

Antibacterial activity of deer musk and *Ziziphus spina-christi* against carbapenem resistant gram negative bacteria isolated from patients with burns and wounds

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Bacteria were isolated from 250 specimens obtained from patients attending the Plastic Reconstructive and Burn Surgery Hospital in the Sulaymaniyah (Kurdistan region) and the burn and wound care units of the Azadi Teaching Hospital (Kirkuk), Iraq. Gram-negative bacteria were isolated from 100 (40%) of the samples which identified by BD phoenix, 66 isolates (66%) were carbapenem-resistant species, Rapidec® Carba NP test and sensitivity tests revealed 17 different genera and species of carbapenem-resistant bacteria. The BD Phoenix system was used to evaluate the susceptibility test of the isolates to 18 different antibiotics. The number of isolates that exhibited resistance to the carbapenem antibiotics, ertapenem, imipenem and meropenem, was 40 (61%), 35 (54%) and 28 (42%) respectively. Meanwhile, the number of isolates resistant to the penicillin antibiotics, ampicillin, amoxicillin-clavulanic acid and piperacillin-tazobactam, was 61 (92%), 54 (82%) and 24 (36%) respectively. The isolates were also evaluated for their resistance to the cephalosporins, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, and ceftolozane-tazobactam; the respective number of resistant isolates were 60 (91%), 60 (91%), 39 (59%), 48 (73%), 42 (64%) and 27(41%). In contrast, a fraction of *Ziziphus spina-christi* leaves that was extracted using ethyl acetate inhibited all of the carbapenem-resistant isolates studied. The zone of inhibition (Zoi) Ø was between 19 and 24 mm. The Zoi Ø of black deer musk was 11–19 mm. The minimum inhibitory concentration (MICs) of ethyl acetate extract of *Ziziphus spina-christi* leaves for *Acinetobacter baumannii* (PDR), CR-*Citrobacter farmeri*, CR-*Escherichia coli*, CR-*Proteus mirabilis*, CR-*Pseudomonas fluorescens*, CR-*Escherichia vulneris*, CR-*Kluyvera ascorbata*, CR-*Pantoea agglomerans*, CR-*Pseudomonas putida* and CR-*Serratia marcescens*, was 6.25 mg/mL. Meanwhile, the MIC for *Acinetobacter calcoaceticus-baumannii* complex, *Citrobacter freundii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Morganella morganii*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* was 12.5 mg/mL. High-performance liquid chromatography (HPLC) was used to analyse the components of the ethyl acetate-extract of *Ziziphus spina-christi* leaves. The analysis found gallic acid (760.3 ppm/mL), caffeine (84.0 ppm/mL) and quercetin (2.2 ppm/mL); the total phenolic content was 846.5 ppm/mL. The evidence obtained from this study indicates that leaves of this plant (named sidr in the Middle East) have the potential to be used as a natural antibiotic against carbapenem-resistant Gram-negative bacteria. Thus, the leaves of this tree present an important opportunity in the development of novel therapeutic agents. The study found the MIC of deer musk for CR-*Citrobacter farmeri*, CR-*Citrobacter freundii*, CR-*Enterobacter cloacae*, CR-*Escherichia vulneris*, CR-*Klebsiella pneumoniae*, CR-*Kluyvera ascorbata*, CR-*Morganella morganii*, CR-*Pantoea agglomerans*, CR-*Proteus mirabilis*, CR-*Pseudomonas fluorescens*, CR-*Pseudomonas putida* and CR-*Serratia marcescens* to be 50 mg/mL. In contrast, the MIC of deer musk was 100 mg/mL for *Acinetobacter baumannii* (PDR), CR-*Acinetobacter calcoaceticus-baumannii* complex, CR-*Escherichia coli*, CR-*Pseudomonas aeruginosa* and CR-*Stenotrophomonas maltophilia*. The results of the gas chromatography–mass spectrometry (GC–MS) indicate that the primary constituents of musk were 1,4,4-tetramethyltetralin, 7-acetyl-6-ethyl-1, diethyl phthalate and tonalid; their contribution to the whole ranged from 12.2% to 19.6%. Black musk exhibits considerable antibacterial activity, able to inhibit seventeen different species of carbapenem-resistant Gram-negative bacteria. The non-natural form of black musk can be used to scent detergent.

Keywords: BD Phoenix; Rapidec® Carba NP; GC-mass spectrometry; HPLC; carbapenemase.

