulfoxide (DMSO) reconstituted extracts were prepared and incorporated into a set of sterile tubes, inoculated with standardized test organisms incubated for 24 h. 500 µl contents from the tube were plated on nutrient media after which the viable counts were enumerated after 24 h of incubation. The minimum inhibitory concentration of the extracts was recorded as the lowest concentration of the extract inhibiting the growth of the test organism (16).

2.8. Determination of phytochemical constituents of date seeds

The qualitative and quantitative phytochemical parameters of date seeds were determined following standard biochemical techniques. The parameters determined were tannin, phytate, oxalate, saponin, alkaloid, terpenoid, steroid, anthocyanin and cardiac glycosides (17-20).

2.8.1. Antioxidant activity of date seeds

The antioxidant activity of date seeds evaluated was total antioxidant activity, total ferric reducing property, total flavonoid and free radical scavenging ability (21).

2.8.2. Total antioxidant activity of date seeds

Total antioxidant activity of date seeds was on the basis of reduction of Mo (VI) - Mo (V) by the sample and subsequent development of a green phosphate/Mo (V) complex at acidic pH. Varied concentrations of the date seeds were added with the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube was incubated at 95^{o}C for 90 min. The mixture was cooled to room temperature against blank and the absorbance was measured at 695 nm. The antioxidant activity was expressed as gallic acid equivalent (18).

2.8.3. Total flavonoid determination

The total flavonoid in the seeds was estimated by colorimeter assay and expressed as milligrams per gram (mg/g) (22).

2.8.4. Ferric reducing property determination

Ferric Reducing Property (FRP) of the sample was determined by mixing the seeds with sodium phosphate buffer pH 6.6 and 1% Potassium ferrocyanide and incubating at 50^{o}C for 20 min. Trichloroacetic acid was afterward added to the preparation and centrifuged at 2000 rpm for 10 min. The supernatant obtained was then mixed with $FeCl_3$ and distilled water and the absorbance was measured at 700 nm (19).

2.8.5. Free radical scavenging ability determination

The free radical scavenging ability of the sample against 1, 1- diphenyl-2-picryhydrazyl (DPPH) was determined as described by Gyamfi et al. (18). Equal volume of the sample was mixed with 0.4 mM methanolic solution of the DPPH and allowed to stand in the dark for 30 min before the absorbance was measured at 516 nm.

3. Results

3.1. Authentication of bacterial identities

Table 1 reveals the identities of the bacterial isolates to be *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Bacillus subtilis*.

3.2. Qualitative phytochemical screening of date seeds extracts

Table 2 shows the qualitative phytochemical constituents of the date seeds extracts. The presence of saponin, tannin, flavonoid, steroid and cardiac glycoside was detected in all the preparations (aqueous, methanol and n-hexane crude extracts) of the extracts. However, phlobatannin and alkaloids were not detected in all the crude extracts evaluated.

3.3. Quantitative phytochemical screening of date seeds extracts

Table 3 shows the quantitative phytochemical constituents of the extracts. Of all the parameters evaluated, saponin had the highest values of 70.53,

34.13 and 170.18 mg/g for methanol, n-hexane and aqueous extracts respectively, while oxalate had the least in all the preparations.

3.4. Quantitative antioxidant properties of date seeds

Antioxidant properties of date seeds is presented in Fig. 1. The TAC, flavonoid, DPPH and FRAP in date seeds were estimated to be 15.04 mg/g, 0.04 mg/g, 49.67% and 7.30 mg/g respectively.

3.5. Percentage yield of date seeds extracts

Fig. 2 reveals the percentage yields of aqueous, methanol and n-hexane date seed extracts. After the extraction process, the methanol seed extract had the highest percentage yield of 9.21%, while yields from aqueous and n-hexane seed extracts were 8.35 and 6.58% respectively.

