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Original Article

ANTIMICROBIAL EFFICACY AND TIME-KILL KINETICS OF PHOENIX DACTYLIFERA L. SEED OIL AGAINST MULTIDRUG RESISTANT PATHOGENS FROM CANCER PATIENTS

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ABSTRACT

Infectious complications are a frequent occurrence in cancer patients, with antimicrobial resistant pathogens contributing significantly to the morbidity burden in this population. This study evaluated the antimicrobial efficacy of *Phoenix dactylifera* L. seed oil (PDEO) against antimicrobial resistant pathogens isolated from cancer patients. *P. dactylifera* seed oil was extracted via steam-distillation technique. The antimicrobial activity of the essential oil was determined using agar disk diffusion, broth microdilution and time-kill kinetics assays, while the mechanism of action was determined by quantifying ion-leakage from the microbial cells. For bacterial isolates, the zone of inhibition ranged from 14.33 ± 0.17 mm in *Klebsiella pneumoniae* to 31.33 ± 0.17 mm in *Streptococcus pneumoniae* and *Staphylococcus aureus*; while for fungal isolates it ranged from 13.67 ± 0.33 mm in *Candida glabrata* to 31.33 ± 0.33 mm in *Candida parapsilosis*. The time-kill kinetics of *P. dactylifera* essential oil revealed levels of concentration and time dependent microbicidal activity against the test pathogens. Respectively, the highest concentration of sodium and potassium ion leakage from the test organisms were observed against *S. aureus* (31.73 ± 0.44 mg/L) and *K. aerogenes* (20.00 ± 0.61 mg/L) for bacterial isolates; and against *C. tropicalis* (38.73 ± 0.20 mg/L; 22.70 ± 0.49 mg/L) ($P \leq 0.05$) for fungal isolates. These results provide reasonable evidence that *P. dactylifera* seed oil could be a veritable source of safe and effective natural antimicrobial with potential utilization in the combat against antimicrobial resistant pathogens.

KEYWORDS: Infection, Antimicrobial resistance, *Phoenix dactylifera* seed oil, Antimicrobial agents

1. Introduction

Cancer remains one of the most dreaded diseases in the modern world, being a significant public health burden [1]. Infection is a frequent occurrence in cancer patients [2,3,4]. In addition to being a leading cause of hospitalization in this population, sepsis represents a frequent route to mortality [5]. The comorbidity of sepsis and cancer poses serious complications with very poor prognosis. Sepsis as a syndrome can result from healthcare-associated or community-acquired infection by microorganisms [6].

Several factors elevate the risk of infection in cancer patients, and it is not unusual for a patient to have multiple risk factors simultaneously [7]. These include disruption of natural anatomic barriers such as the skin and mucosal surfaces, obstruction (most often adduced to the tumor progression), and treatment-related factors such as chemotherapy, radiation, diagnostic and/or therapeutic surgical procedures, and the increasing use of medical devices such as various catheters, stents, and prostheses [7,8]. Common sites of infection include the skin and skin structures (including surgical site infections), the bloodstream (including infections associated with central venous catheters), the lungs, the hepato-biliary and intestinal tracts, and the urinary tract, and include distinct clinical syndromes such as neutropenic enterocolitis, post-obstructive pneumonia and obstructive uropathy [7,9].

The epidemiology of most of these infections is changing with resistant organisms being isolated more frequently than in the past [4,10-2], consequently limiting the effectiveness of antimicrobial agents. The empirical use of antimicrobials has contributed to the menace of multidrug-resistant organisms [6,4]. In the last decades, the incidence of human pathogens resistant to several antimicrobials has increased worldwide. Management of infections attributable to (multidrug resistant) MDR pathogens is a critical challenge in health systems.

Natural products from plants offer a vast reservoir for

the investigation of novel therapeutics [13]. The use of plants for therapeutic purposes is an ancient practice. Medicinal plants have been an integral part of human life all through civilization history. Plants produce a plethora of structurally varied compounds which are diversity oriented and exert a range of biological effects. The therapeutic properties of medicinal plants have been adduced to the presence of secondary metabolites [14], which, besides playing crucial physiological roles in these organisms, interfere with pharmacological targets in humans and many other species. Burgeoning quest for the discovery of novel therapeutics has made essential oils (EOs) from plants an attractive area of study in recent decades [15]. Scientific evidences have demonstrated that, among natural compounds, EOs showed great potential for the management of a number of diseases [13,16].