Introduction

Carbapenems are β -lactam antibiotics that exert diverse bactericidal activities and are largely unaffected by β -lactamases, including ESBLs. Due to these attributes, carbapenems are generally reserved for the 'last resort' treatments of infections caused by MDR Gram-negative bacteria. Patel & Bonomo (2010) explain that Gram-negative bacteria that synthesise carbapenemase are associated with severe hospital-acquired infections and increased mortality. Such bacteria present a significant challenge to controlling and treating infection.

There is significant global concern about the rise of carbapenem-resistant pathogens (Hamed & Hasoon, 2019). The mechanism by which resistance is most frequently conferred is the synthesis of carbapenemases,

which are enzymes that hydrolyse carbapenem, a member of the β -lactam group of antibiotics (Tarashi et al., 2016). Multiple genes are responsible for carbapenem resistance encoding for carbapenemases; these genes that encode for carbapenemases are associated with various kinds of mobile genetic elements (Nordmann, 2014). Gram negative bacteria with transmissible carbapenemase genes are considered as a major threat to public health globally, because these severely limit options for effective antibacterial treatments causing high mortality (Bush et al., 2013). Carbapenemase genes are transmitted among various kinds of nosocomial pathogens, such as *Acinetobacter baumannii*, Enterobacteriaceae family members and *Pseudomonas aeruginosa* (Thomson, 2010).

There are different kinds of β -lactamases that hydrolyse carbapenem, examples of these β -lactamases include metallo- β -lactamases (MBLs),

such as New Delhi metallo- β -lactamase (NDM) Verona imipenemase (VIM) and imipenemase (IMP), furthermore Ambler class A member, *Klebsiella pneumoniae* carbapenemase (KPC), and class D member, oxacillinase-48 (OXA-48) (Iraz et al., 2015). Globally, there is an urgent need to create new antimicrobial agents capable of treating infections caused by gram negative MDR, EDR and PDR species resistant to carbapenems, in order to solve this issue, natural products with various biologically active compounds attracted researchers' attention in order to identify new antibacterial agents capable of controlling carbapenem resistant pathogens.

Nasim et al. (2022) describe how the nature is a rich source of various kinds of compounds that could be act as an active biologic drugs such as small bio-molecules, plant-made and phytopharmaceuticals drugs.

Natural sources of antibacterial compounds, such as those found in *Ziziphus spina-christi*, a tropical plant, have been used in conventional medicine against infections because of their content of various natural antibacterial compounds (Al-Mutairi et al., 2016). This plant is also used in treating different diseases such as eye diseases, dandruff, fever and inflammation (Sarjito et al., 2021). A large amount of research indicates that *Z. spina-christi* leaves have excellent antibacterial properties that inhibit the growth of *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella* sp. and *Klebsiella* spp., and also inhibit the bacterial pathogens of aquaculture organisms (Al-Mutairi et al., 2016; Sarjito et al., 2021). There are also numerous reports of inhibition of various kinds of gram positive and gram negative bacteria by using methanol and ethanol as good solvents in extraction of different compounds from *Z. spina-christi* leaves (Temerk et al., 2017; Khaleel et al., 2019). These inhibitory effects belong to the various phytochemical compounds in leaves, such as phenols, saponins, flavonoids and alkaloids (Asgarpanah & Haghighat, 2012). These leaf compounds are also considered as source of antioxidants for pharmaceutical preparations (Khaleel et al., 2019). The diversity of phytochemical contents, *Z. spina-christi* leaves can be considered as an important natural source of antimicrobial drugs.

Deer musk is an animal natural product. Whilst numerous researchers reported the effectiveness of this natural product in inhibiting various kinds of pathogenic microorganisms, the current study is the first study that used black musk against various kinds of carbapenem-resistant Gram-negative bacteria. Various kinds of compounds present in deer musk responsible for its great scent, muscone (3-methylcyclopentadecan-one-1), are reported by Lin et al. (2004) to have medicinal properties. Musk also contains fatty acids, steroids, muco pyridine, other nitrogenous substances, paraffin, triglycerides and waxes (Thevis et al., 2013; Aljuriss et al., 2020).

The antibiotic effects of natural musk are attributed to the various alkaloid, flavonoid and sterol compounds and metabolites (Shami & Almasri, 2018). A possible mechanism by which these compounds might act is to increase cell-membrane permeability, thereby disrupting the cell environment, which in turn can result in the death of the bacterial or fungal cell. Another potential mechanism of inhibitory action is interference by some of the compounds in musk, such as volatile oils, with the synthesis of nucleic acids, leading the microbes to produce malformed or malfunctioning proteins (Saddiq, 2011; Aljuriss et al., 2020).

Natural musk is biosynthesised by animals, but the level of production is very low (Han et al., 2022). Since the demand for musk outstrips the natural supply, synthetic musk has been created to meet the huge demand (Sytniczuk et al., 2018).