3.6. Sensitivity patterns of bacterial isolates to commercial antibiotics

The antibiotic sensitivity profile of the Gram-positive bacterial isolates is shown in Table 4. Zinnacef exerted the highest inhibition on *Bacillus subtilis,* followed by Ciprofloxacin with a zone of inhibition of 14.66 mm, while there was no inhibition with Pefloxacin, Streptomycin, Rocephin and Erythromycin. It indicates that *B. subtilis* was resistant to Pefloxacin, Streptomycin, Rocephin and Erythromycin. *Staphylococcus aureus* was susceptible to 80 % of the commercial antibiotic used. Pefloxacin and Septrin had the highest zone of inhibition of 20.00 mm followed by Gentamycin with a value of 12.00 mm, while Streptomycin and Erythromycin exerted no inhibitory effect.

Table 1. Morphological characteristics and biochemical characterization of the bacterial isolates

colour	Elevation	Gram reaction	shape	catalase	oxidase	citrate	motility	mannitol	coagulase	glucose	sucrose	lactose	suspected bacteria
metallic Sheen	Convex	-	rod	+	-	-	+	+	-	AG	AG	AG	*Escherichia coli*
cream	slightly raised	+	cocci	+	-	+	-	+	+	AG	AG	AG	*Staphylococcus aureus*
pink	raised	-	rod	+	-	-	+	-	+	AG	AG	AG	*Shigella fexneri*
black	raised	-	rod	+	-	-	+	+	-	AG	-	-	*Salmonella typhimurium*
cream	flat	+	rod	+	-	+	+	ND	ND	AG	AG	-	*Bacillus subtilis*
cream	raised	-	rod	+	-	+	-	-	ND	AG	-	AG	*Klebsiella pneumoniae*

+ = Positive, - = Negative, AG = Acid and Gas production, ND- not determined

Table 2. Qualitative phytochemical contents of date seed crude extracts

Phytochemicals	aqueous extract	N-hexane extract	methanol extract
Tannin	+	+	+
Alkaloid	-	-	-
Saponin	+	+	+
Terpenoid	+	+	+
Steroid	-	-	-
Flavonoids	+	+	+
Plobatannin	-	-	-
Legal's test	+	+	+
Liberman's test	+	+	+
Salkwoski'd test	-	-	-
Cardiac glycosides	+	+	+
Keller-Killiani's test	+	+	+

+ = Present, - = Absent

Table 3. Quantitative phytochemical properties of date seed extracts (mg/g)

Samples	Oxalate	Phytate	Alkaloid	Steroid	Saponin	Tannin	Glycoside	Terpenoid
Methanol	0.91 ± 0.54^{b}	13.18 ± 0.00^{e}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	70.53 ± 0.18^{f}	3.41 ± 0.00^{c}	0.13 ± 0.23^{a}	4.1 ± 0.26^{d}
N-hexane	0.47 ± 0.33^{b}	10.8 ± 0.90^{e}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	34.13 ± 0.16^{g}	4.55 ± 0.06^{c}	11.12 ± 00.03^{f}	10.54 ± 00.02^{d}
Aqueous	1.98 ± 0.52^{b}	86.45 ± 0.47^{e}	00.00 ± 00.00^{a}	00.00 ± 00.00^{a}	170.18 ± 0.18^{f}	2.30 ± 0.06^{b}	8.55 ± 0.30^{c}	12.07 ± 0.02^{d}

Data are represented as mean ± standard deviation. Means with the same superscript across the row are not significantly different from each other ($p>0.05$).