The date palm (*Phoenix dactylifera* L.) is an angiosperm, a monocotyledonous plant belonging to the family Arecaceae. It is an iconic fruit plant species having one of the oldest cultivation histories in the Middle East and African regions, with viable economic returns and a robust source of nutrition and livelihood. Folkloric medicine noted its parts are used for the treatment of various diseases such as cough, rheumatism, respiratory infections, nephropathy, gastropathy, asthma, cancer and high blood pressure [17,18].

Although studies have documented the bioactivities of extracts from various date palm parts [18,19], nevertheless, there is dearth of data on the bioactivities of the seed oil. This study therefore investigated the antimicrobial potency of *P. dactylifera* seed oil against MDR pathogens isolated from cancer patients (in previous study) [4].

2. Materials and Methods

2.1. Test Organisms and Standardization of Inoculum

The test microorganisms used in this study were antimicrobial resistant strains isolated from cancer patients in a previous study [4]. McFarland standard (0.5) was prepared by combining 0.05 ml of 1% barium chloride Dihydrate ($BaCl_2 \cdot 2H_2O$) with 9.95 ml of 1% Sulfuric acid (H_2SO_4) to yield 1.0%w/v barium sulphate suspension. In preparing the standard inoculum of the bacterial isolates, the 18 hours old bacterial colonies were transferred to a tube of sterile saline. The bacterial suspension was adjusted to the proper density as the 0.5 McFarland by adding sterile saline or more bacterial cultures. Then bacterial suspension was diluted to obtain 106 cfu/ml. For yeast cells, inoculum was prepared by picking five distinct colonies of approximately 1 mm in diameter from a 24-hour-old culture. Colonies were suspended in 5 mL of sterile 0.85% saline. The resulting suspension was vortexed for 15 seconds and its turbidity was adjusted to 0.5 McFarland standard. This procedure yielded a yeast stock suspension of 1-5 x 10^6 cells per mL. For mould, isolates were subcultured on Sabauraud dextrose agar tubes and incubated at 35 ºC for 7 days. Using a sterile Pasteur-pipette the surface of colony was gently washed with 3 ml sterile distilled water or 0.85% sterile saline. The suspension was then pipetted out over the slant, transferred to a sterile tube and vortexed for 10 to 15 seconds. The inoculum was adjusted using spectrophotometer (625 nm) to optical density 0.09 - 0.11 with test inoculum in range 0.2 to 2.5×10^5 CFU/mL (CLSI, 2020) [20].

2.2. Collection of Plant Material and Extraction of Oil from *Phoenix dactylifera* Seed

The fruits of *Phoenix dactylifera* L. (dates) used for this study were sourced from a local market. The fruit samples were authenticated and designated with voucher specimen number PSBH-240. After manual separation from the fruits, the seeds were milled into powdery form. Oil from the seed was extracted using the Clevenger steam-distillation method as previously described [13]. The stock essential oil was sterilized by filtration using sterile membrane filters (Millex-GP 0.22 µm, Merck) and stored at -18 ºC until further use.

2.3. Determination of Antimicrobial Activity of *Phoenix dactylifera* Seed Oil

Agar disc diffusion method as described by Li et al. [22] was employed for the determination of antimicrobial activities of PDEO with some modifications. The appropriate solidified medium (Mueller Hinton Agar and Sabouraud Dextrose Agar plates) was inoculated with 100 µl of the standardized bacterial and fungal inoculum respectively. The inoculum was spread over the plates using a sterile glass rod in order to get a uniform microbial growth on both control and test plates. After inoculum absorption by agar, sterile filter discs (Whatman no 1, England, 6 mm diameter) impregnated with 10 µl of stock solution of the essential oil were placed on the agar surface using sterile forceps. The growth cultures were accompanied by 1% DMSO solution as a negative control, while ciprofloxacin or gentamycin; and amphotericin-B or voriconazole were used as positive controls for bacterial and fungal plates respectively. All Petri dishes were sealed with sterile laboratory paraffin to avoid eventual evaporation of the essential oils. The dishes were left for 30 minutes at room temperature to allow the diffusion of oil, and then incubated at appropriate temperature and time (37 ºC for 24 hours for bacterial plates; and 35 ºC for 48 hours for fungal plates). After the incubation period, the mean diameter of inhibition zone was measured in millimeters and recorded.