Multiple researchers have explored the inhibitory effect of musk upon the growth of diverse microorganisms that are pathogenic to animals, humans and plants (Saddiq, 2011; Shami & Almasri, 2018). According to Badawy & Elleboudy (2014), not only did musk inhibit the growth of *Candida albicans*, *Cryptococcus neoformans* and *Saccharomyces cerevisiae*, but the extent to which musk inhibited growth exceeded inhibition by antibiotics. Other researchers, who state musk is a safe and natural product, also report upon musk's antifungal properties; furthermore, they describe it as being effective against *Trichomonas vaginalis* parasites (Jobori et al., 2016). Shami & Almasri (2018) reported that deer musk had antibacterial activity against several MDR bacteria.

The aim of this study was to evaluate the effectiveness of deer musk and extract of *Z. spina-christi* leaves against carbapenem-resistant Gram-negative bacteria to indicate whether these natural products offer therapeutic potential against globally prevalent carbapenem-resistant 'superbugs'.

Material and methods

This cross-sectional study used bacterial specimens from consenting patients admitted between January and July 2023 to the Plastic Reconstructive and Burn Surgery Hospital in the Sulaymaniyah/Kurdistan region and the burn and wound care units of the Azadi Teaching Hospital in Kirkuk, Iraq. Sterile cotton swabs were used to collect the specimens; 140 wound swabs and 110 burn wound swabs (total of 250 specimens) were collected. Maintaining aseptic conditions to avoid contamination, all swabs were transferred immediately to a microbiology laboratory for processing. Once the swabs reached the lab, they were immediately cultured on cetrimide agar, MacConkey agar and Drigalski lactose agar. The latter is a specialist medium that exclusively isolates the Enterobacteriaceae and various non-fermenting pathogens obtained from clinical samples. Cultures were incubated at 37 °C for 18–24 hrs then plates were reviewed and the colour, morphology and overall appearance of the colonies were evaluated.

This was performed using a BD Phoenix™ M50 device. Clinicians use the identification and susceptibility information generated by the device to help make decisions about managing and treating the patient.

Two methods were used to detect carbapenem. Firstly, a Rapidec® Carba NP kit was used. This medical kit reliably and quickly tests for bacteria that produce carbapenemase in agar cultures. Reliable test results become available within 30 to 120 mins (Hombach et al., 2015; Poirel & Nordmann, 2015; Mancini et al., 2017). The second method was to determine the susceptibility by using meropenem discs (10 µg). The Kirby Bauer disc diffusion method was employed in accordance with CLSI 2018 guidelines.

Ziziphus spina-christi leaves were collected from plants growing in Kirkuk city, Iraq. They were left in the laboratory for seven days to air dry at room temperature (37 °C). Once dried, the leaves were crushed with a mortar then put into an electric blender to turn them into a fine powder.

Precision scales were used to measure out 10 g of ground plant material with 250 mL ethyl acetate solvent, the Soxhlet extraction method was used, this apparatus consists of a Soxhlet extractor thimble with powdered leaves added to it and ethyl acetate was added into the round-bottomed-flask, condenser and reflux setup also considered as parts of Soxhlet apparatus.

A rotary evaporator was used in evaporation of solvent. The drying process was made by using the desiccator, then the vials with the extract were stored in a dark and cool place for further studies. HPLC instrument. Shimadzu HPLC was used with a C18 column and a detector with 280 nm for detection the phenolic compounds. A solution of methanol: water (80:20) was used, in order to separate the compounds in the studied extract. To obtain effective separation, the flow rate of the mobile phase kept at 1 mL/min. The volume of the studied extract by HPLC was 20 µL.

Depending on the compounds' hydrophobicity and their interactions, the C18 column performed the separation process of these compounds. For obtaining an effective detection of phenolic compounds of the studied extract, optimal HPLC chromatographic conditions existed: column pressure, temperature and other parameters.

The peak of UV absorbance and retention time were displayed by using the spectroscopy graph. For identification of the compounds of studied extract, the peaks were compared with the peaks of known phenolic standards, the final concentration of phenolic compounds present in the studied extract were calculated by making a comparative analysis against the standard solutions with known concentrations, depending on their peak areas and the recognised relationships between signal intensity and concentration. Calibration curves determined the concentration of individual phenolic compounds, by measuring the precise concentrations of the phenolic compounds present in the *Z. spina-christi* leaves' extract.