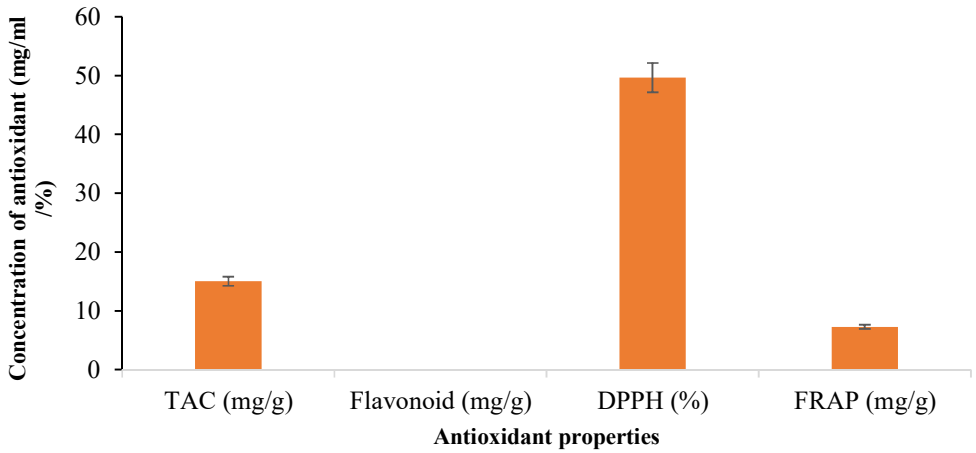

Figure 1. Quantitative antioxidant properties of date seeds

TAC- Total Antioxidant Capacity; DPPH- 1,1-diphenyl-2-picryhydrazyl (free radical scavenging ability); FRAP- Ferric Reducing Antioxidant Property.
Data are represented as mean ± standard deviation. Means with the same superscript across the row are not significantly different from each other (p>0.05).

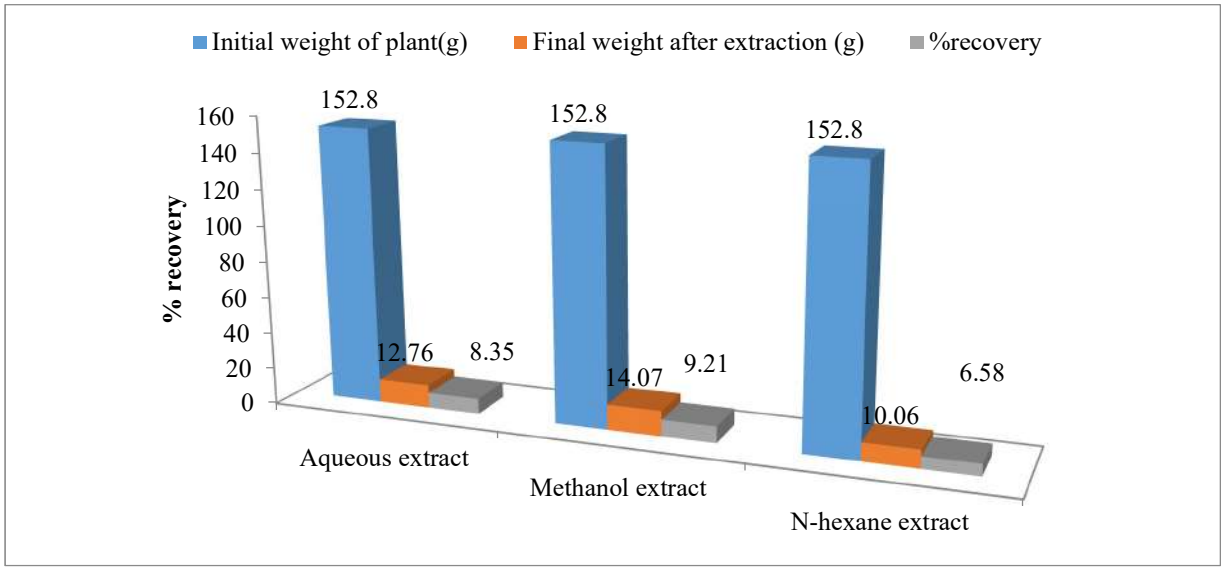

Figure 2. Percentage yields of date seed crude extracts

Table 4. Sensitivity patterns of Gram-positive bacterial isolates to commercial antibiotics

