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IN-VITRO ANTIBACTERIAL ACTIVITIES OF AJWA DATE FRUIT (*PHOENIX DACTYLIFERA* L.) EXTRACT AGAINST SELECTED GRAM-NEGATIVE BACTERIA CAUSING GASTROENTERITIS

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ABSTRACT: Gastroenteritis, especially those caused by bacterial pathogens, remains as one of the major health issues in developing countries. Despite the availability of treatment, people are turning their interest in consuming certain food for its therapeutic value given the adverse effects of medications. This includes the consumption of date fruits (*Phoenix dactylifera* L.). This study aims to determine the antibacterial effect of two methods of extraction, *i.e.*, hot aqueous extract and methanol extract of Ajwa date fruit against selected gram-negative bacteria causing gastroenteritis. The antimicrobial activities were determined quantitatively using well diffusion assay together with the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). Results showed that both hot aqueous and methanol extracts have the potential to suppress the growth of all tested bacteria. The methanol extract had higher inhibitory zones compared to hot aqueous extract, and the zones of inhibition were noted to increase with increase in the extract concentration. *S. flexneri* was the most sensitive bacteria to both types of extract. The methanol extract was also more effective in suppressing the bacterial growth and has a higher potential in killing the bacteria compared to hot aqueous extract. These results suggest that Ajwa date extract has a bacteriostatic property at lower concentration and bactericidal property at higher concentration. In conclusion, Ajwa date fruit extract has the antibacterial potential against selected bacterial causing gastroenteritis. Further study on the phytochemical properties that contribute to this effect is currently in progress.

INTRODUCTION: Gastroenteritis is an infection of the intestine usually presented as diarrhea and may be accompanied by nausea, vomiting, abdominal pain, and fever. It can be caused by pathogenic bacteria, viruses, and parasites and remains a significant global health concern with an estimate of up to 2.5 million deaths per year [1].

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The incidence of bacterial gastroenteritis is commonly reported in developing countries where food and water sources are contaminated in addition to poor hygiene practices that lead to fecal-oral transmission [2].

Among the common bacteria causing gastroenteritis reported in the community include *Vibrio cholerae*, *Clostridium difficile*, *Staphylococcus aureus*, *Salmonella typhi*, *Campylobacter jejuni*, *Shigella* and *Escherichia coli* [2, 3]. These bacteria often disrupt the normal intestinal functions and cause diarrhea by adhering to the mucosal epithelium, produce toxin and may even invade the epithelial cell to produce dysentery [1].

Most of the cases of bacterial gastroenteritis are, however, self-limited and definitive treatment with antibiotics is rarely warranted. However, supportive treatments are often necessary to prevent further morbidity and mortality, especially in the susceptible age group [3]. The concern on the adverse effects of some medications has led to an increase in the recognition of certain food for its therapeutic values. This includes the consumption of date fruits (*Phoenix dactylifera* L.) which are stapled in some countries and continue to be cultivated worldwide [4, 5]. Date fruits are delicious, popular and very significant in Muslim culture and history, being mentioned in many verses of the Quran and also known to be regularly consumed by the Prophet Muhammad SAW for energy and treatment of various ailments [6, 7, 8].

Among the various types of date fruits, Ajwa date, which is indigenously grown in Madinah, Saudi Arabia [7] is of exceptional values. It is known to contain a higher content of fibers, sugars, amino acids, minerals such as calcium, magnesium, sodium, potassium, and vitamins as compared to another type of dates [9, 10]. Also, the flesh of Ajwa dates is rich with phytochemicals such as flavonoids and phenolic, which contributes to its therapeutic effects such as anti-oxidant, anti-inflammatory, antiproliferative, anti-viral, anti-fungal and anti-cancer activities [5].

Although previous studies have shown the antibacterial effects of *Phoenix dactylifera* L., they have not specified the type of date fruit used. A study which had used Ajwa date fruit had chilled and stored the dates in low temperature before extracting them using methanol and acetone [11]. Because the phytochemicals content may vary according to the method of handling and extraction [12], therefore we conducted this study to determine the antibacterial effects of two methods of extractions *i.e.* hot aqueous extract and methanol extract of Ajwa date fruit against gram-negative pathogenic bacteria such as *Salmonella typhi*, *Salmonella typhimurium*, *Escherichia coli*, *Vibrio cholerae* and *Shigella flexneri*.

MATERIALS AND METHODS:

Drying and Storage of Ajwa Dates: Ajwa dates which were cultivated in Medina, Saudi Arabia were purchased from a local supplier. The fruits' seeds were removed, and the pulps were rinsed with distilled water. They were then dried in the oven and later stored in a tight glass container at 4 °C until used.