USA Agilent Technologies (5977E) device was used in identification of active elements in black musk oil. This device consists of an analytical column (Agilent HP-5ms Ultra inert (30 m length x 250 µm bore x 0.25 µm film thickness) and helium gas. The injection volume was 1 µL. The injector temperature was set to 250 °C. The oven program-temperature ramp process was as follows. Ramp 1: 3 min at 60 °C; Ramp 2: increase to 180 °C at a rate of 5 °C/min, Ramp 3: increase to 280 °C at a rate of 6 °C/min. Ramp 4: 280 °C with a 5-minute hold.

To detect and identify the active constituents of the musk oil, the samples were diluted to an appropriate concentration in accordance with established protocols before they were injected into the GC-MS equipment. The GC-MS was setup carefully as per the settings described previously. To ensure the analysis results were accurate and reliable, the analytical column, carrier gas, injection volume and temperature conditions were calibrated with precision. The separation of constituents of the sample was achieved by injecting a 1- μ L volume of musk oil into the gas chromatograph at the specified injector temperature and pressure. Then the GC oven temperature program was implemented. The mass spectrometer captured and analysed the spectra generated by the black musk oil as it moved through the chromatographic column. Spectra were within the designated m/z range. The active ingredients present within the musk oil were established by examining and interpreting the GC-MS analysis output data.

Data analysis was performed with Statistical Package for Social Science (23rd edition) using chi-square test. The threshold for statistical significance was set to $P < 0.05$.

Results

Bacteria were isolated from 250 specimens obtained from patients attending the Plastic Reconstructive and Burn Surgery Hospital in the Sulaymaniyah/Kurdistan region and the burn and wound care units of the Azadi Teaching Hospital in Kirkuk, Iraq. Gram-negative bacteria were isolated from 100 (40%) of the samples; of these, 66 (66%) were carbapenem-resistant species. As depicted in Figure 1, 17 different genera and species were reported as carbapenem-resistant bacteria. The most common was CR-*Pseudomonas aeruginosa* ($n = 24$). The other isolates identified were CR-*Escherichia coli* ($n = 10$), CR-*Enterobacter cloacae* ($n =$

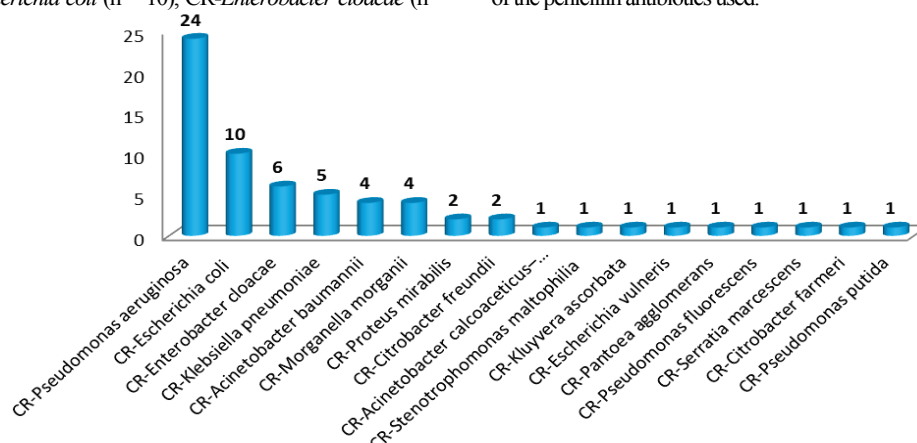


Fig. 1. Quantity and diversity of carbapenem-resistant Gram-negative bacteria recovered from specimens obtained from burn and wound patients

The isolates were also evaluated for their resistance to the cephalosporins, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime and ceftolozane-tazobactam; the respective number of resistant isolates were 60 (91%), 60 (91%), 39 (59%), 48 (73%), 42 (64%) and 27(41%). Exceptions to cephalosporin resistance were as follows: CR-*Acinetobacter calcoaceticus-baumannii* complex was sensitive to cefepime; CR-*Enterobacter cloacae* and CR-*Escherichia vulneris* were sensitive to ceftolozane-tazobactam; CR-*Pantoea agglomerans* was sensitive to ceftazidime and ceftolozane-tazobactam; CR-*Pseudomonas fluorescens* and CR-*Citrobacter farmeri* were sensitive to ceftazidime, cefepime and ceftolozane-tazobactam. Meanwhile, CR-*Serratia marcescens* was sensitive to ceftazidime, ceftriaxone and ceftolozane-tazobactam, and CR-*Morganella morganii* and CR-*Pseudomonas putida* was sensitive to ceftazidime, ceftriaxone, cefepime and ceftolozane-tazobactam. CR-*Proteus mirabilis* showed the greatest vulnerability, being sensitive to all of the cephalosporin antibiotics.