Bacterial isolates	APX	CN	PEF	AM	CPX	S	SXT	R	E	Z
Bacillus subtilis	8.33±0.57^{b}	9.33±0.57^{b}	0.00±0.00^{a}	9.33±0.57^{b}	14.66±1.15^{c}	0.00±0.00^{a}	13.33±1.15^{c}	0.00±0.00^{a}	0.00±0.00^{a}	27.33±1.15^{d}
Staphylococcus aureus	2.00±0.57^{a}	12.00±0.00^{c}	20.00±1.73^{d}	9.33±0.05^{c}	11.33±1.15^{c}	0.00±0.00^{a}	20.00±0.50^{d}	10.66±1.51^{c}	0.00±0.00^{a}	8.00±1.00^{b}

CPX-Ciprofloxacin, CN-Gentamycin, S-Streptomycin, AM-Amoxacillin, PEF-Pefloxacin, SXT-Septrin, APX-Ampiclox, R-Rocephin, E-Erythromycin, Z-Zinnacef
Data are represented as mean ± standard deviation. Means with the same superscript across the row are not significantly different from each other ($p>0.05$).

3.7. Sensitivity patterns of Gram-negative bacterial isolates to commercial antibiotics

In Table 5, the sensitivity patterns of the bacterial pathogens to commercial antibiotics were revealed. *Salmonella typhi* was susceptible to all the antibiotics employed with none having less than a 13.00 mm zone of inhibition. *Escherichia coli* was resistant to Streptomycin, Chloraphenicol, Amoxacillin and Augmentin, *Shigella flexneri* was also resistant to Streptomycin and Augmentin. *Klebsiella Pneumonia* was not susceptible to Ofloxacin, Streptomycin, Septrin, Chloraphenicol, Amoxacillin, Gentamycin and Pefloxacin.

3.8. *In-vitro* antibacterial activity of date seed extracts against bacterial pathogens

The *in-vitro* antibacterial activity of different concentrations of date seed extracts against selected bacterial pathogens are presented in Fig. 3, 4 & 5. In Fig. 3, *E. coli* was not sensitive to all the concentrations of methanol extract of date seeds except for 200 mg/ml where a zone of inhibition of 3.50 mm was observed. The inhibitory effect of the extract was only recorded at 100 and 200 mg/ml on *S. flexneri*. The inhibitory potential of the extract on *K. pneumoniae* varied with the concentration applied, 100 mg/ml had the highest zone of inhibition on the test isolate. *Salmonella typhi* was resistant to all the concentrations except for 200 mg/ml where inhibition was recorded, while 50, 100 and 200 mg/ml of the extract had 3.66, 7.33 and 1.33 mm zones of inhibition respectively on *B. subtilis*. None of the prepared concentrations of n-hexane extract had antibacterial activity on S. flexneri. 50 and 200 mg/mL of the extract exerted varied degrees of inhibitions on *E. coli*, 100 and 400 mg/mL produced zone of inhibitions of 1.66 and 4.66 mg/mL respectively on *S. typhi*, while only 100 mg/ml had inhibitory action on K. pneumoniae (Fig. 4). The concentration of the extract at 100 and 200 mg/mL had zone of inhibitions of 5.66 and 0.66 mg/mL respectively on *B. subtilis*.

In Fig. 5, the antibacterial activities of different concentrations of aqueous extract of date seeds are presented. It was only 100 mg/mL out of the entire concentrations that inhibited *E. coli*, *S. flexneri* and *K. pneumoniae*, while 50 and 100 mg/mL exerted inhibition on *S. typhi*. At 50, 100 and 200 mg/mL concentrations, zone of inhibitions were 5.83, 5.66 and 2.66 mm on *B. subtilis* respectively. 3.9. Minimum inhibitory concentration of the extracts The comparative minimum inhibitory concentration (MIC) of the date seeds extracts are presented in Table 6. The MIC of aqueous extract against *S. flexneri*, *E. coli*, *B. subtilis*, *K. pneumoniae*, *S. aureus* and *S. typhi* were 50, 100, 100, 100, 50 and 100 mg/mL respectively. The MIC of methanol and n-hexane extracts against *S. flexneri*, *E. coli*, *B. subtilis*, *K. pneumoniae*, *S. aureus* and *S. typhi* were 200, 200, 100, 100, 200 and 50 mg/mL, and 100, 50, 50, 50, 100 and 50 mg/mL respectively. 3.10. Minimum bactericidal concentration of the extracts