2.4. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC) of *P. dactylifera* Seed Oil

The MIC of the oil was determined by broth microdilution method utilizing 96-well microtiter plates [22]. The stock of the oil was diluted in 1% (v/v) DMSO (Merck KGaA, Germany). No detrimental effect on microbial growth was observed at this concentration. A 100 µl of the essential oil suspension was dispensed into the first well of the microtiter plate, while the second to twelfth wells were liquated with 50 µL of the appropriate sterile medium, Mueller Hilton broth (MHB) and Sabauraud Dextrose broth (SDB) for bacterial and fungal isolates respectively. A two-fold serial dilution of the essential oil ranging from 500 to 0.97 µg/mL was performed by transferring 50 µL suspension from the first well to the second well and subsequently to the 10th well. Ten (10) µL of 0.5 McFarland standard of each inoculum was added into the wells (second to eleventh). Ciprofloxacin or gentamycin; and amphotericin-B or voriconazole were used

as positive control for bacterial and fungal plates respectively, while the negative control contained inoculated broth only. The microtitre plates were placed in a rotary agitator (120 rpm), for 30 minutes, followed by incubation of the bacterial plates at 37 ºC for 24 hours and fungal plates at 35 ºC for 48 hours. The lowest concentration where there was no visible microbial growth was taken as the MIC. The minimum bactericidal and fungicidal concentration (MBC/MFC) were determined by subculturing 50 µL from each well with no visible microbial growth on MHA and SDA plates and incubating at 37 ºC for 24 hours and at 35 ºC for 48 hours respectively for bacterial and fungal isolates.

2.5. Time-Kill Kinetics Assay of *Phoenix dactylifera* Seed Oil

Time-kill assay as described by Singh and Katoch [23] was used to investigate the microbicidal activity of the oil. Standard inoculum of the bacterial and fungal cultures respectively in MHB and SDB containing the essential oil at different concentrations of MIC, 2xMIC, 4xMIC were incubated respectively at 37 ºC and 35 ºC. Aliquots of 20 µL were taken from the culture (at every 2-hourly time interval for 12 hours and at 24 hours for bacterial cultures; and at 6-hourly time interval until 48 hours for fungi) and cultured on MHA and SDA respectively for bacteria and fungi, followed by incubation at appropriate temperature. Tubes containing inoculum plus antimicrobial agents served as positive control, while tubes containing sterile broth plus inoculum served as negative control. After incubation at appropriate temperature, the number of colonies were counted and expressed as CFU/mL and SFU/mL respectively for bacteria and fungi. Time–kill curves were then constructed by plotting the log10 CFU/mL and log10 SFU/mL against the exposure time (hours).

2.6. Determination of Mechanism of Action of *Phoenix dactylifera* Seed Oil

The mechanism of action was determined by assessing the integrity of cell membrane through leakage of vital cellular ions from microbial cells into solution. This was carried out as described by Falade et al. [24] with minor modifications. From a 100 mL broth culture medium of the test organisms, microbial cells were collected by centrifuging at 1500 rpm for 5 min, washed three times using sterile distilled water and re-suspended in 0.1 M Phosphate buffered saline (PBS pH 7.4). The final cell suspension of tested microorganisms were adjusted to 0.5 Mcfarland's solution. The cells were then introduced into 100 mL (MHB and SDB respectively for bacterial and fungal isolates) medium containing MIC concentration of the essential oil, MIC concentration of the essential oil plus antibiotics, and accompanied with the control (10 mM EDTA). The microbial suspensions were incubated at appropriate temperature under agitation. Afterwards, the supernatant solution obtained after centrifuging at 3000 rpm for 5 min was analyzed for the presence of sodium and potassium ion using a flame photometer (Jenway PFP7) at 589 nm and 766 nm respectively.

2.7. Statistical Analysis

All data obtained were analyzed using Statistical Package for Social Sciences (SPSS) version 22.0 (IBM Corp. Armonk, NY, USA); data were subjected to one way analysis of variance (ANOVA) and differences between means were determined by Duncan's New Multiple Range Test at ($p \leq 0.05$).

3. Results

3.1. Antimicrobial Activity of *P. dactylifera* Seed Oil against Microorganisms Isolated from Cancer Patients

The results of the antimicrobial activity of PDEO against the test isolates are presented in Tables 1 and 2. The zone of inhibition (ZOI) of PDEO against the pathogens ranged from 14.33 to 31.33 mm for bacterial isolates and 13.67 to 31.33 mm for fungal isolates; while the Minimum inhibitory concentration (MIC) ranged from 3.91 µg/ml to 125 µg/ml for both bacterial and fungal isolates. Amongst the fungal isolates, *Candida parapsilosis, Candida tropicalis, Aspergillus niger* and *Aspergillus terreus* were the most susceptible to the seed oil.