Extraction: Two methods of extraction were used in this study, *i.e.* hot aqueous extraction and methanol extraction.

Hot Aqueous Extraction: 100 g of Ajwa date fruit was extracted with 1000 ml of distilled water, and the mixture was heated at 60 °C for 2 h. The mixture was then filtered through cotton gauze and filtered again through Whatman filter paper no. 1. The filtrates were later dried at 60 °C in the oven. The extract yields were stored at -20° until further analysis.

Methanol Extraction: 100 g of Ajwa date fruit was extracted with 1000 ml of methanol, and the mixture was left in room temperature for 24 h. The mixture was then filtered through cotton gauze and filtered again through Whatman filter paper no. 1. The filtrates were evaporated and dried at 40 °C under reduced pressure using rotatory vacuum evaporator and then further reduced at 60 °C in the oven until dry. The extract yields were stored at -20°C until further analysis.

Antibacterial Activity of Ajwa Date:

Test Organisms: The microorganisms used in this study represent among the common pathogenic gram-negative bacteria causing gastroenteritis. They consisted of *Escherichia coli*, *Salmonella typhi*, *Salmonella typhimurium* (ATCC 14028), *Shigella flexneri* (ATCC 12022), and *Vibrio cholerae*. All the bacterial strains were stored in 10% glycerol stock at -80 °C and sub-cultured on nutrient plate agar (Oxiod) at 37 °C for 24 h before any screening.

Antibacterial Activity of Ajwa Date Extract: The antibacterial activity of Ajwa date extract was assessed quantitatively using the well diffusion assay method on Mueller-Hinton agar (Oxoid). Four wells (diameter of 7 mm) were made using sterile cork borer, and the agar plates were inoculated with bacterial suspensions with a cell density adjusted at 1.5×10^8 colony forming units @ McFarland standard.

Three of the wells were filled with the test samples, which were diluted serially at the following concentrations: 500, 400, 300, 200, and 100 mg/mL. A well was filled with the positive control of 10 µl of ampicillin antibiotic (concentration of 1mg/mL). All plates were incubated at 37 °C for 24 h. After the incubation period, the diameter of inhibition zones was measured by Vernier caliper and compared with the positive control. All tests were performed in triplicate.

Determination of the Minimum Inhibitory Concentration (MIC) of the Ajwa Date Extract: Different concentrations of the Ajwa date extracts (1000, 500, 250, 125, 6.25 mg/mL) were prepared separately by dissolving the extract in nutrient broth. The bacterial suspension was adjusted at 1.5 × 10^8 CFU @ McFarland standard and diluted to 1:100 adapting the microdilution method by Weigand *et al*., in 2008. To determine the MIC, 50 µl of the different extract concentrations were added in sterile 96-well plate nutrient agar (Oxoid) (Corning Costar Ltd., USA) followed by 50 µl of the bacterial suspensions. The plates were then incubated at 35 °C for 16 to 20 h. The lowest concentration of extract in the well with no bacterial growth (shown as no turbidity) was taken as the MIC.

Determination of the Minimum Bactericidal Concentration (MBC) of the Ajwa Date Extract: The dilutions of three different concentrations (*i.e.*, MIC concentration and those above and below the MIC) of Ajwa date extract that inhibit the growth of bacterial organism were taken and sub-cultured on fresh nutrient agar plates (Oxoid). The plates were further incubated at 37 °C for 24 h. The lowest concentration of the extract that showed no colony growth on the agar plates was taken as the MBC.

RESULTS AND DISCUSSION:

Antibacterial Activity of Ajwa Date Extract: Two methods of extraction of the Ajwa date fruit were evaluated for their antibacterial activities against the gram-negative bacterial, causing gastro-enteritis using the well-diffusion assay method. The antibacterial activities were recorded in **Table 1**. Based on the results, none of the 100 mg/mL of Ajwa date extracts exhibited any antibacterial activities. The lowest concentration of hot aqueous and methanol extract of Ajwa date fruit that could inhibit bacterial growth was 200 mg/mL. This suggests that the potency of hot aqueous extract was almost similar to methanol extract against all tested bacteria. The methanol extract had shown higher inhibitory zones with the average zones of inhibition ranging between 15 mm to 34 mm compared to the average zones of inhibition between 13 mm to 17 mm for hot aqueous extract.

The zones of inhibition were increased with increased extract concentrations. Among the five tested bacteria, *S. flexneri* was the most sensitive bacteria to both hot aqueous and methanol extract, as shown by the highest zone of inhibition for almost all concentrations. The results revealed that both methods of Ajwa date extraction were potentially effective in suppressing the growth of the tested bacteria.