The comparative minimum bactericidal concentration (MBC) of the extracts is revealed in Table 7. The MBC of the aqueous and n-hexane extracts against all the test organisms was 50 mg/mL, while the MBC of the methanol extract against *S. flexneri*, *E. coli* and *S. aureus* were 100 mg/ml and 50 mg/mL.

Table 5. Sensitivity patterns of Gram-negative bacterial isolates to commercial antibiotics

Bacterial isolates	OFX	S	SXT	CH	SP	CPX	AM	AU	CN	PEF
Escherichia coli	14.00±0.00^{b}	0.00±0.00^{a}	14.00±1.15^{c}	0.00±0.00^{a}	15.33±1.15^{c}	12.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{a}	14.66±1.15^{c}	16.00±1.15^{c}
Shigella flexneri	19.33±0.57^{d}	0.00±0.00^{a}	13.33±0.57^{b}	15.33±0.57^{b}	18.00±1.15^{c}	26.66±0.57^{e}	11.33±0.57^{b}	0.00±0.00^{a}	11.33±1.15^{b}	16.66±1.15^{d}
Salmonella typhi	21.33±0.57^{d}	18.66±0.28^{d}	14.33±0.50^{a}	13.66±0.15^{a}	21.33±0.57^{d}	19.33±0.28^{d}	20.00±0.57^{d}	15.66±0.28^{a}	22.00±0.57^{d}	22.33±0.28^{d}
Klebsiella Pneumonia	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	7.33±1.15^{b}	4.66±1.15^{b}	0.00±0.00^{a}	11.33±1.15^{c}	0.00±0.00^{a}	0.00±0.00^{a}

CPX-Ciprofloxacin, CN-Gentamycin, CH-Chloraphenicol, SP-Sparfloxacin, S-Streptomycin, OFX-Ofloxacin, AU-Augmentin, AM-Amoxacillin, PEF-Pefloxacin, SXT-Septrin

Data are represented as mean ± standard deviation. Means with the same superscript across the row are not significantly different from each other ($p>0.05$).

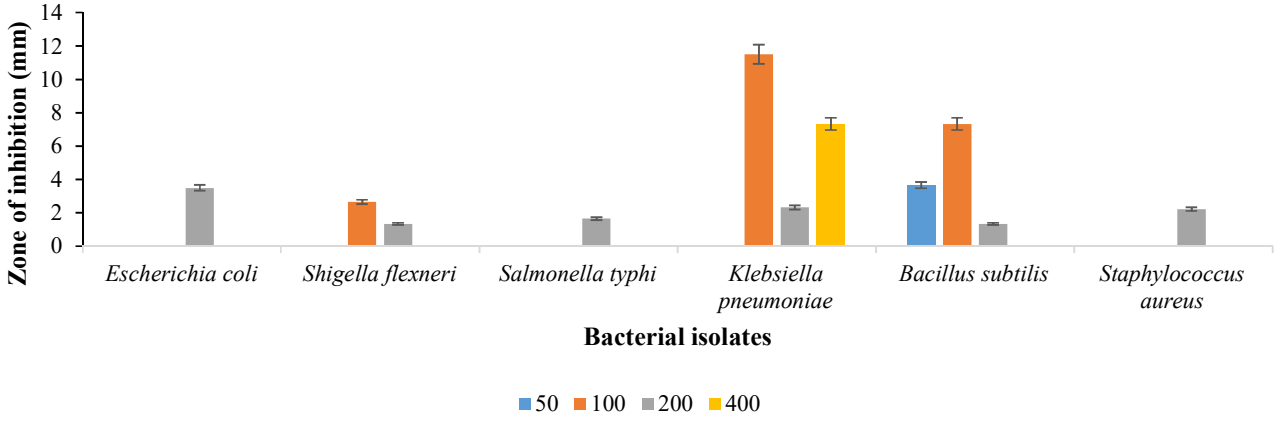

Figure 3. Sensitivity patterns of bacterial isolates to methanol extract of date seeds (mg/ml)