Table 1. Antibacterial activity of *P. dactylifera* seed oil against antibiotic resistant test isolates

Organism	ZOI range (mm)	Mean ZOI (mm)	MIC range (µg/mL)	Mean MIC (µg/mL)	MBC ran-ge (µg/mL)	Mean MBC (µg/mL)
K. aerogenes (n=4)	25.67 ± 0.33 - 30.17 ± 0.17	27.92 ± 0.50c	7.81 – 31.25	15.63 ± 2.89a	15.63 - 62.5	31.25 ± 5.77a
K. pneumoniae (n=4)	14.33 ± 0.17 - 17.67 ± 0.17	15.92 ± 0.38a	62.5 – 125	85.94 ± 12.24c	125 – 250	171.88 ± 12.48c
E. coli (n=3)	24.33 ± 0.33 - 30.17± 0.17	27.72 ± 0.89c	15.63 – 31.25	26.04 ± 2.60a	31.25 - 62.5	52.08 ± 5.21a
S. dysenteriae (n=3)	24.00 ± 0.58 - 25.00 ± 0.58	24.56 ± 0.29b	15.63 – 31.25	26.04 ± 2.60a	31.25 - 62.5	52.08 ± 5.21a
S. typhi (n=2)	24.00 ± 0.58 - 26.00 ± 0.58	25.00 ± 0.57b	15.63 – 31.25	23.44 ± 3.49a	31.25 - 62.5	46.88 ± 6.99a
P. mirabilis (n=2)	22.00 ± 0.58 - 25.00 ± 0.58	23.50 ± 0.76b	15.63	15.63 ± 0. 00	31.25	31.25 ± 0.00a
S. pneumoniae (n=3)	26.17 ± 0.17 - 31.33 ± 0.17	28.56 ± 0.76c	3.91 – 15.63	11.72 ± 1.95a	7.81 - 31.25	23.44 ± 3.91a
S. aureus (n=3)	25.67 ± 0.33 - 31.33 ± 0.17	28.22 ± 0.83c	31.25 – 62.5	52.08 ± 5.21b	62.5 – 125	104.17 ± 10.42b

Legend : ZOI = Zone of inhibition, MIC = Minimum inhibitory concentration, MBC = Minimum bactericidal concentration.

Table 2. Antifungal activity of *P. dactylifera* seed oil against fungal test isolates

Organism	ZOI Range (mm)	Mean ZOI (mm)	MIC Range (µg/mL)	Mean MIC (µg/mL)	MFC range (µg/mL)	Mean MFC (µg/mL)
A. fumigatus (n=7)	14.00 ± 0.58 - 20.00 ± 0.58	16.52 ± 0.51a	7.81 - 62.5	34.60 ± 4.37b	15.63 - 125	69.20 ± 8.73b
A. flavus (n=5)	16.67 ± 0.88 - 21.00 ± 0.58	18.33 ± 0.48a	15.63 - 62.5	40.63 ± 5.01b	31.25 - 125	81.25 ± 10.02b
A. terreus (n=2)	23.33 ± 0.33 - 26.00 ± 0.58	24.67 ± 0.67c	3.91 - 7.81	5.86 ± 0.87a	7.81 -15.63	11.72 ± 1.75a
A. niger (n=2)	21.33 ± 0.88 - 26.33 ± 0.33	23.83 ± 1.19bc	3.91	3.91 ± 0.00a	7.81	7.81 ± 0.00 a
C. albicans (n=7)	17.00 ± 0.58 - 25.66 ± 0.66	21.52 ± 0.71b	15.63 - 62.5	33.48 ± 4.35b	31.25 - 125	66.96 ± 8.70b
C. glabrata (n=5)	13.67 ± 0.33 - 24.00 ± 0.58	18.53 ± 0.93a	15.63 - 62.5	31.25 ± 4.57b	31.25 - 125	62.50 ± 9.15b
C. tropicalis (n=3)	27.33 ± 0.33 - 29.67 ± 0.33	28.33 ± 0.41d	3.91	5.21 ± 0.65a	7.81	10.42 ± 1.30a
C. parapsilosis (n=2)	27.00 ± 0.58 - 31.33 ± 0.33	29.17 ± 1.01d	3.91	3.91 ± 0.00a	7.81	7.81 ± 0.00a

3.2. Time Kill Kinetics of *P. dactylifera* Essential Oil Against Microorganisms Isolated from Cancer Patients

P. dactylifera seed oil showed concentration and time dependent microbicidal activities against the test pathogens. Generally, the killing rate was faster against Gram-positive bacteria compared to other test isolates. The results are presented in Figures 1 to 4.