TABLE 1: DIAMETER OF INHIBITION ZONE OF HOT AQUEOUS EXTRACT AND METHANOL EXTRACT AGAINST THE MICROORGANISM USING WELL DIFFUSION ASSAY METHOD

Types of extraction	Conc. (mg/mL)	Zone of inhibition (mm)				
		E. coli	***S. typhi***	***S. typhimurium***	***S. flexneri***	***V. cholerae***
Hot aqueous extract	500	27.33(±0.58)	28.67(±0.58)	27(±0)	32.33(±0.58)	27.33(±1.15)
	400	24.67(±0.58)	25.67(±0.58)	20.67(±0.58)	32.00 (±1)	25.00 (±0)
	300	22.67(±0.58)	20(±0.58)	20.67(±0.58)	29.67(±0.58)	21.00 (±1)
	200	17.33(±0.58)	16.33 (±0)	17.67(±0.58)	13.33(±0.58)	16.33(±0.58)
	100	N.D.	N.D.	N.D.	N.D.	N.D.
Methanol extract	500	27.67(±0.58)	29 (±0)	25.67(±0.58)	34.00 (±0)	28.67(±0.58)
	400	24.67(±0.58)	25.33(±0.58)	21.33(±0.58)	31.67(±0.58)	24.67(±0.58)
	300	21.67(±0.58)	21.33(±1.15)	17.67(±0.58)	29.33(±1.15)	22.33(±0.58)
	200	17.33(±0.58)	15.33(±0.58)	17.33(±0.58)	24.33(±1.15)	17.33(±1.15)
	100	N.D.	N.D.	N.D.	N.D.	N.D.
Positive control (Ampicillin)	1	33(±0)	31(±0)	32(±0)	31(±0)	31(±0)

Note: mean (±SD), N.D: not definite, Conc: concentration

Minimum Inhibitory Concentration (MIC) of the Ajwa Date Extract: The minimum inhibitory concentrations (MIC) were evaluated to test the bacteriostatic property of Ajwa date extracts. The minimum concentration that exhibited inhibition of bacterial growth was presented in **Table 2**. When compared between the 2 types of extraction, methanol extract was more effective in suppressing the bacterial growth with the MIC of 250 mg/mL for 3 different strains of bacteria *i.e. E. coli*, S. *typhimurium*, and *V. cholerae* while the MIC for the rest of the bacterial strain was 500 mg/mL. In contrast, the MIC of hot aqueous extract was 500 mg/mL for all bacteria except for *V. cholerae*.

TABLE 2: MINIMUM INHIBITORY CONCENTRATION (MIC) OF THE HOT AQUEOUS AND METHANOL EXTRACT OF AJWA DATE FRUIT

Bacterial strain	Concentration(mg/mL)	
	Hot aqueous extract	Methanol extract
E. coli	500	250
S. typhi	500	500
S. typhimurium	500	250
S. flexneri	500	500
V. cholerae	250	250

Note: Data provided as means of triplicate with the SD of 0.0

Minimum Bactericidal Concentration (MBC) of the Ajwa Date Extract: The MBC was determined by the minimum concentration where there was the absence of bacterial growth taken from the MIC plate to test the bactericidal property of Ajwa date extracts.

TABLE 3: MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF THE HOT AQUEOUS AND METHANOL EXTRACT OF *AJWA* DATE FRUIT

Bacterial strain	Concentration (mg/mL)	
	Hot aqueous extract	Methanol extract
E. coli	1000	500
S. typhimurium	1000	500
S. typhi	500	500
S. flexneri	500	500
V. cholerae	500	250

Note: Data provided as means of triplicate with the SD of 0.0

Both methods of extraction showed potential bactericidal activity with methanol extract showed the higher potential in killing the bacteria with the MBC range of 250 to 500 mg/mL in almost all tested bacteria whilst the MBC using hot aqueous extract range from 500 to 1000 mg/mL. *V. cholerae* was found to be the most sensitive organism to methanol extract with both the MIC and MBC of 250 mg/mL. On the other hand, *E. coli* and *S. typhimurium* were the least sensitive bacteria with the MBC of 1000 mg/mL using the hot aqueous extract. From the both MIC and MBC test, it was shown that for both methods of extraction, Ajwa date extract has the bacteriostatic property at lower concentrations and bactericidal property at higher concentrations.

CONCLUSION: In this present study, both hot aqueous and methanol extracts of Ajwa date showed potential antibacterial activities towards selected gram-negative bacteria causing gastro-enteritis. Also, it has both bacteriostatic and bactericidal effects of the bacteria tested. The antibacterial activities of the methanol extract were higher than those seen with hot aqueous extract, suggesting that different methods of extractions yield different types or concentrations of phytochemicals to produce the bactericidal effect. Further study of the biochemical composition of Ajwa date is currently in progress.

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