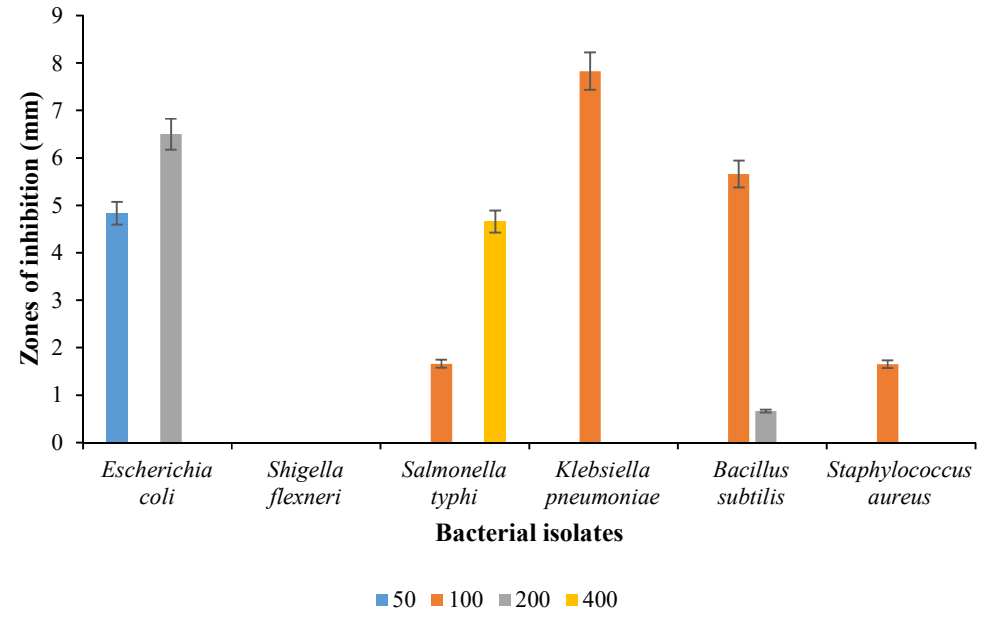

Figure 4. Sensitivity patterns of bacterial isolates to n-hexane extract of date seeds (mg/ml)

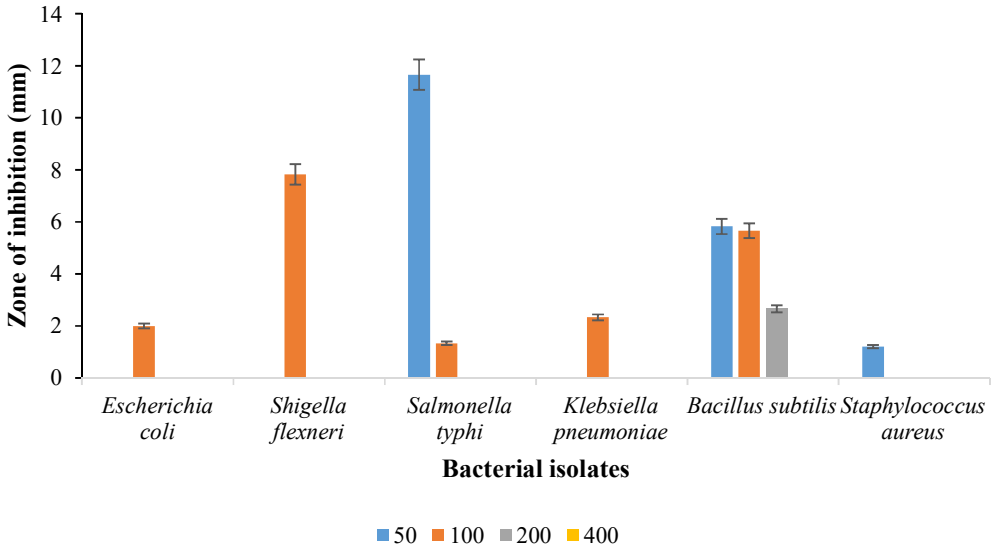

Figure 5. Sensitivity patterns of bacterial isolates to aqueous extract of date seeds (mg/ml)