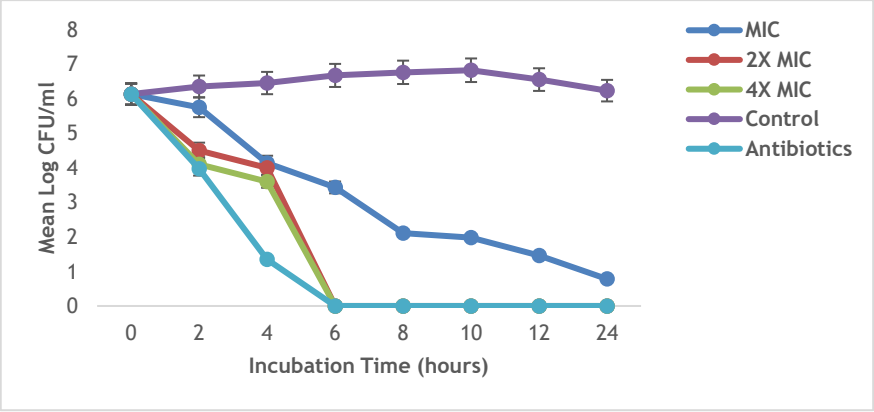

Fig. 1. Time kill kinetics of *P. dactylifera* seed oil against *S. pneumoniae*

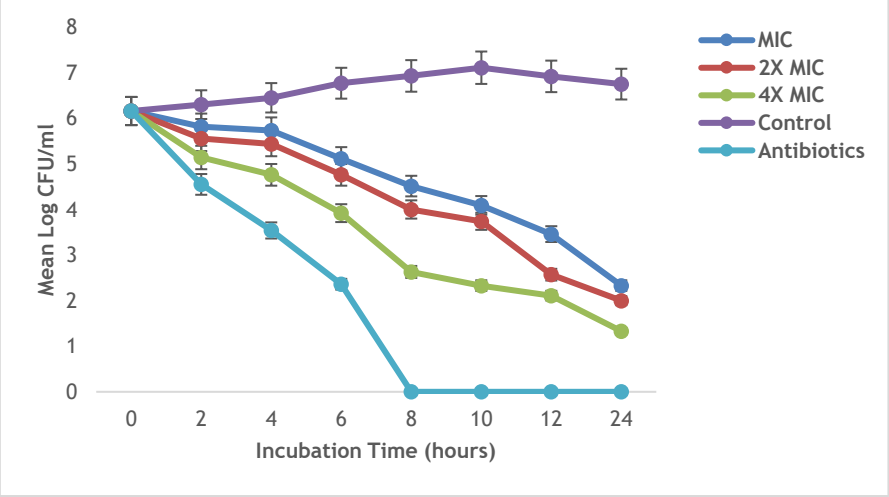

Fig. 2. Time kill kinetics of *P. dactylifera* seed oil against *K. pneumoniae*

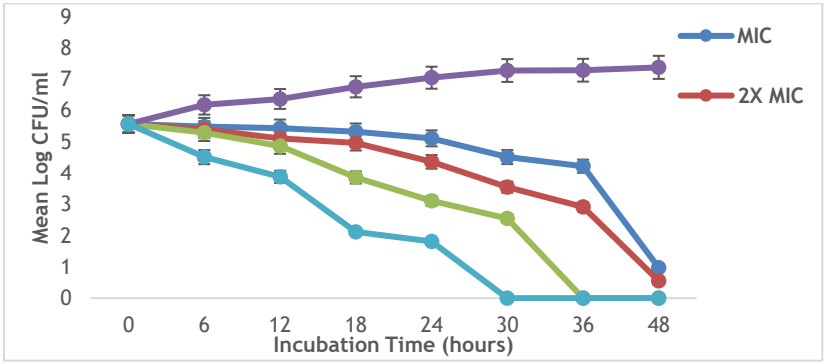

Fig. 3. Time kill kinetics of *P. dactylifera* seed oil against *C. glabrata*

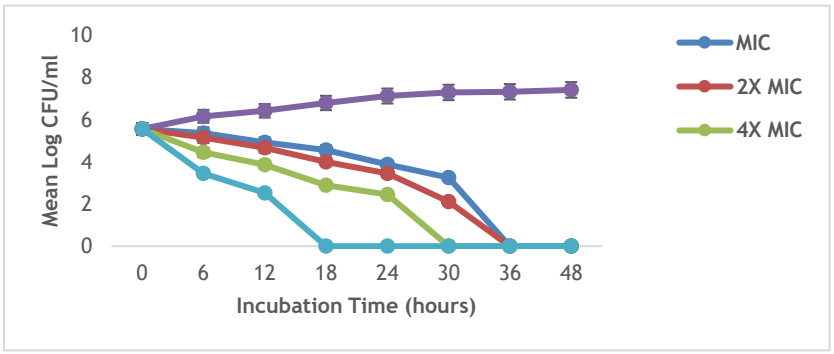

Fig. 4. Time kill kinetics of *P. dactylifera* seed oil against *C. parapsilosis*

3.3. Effect of *P. dactylifera* Seed Oil on Cellular Integrity of Test Microbial Isolates

The seed oil caused leakage of sodium and potassium ions from the test pathogens. Respectively, the highest concentration of sodium and potassium ion leakage