Table 6. Minimum inhibitory concentration of aqueous, methanolic and n-hexane extracts of date seeds (mg/ml)

Bacterial isolates	positive control (CPX)	aqueous	methanol	N-hexane
Shigella flexneri	1.25	50	200	100
Escherichia coli	1.25	100	200	50
Bacillus subtilis	1.25	100	100	50
Klebsiella pneumoniae	1.25	100	100	50
Staphylococcus aureus	0.625	50	200	100
Salmonella typhi	1.25	100	50	50

Data are means of replicate determinations
CPX = Ciprofloxacin

Table 7. Minimum bactericidal concentration of aqueous, methanolic and n-hexane extracts of date seed crude extracts (mg/ml)

Bacterial isolates	aqueous	methanol	n-hexane
Shigella flexneri	50	100	50
Escherichia coli	50	100	50
Bacillus subtilis	50	50	50
Klebsiella pneumoniae	50	50	50
Staphylococcus aureus	50	100	50
Salmonella typhi	50	50	50

Data are means of replicate determinations

4.Discussion

Cooperate scientific investigations are required globally to safeguard mankind from bacterial food poisoning and thus, ameliorate the menace associated with ingestion of unsafe foods that often result in different food-borne diseases and intoxication.

The authenticated bacterial pathogens worked with in this study have been reported to have links to a series of disease outbreaks worldwide. Certain strains of *Shigella* spp. are well known etiological agents of shigellosis, a condition of distress that developed in humans and primates after ingestion of foods containing live species of *Shigella* or enteric reactions to their toxic metabolites (23,24). The ingestion of a significant population of *Salmonella* spp. in foods was reported to cause enteric infection normally characterized by local damage to the mucosal wall (25). The presence of fecal indicator organisms such *E. coli* in foods is a serious health issue associated with virulent genes. A strain of *E. coli* called Enterohemorrhagic *E. coli* (EHEC) is reported to produce cytotoxins commonly known as Shiga-like toxins (SLTs) that mortgage cell secretion and paralyzes colonic epithelial cells (23). In the studies of Derbew et al. (26) and Kwiri et al. (27), *S. aureus* was isolated from foods vended in Gondar town of Ethiopia and it was reported that selected strains of the organism could cause food-borne diseases called staphylococcal food poisoning. The selected strains of this organism have been reported to produce enterotoxin on unprotected foods and cause gastroenteritis, and inflammation of gastrointestinal tract (14).

Recently, concerted efforts have been put in place to curtail the emergence of antibiotic- resistant food and clinical bacterial pathogens, especially in developing countries. In developing countries, children and individuals with compromised immunity are most vulnerable to these pathogens (28). The test bacterial pathogens showed varied sensitivity to commercial antibiotics used in this study. According to the report of Amare et al. (29), 100% sensitivity was shown by *Citrobacter* spp. to ceftriaxone, gentamicin and tetracycline, while *S. aureus* and *E. coli* showed 73.53 and 86.67% resistance to penicillin and ampicillin respectively. The values obtained in this study are comparable with a study conducted by Eromo et al. (30) who reported 86.67, 70 and 75% resistance to ampicillin by *E. coli*, *Enterobacter* and *Citrobacter* spp. respectively. In support of the current study, Olaniyi et al. (31) studied the susceptibility pattern of bacteria isolated from non-alcoholic beverage, 'burukutu' to some commercial antibiotics and reported that *E. coli* was resistant to Amoxicillin and Erythromycin, while it was sensitive to other antibiotics. Furthermore, *B. subtilis* and *S. aureus* were resistant to Septrin and Zinacef respectively. In another study conducted by Bodunde et al. (32), *Shigella* sp., *E. coli* and *S. aureus* isolated from muscle foods had approximately 29% of all the commercial antibiotics tested. Also, the prevalence of antibiotic-resistant *S. aureus* and *E. coli* from raw seafood and fish imported to Switzerland was reported by Boss et al. (33). *S. aureus* was isolated from retail meat and meat products in China and it was observed that it had 85.4, 84.6, 52.7, 49.3, 21.1, 20.4 and 19.4% resistance to ampicillin, penicillin, erythromycin and tetracycline, streptomycin, norfloxacin and gentamicin respectively (34). The resistance of these bacteria to some of the commercial antibiotics might be attributed to acquired antibiotic-resistant genes and mobile genetic elements via horizontal gene transfer together