from the test organisms were observed against *S. aureus* (31.73 ± 0.44 mg/L) and *K. aerogenes* (20.00 ± 0.61 mg/L) for bacterial isolates; and against *Candida tropicalis* (38.73 ± 0.20 mg/L; 22.70 ± 0.49 mg/L) (P≤0.05) for fungal isolates. The results are presented in Figures 5 to 8.

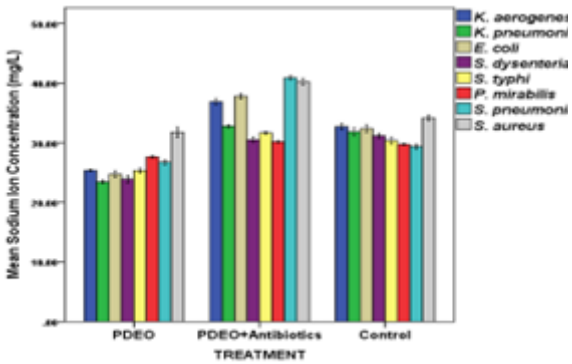

Fig. 5. Effects of *P. dactylifera* seed oil on leakage of sodium ion from antibiotic resistant bacterial isolates

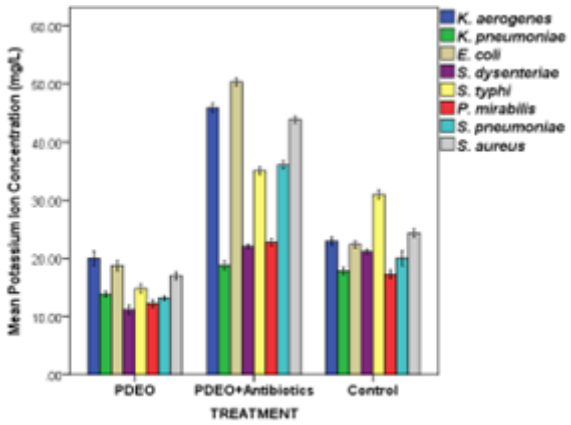

Fig. 6. Effects of *P. dactylifera* seed oil on leakage of potassium ion from antibiotic resistant bacterial isolates

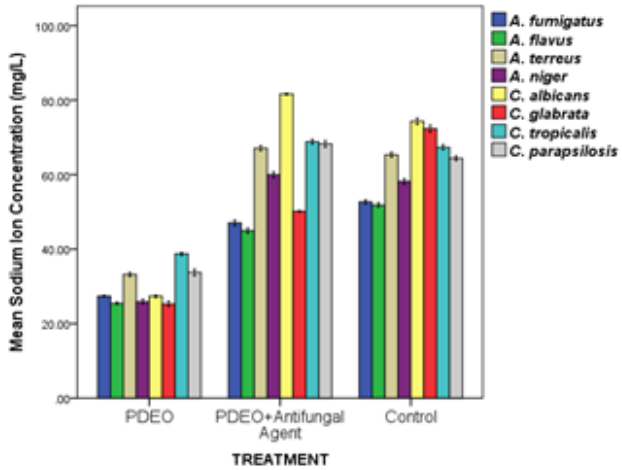

Fig. 7. Effects of *P. dactylifera* seed oil on leakage of sodium ion from fungal isolates

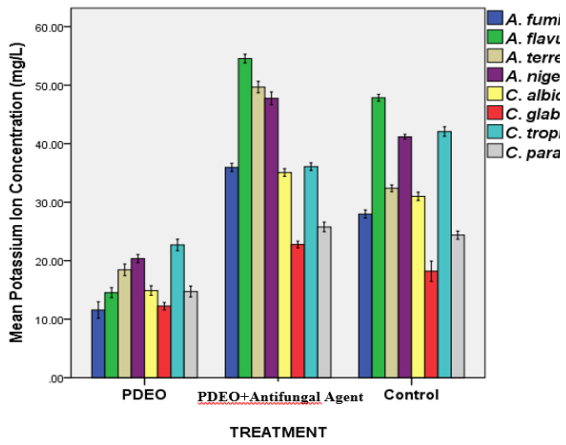

Fig. 8. Effects of *P. dactylifera* seed oil on leakage of potassium ion from antifungal resistant isolates.

4. Discussion

There is a growing interest in exploring various plant groups to uncover their potential therapeutic benefits. This surge is driven by a rich historical tradition of using plants in ethno-medicine to treat a wide range of ailments [25,26]. Essential oils represent a unique category of possible novel antimicrobial agents which is currently garnering special interest due to their chemical variety. *P. dactylifera* seed oil showed broad spectrum antimicrobial activity against the test pathogens in a concentration-dependent manner with significant differences within the bacterial and fungal species ($P<0.05$). A plethora of studies have also affirmed the activity of essential oil from diverse plants against a wide range of bacterial and fungal species [27-29]. Respectively for bacterial and fungal isolates, the highest zone of inhibition of PDEO (31.33 mm) was observed against *S. pneumoniae*, *S. aureus*, and *C. parapsilosis*. Overall, lower MIC range of values (3.91 µg/ml - 62.5 µg/ml) of the essential oil was observed against Gram-positive bacterial and fungal isolates compared to Gram-negative bacterial isolates (7.81 µg/ml – 125 µg/ml) ($P<0.05$). Amongst the Gram-positive bacterial isolates, the lowest and highest MIC values were respectively observed against *S. pneumoniae* (3.91 µg/ml) and *S. aureus* (62.5 µg/ml); while for Gram-negative bacteria, the lowest and highest MIC values were respectively observed against *K. aerogenes* (7.81 µg/ml) and *K. pneumoniae* (125 µg/ml). Whereas, amongst the fungal isolates, *C. parapsilosis, C. tropicalis, A. niger* and *A. terreus* were the most susceptible to the essential oil with MIC value of 3.91 µg/ml.

This is in agreement with the study of Elgammal et al. [30] who screened essential oil from *Cinnamomum zeylanicum* against *B. subtilis, S. aureus, E. coli, S. typhimurium, K. pneumoniae, P. vulgaris, C. albicans* and *A. niger*, and observed that MIC of the essential oil ranged from 3.3 µl/ml for Gram-positive bacteria and fungi to 10 µl/ml for Gram-negative bacterial isolates. This is further in consonance with the susceptibility trend reported by Sripahco et al. [31] on the antimicrobial efficacy of the *Elsholtzia beddomei* essential oil against seven pathogenic microorganisms (*E. coli, P. aeruginosa, E. aerogenes, S. aureus, S. epidermidis, B. subtilis* and *C. albicans*). The results from their study indicated that Gram-positive bacteria were the most significantly sensitive to *E. beddomei* essential oil with zone diameter ranging between 25.17 and 29.91 mm and MIC of 3.91 µg/mL, compared to those obtained from other tested pathogens (ZOI ranging between 19.34 and 22.23 mm, and MIC 7.81 to 15.62 µg/mL). In addition, studies on the antimicrobial activity of *Achillea setacea* essential oil against clinically important microbial strains showed that the oil was more active against Gram-positive bacteria (*S. aureus* 13.00 ± 1.00, *B. cereus* 20.00 ± 2.00) than Gram-negative bacteria (*E. coli* 8.33 ± 0.58, *S. flexneri* 8.00 ± 2.00, *S. enterica* 6.33 ± 0.58) and fungi (*C. glabrata* 10.00 ± 1.00, *A. niger* 8.00 ± 1.00) [30].