with the loss of antibiotic target sites of the organisms (31).

In vitro antibacterial activity of different concentrations of date seeds crude extracts had varied inhibition on selected food bacterial pathogens. Antibacterial activities of many plants and their secondary metabolites have been documented (14,35). The water and methanol extracts of date seeds inhibited the growth of all tested bacterial pathogens at concentrations of 100 and 200 mg/ml respectively with variable potency. The findings of Mostafa et al. (14) and Perveen and Bokahri (35) validate the current investigation. Antibacterial activity of five plant extracts against bacterial pathogens causing food poisoning was investigated and all the plant extracts exhibited a suppressive effect on the test organisms with variable potency (14). Similarly, Perveen and Bokahri (35) evaluated the antibacterial activity of crude extracts of dates and reported that the extracts exhibited varied antibacterial properties against *S. flexeneri*, *S. aureus*, *E. coli*, *S. pyogenes*, *P. aeruginosa* and *B. subtilis*. In another study, methanol and aqueous extracts of pits and leaves of three varieties of *P. dactylifera* showed good inhibitory action against *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. aureus*, *S. flexeneri* and *S. pyogenes* (36). Antibacterial activity exhibited by date seed extracts against bacterial pathogens might be due to the synergistic effect of its various phytochemical/antioxidant constituents (37-39). These chemicals suppress the growth of test isolates by disrupting the architectural structure of bacterial cell membranes or inhibiting enzymes required for the biosynthesis of amino acids (39-41). Other investigators linked the antibacterial activity of these plant extracts to their hydrophobicity characteristics which enhance their performance in reacting with bacterial cell membrane protein and mitochondria causing various degrees of damage to their structure and altering their permeability (14,42). Some researchers have reported that date plant contains a good number of flavonoids, alkaloids, tannins, terpenoids and some vitamins (43-45).

Varied MIC and MBC of different date seed extracts showed potential bacteriostatic and bactericidal action against the tested food bacterial pathogens. These results are in agreement with the reports of Mostafa et al. (14), Mahboubi et al. (46) and Qader et al. (47). The variations in MIC and MBC of the extracts might be due influence of extraction solvents on chemical constituents, volatile nature of their constituents and as well as bacterial strains on which the preparation was tested (14). Previous investigations with respect to the variations in MIC and MBC exhibited by different extracts on bacterial pathogens had also been linked to the differences in the antioxidant constituents of the extracts (48,49).

5. Conclusion

Food poisoning or infection is often caused by the elaboration of toxic metabolites of many strains of pathogenic bacteria. The adopted preventive measure to control these bacterial pathogens in foods is centered on the application of chemical preservatives with damnable consequences on human health and this has necessitated for search for novel biological food preservatives. Natural biological food preservatives are competing well with their chemically synthesized counterpart and are safer with no adverse effects on consumers' health. The date seed extracts possess

antibacterial potential against some food bacterial pathogens. So, date seeds can be considered as an alternative bio-preservative to a chemical antibacterial agent to control causative agents of food poisoning.

Conflict of interest

Authors declare no conflict of interests.

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