The difference in susceptibility to the essential oil might be attributed to the presence of the outer membrane (made of phospholipids and lipopolysaccharides) in Gram-negative bacteria. The passage through the outer membrane is regulated by the presence of hydrophilic channels called porins, which generally extrudes the entry of hydrophobic substances consequently restricting the diffusion of lipophilic based substances of the essential oil into the cell [21,33]. The antimicrobial efficacy of PDEO observed in this study may be attributed to the concerted activity of its constituents. Essential oils are complex mixtures of a diversity of components and their bioactivity is therefore related to their chemical nature and the amount of each constituent [34].

The time-kill kinetics of PDEO at the evaluated concentrations showed similar trend for both the bacterial and fungal species tested, with the microbial population declining with time in contrast to the negative control. However, it was observed that at the highest PDEO concentration, viable Gram-positive (*S. pneumoniae*) bacterial count reduced most rapidly between 4 to 6 hours, reaching an end point at same time (6 hours) with the positive control. Whereas, Gram-negative (*K. pneumoniae*) bacterial count declined most rapidly between 6 to 8 hours which was proceeded by a gradual decline but without an end point at 24 hours unlike the positive control which reached an end point at 8 hours. The existence of viable microbial colonies after the incubation period with PDEO could be due to the occurrence of mutant forms which survived in the presence of the essential oil concentrations and changed to vegetative forms when PDEO was withdrawn. For the fungal isolates, a faster fungicidal endpoint was attained against *C. parapsilosis* (30 hours) compared to *C. glabrata* (36 hours) at the highest PDEO concentration, their positive controls however reached an end point within a shorter time frame of 18 and 30 hours, respectively.

PDEO caused leakage of sodium and potassium ions from the test microbial cells, indicating breach of cell membrane integrity. Many biosynthetic processes within cells are mediated by enzymes activated by sodium and potassium ions [35], therefore, the release of these ions might be responsible for the observed antimicrobial activity of PDEO. The antimicrobial activity of essential oils has been attributed mainly to their hydrophobicity. This property allows them to partition within the cell membrane, increasing membrane fluidity and permeability consequently leakage of cellular constituents and compromise of vital cellular functions such as energy conversion, nutrient processing, synthesis of structural macromolecules, and the secretion of growth regulators [36].

Generally, the amount of sodium ion leaked from the microbial cells in this study is higher than its potassium ion counterpart. Although, the amount of these leaked ions varied from one organism to the other, the highest sodium ion concentration leaked was seen in *S. aureus* (31.73 ± 0.44 mg/L) and *C. tropicalis* (38.73 ± 0.20 mg/L); while the highest potassium ion concentration leaked was seen in *K. aerogenes* (20.00 ± 0.61 mg/L) and *C. tropicalis* (22.70 ± 0.49 mg/L). The differences observed in the amount of leaked sodium and potassium ion is traceable to the difference in the molecular size of these two ions, nature of the cell wall composition, as well as the size of pores on the microbial cell membrane [24,37,38].

The bioactivity elicited by PDEO against the test microorganisms in this study is remarkable, considering that these organisms are MDR strains from clinical patients. Isolates from clinical settings have been reported as notorious with reference to antibiotics resistance [39,40].

5. Conclusions

The results from this study provide reasonable evidence that *P. dactylifera* seed oil might be a veritable source of safe and effective natural antimicrobial compounds, with significant potential for use in combating antimicrobial-resistant pathogens. The findings suggest that PDEO exhibits antimicrobial activity against a range of harmful microorganisms, which is crucial in the current context of rising antimicrobial resistance (AMR). Given the growing concern over the diminishing effectiveness of conventional antibiotics, PDEO could offer an alternative or adjunct to traditional treatments, potentially alleviating some of the pressures on healthcare systems worldwide. Moreover, PDEO's natural origin and minimal toxicity further underscore its appeal as a safe therapeutic option. It could be particularly useful in developing natural-based antimicrobial formulations, offering an eco-friendly alternative to synthetic chemicals. However, to fully harness the potential of PDEO in clinical and pharmaceutical applications, additional research is necessary to explore its full antimicrobial spectrum, optimal dosages, and possible mechanisms of action. Furthermore, in order to enhance the effectiveness of both current and future antimicrobial therapies, it is important to investigate the synergistic effects of PDEO when combined with conventional antimicrobial agents. This combinatorial approach could improve the potency of existing treatments, potentially overcoming the challenges posed by antimicrobial resistance. Future studies should focus on in vitro and in vivo investigations to evaluate these synergistic interactions and establish the clinical relevance of PDEO as part of a comprehensive antimicrobial strategy.

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