Fifty-one isolates (77%) were resistant to trimethoprim-sulfamethazazole, though CR-*Citrobacter farmeri*, CR-*Escherichia vulneris*, CR-*Pantoea agglomerans*, CR-*Proteus mirabilis* and CR-*Serratia marcescens* were still sensitive to this antibiotic. In total, 39 (59%) isolates were resistant to tigecycline, though CR-*Acinetobacter calcoaceticus-baumannii* complex, CR-*Citrobacter farmeri*, CR-*Enterobacter cloacae*, CR-

6), CR-*Klebsiella pneumoniae* ($n = 5$), CR-*Acinetobacter baumannii* ($n = 4$), CR-*Morganella morganii* ($n = 4$), CR-*Proteus mirabilis* ($n = 2$), CR-*Citrobacter freundii* ($n = 2$). Also, solitary isolates of the following were recovered: CR-*Acinetobacter calcoaceticus-baumannii* complex, CR-*Citrobacter farmeri*, CR-*Escherichia vulneris*, CR-*Kluyvera ascorbata*, CR-*Pantoea agglomerans*, CR-*Pseudomonas fluorescens*, CR-*Pseudomonas putida*, CR-*Serratia marcescens* and CR-*Stenotrophomonas maltophilia*. The BD Phoenix system was used to evaluate the susceptibility test of the isolates to 18 different antibiotics (Table 1). The number of isolates that exhibited resistance to the carbapenem antibiotics, ertapenem, imipenem and meropenem, were 40 (61%), 35 (54%) and 28 (42%) respectively. However, there were some exceptions with some species being sensitive to just one antibiotic, such as CR-*Kluyvera ascorbata* and CR-*Pseudomonas putida*, which were sensitive to imipenem, and CR-*Proteus mirabilis* and CR-*Serratia marcescens* which were sensitive to meropenem. CR-*Enterobacter cloacae* and CR-*Morganella morganii* were sensitive to both ertapenem and meropenem, while CR-*Escherichia vulneris* and CR-*Pseudomonas fluorescens* were sensitive to imipenem and meropenem. Meanwhile, CR-*Citrobacter farmeri* was sensitive to all studied three types of carbapenem antibiotics.

The number of isolates recovered from the burn and wound patients that were resistant to the penicillin antibiotics, ampicillin, amoxicillin-clavulanic acid and piperacillin-tazobactam, was 61 (92%), 54 (82%) and 24 (36%) respectively. However, there were exceptions, with CR-*Enterobacter cloacae*, CR-*Escherichia vulneris*, CR-*Morganella morganii*, CR-*Pseudomonas fluorescens*, CR-*Serratia marcescens* and CR-*Pseudomonas putida* exhibiting sensitivity to piperacillin-tazobactam. CR-*Citrobacter farmeri* was sensitive to both amoxicillin-clavulanic acid and piperacillin-tazobactam, only CR-*Citrobacter farmeri* was sensitive to all of the penicillin antibiotics used.

Escherichia coli, CR-*Pantoea agglomerans*, CR-*Pseudomonas putida* and CR-*Stenotrophomonas maltophilia* exhibited sensitivity.

Meanwhile, 43 isolates (65%) were resistant to the quinolone antibiotic, ciprofloxacin, and 36 isolates (55%) were resistant to levofloxacin, also a quinolone. However, CR-*Escherichia vulneris*, CR-*Kluyvera ascorbata* and CR-*Stenotrophomonas maltophilia* demonstrated sensitivity to levofloxacin, and CR-*Pantoea agglomerans*, CR-*Proteus mirabilis*, CR-*Pseudomonas fluorescens*, CR-*Pseudomonas putida* and CR-*Serratia marcescens* were sensitive to both ciprofloxacin and levofloxacin.

Finally, isolates were assessed for their resistance to the aminoglycosides antibiotics, amikacin ($n = 11$; 17%) and gentamycin ($n = 36$; 55%). The following isolates were sensitive to aminoglycoside: CR-*Pantoea agglomerans*, CR-*Proteus mirabilis*, CR-*Pseudomonas fluorescens*, CR-*Pseudomonas putida* and CR-*Serratia marcescens*. Finally, we found amikacin to be the most active antibiotic against the carbapenem-resistant Gram-negative bacteria isolates. CR-*Acinetobacter baumannii*, *Acinetobacter calcoaceticus-baumannii* complex and CR-*Stenotrophomonas maltophilia* were completely resistant to amikacin. Two (8%) isolates of CR-*Pseudomonas aeruginosa* and three (60%) isolates of CR-*Klebsiella pneumoniae* were 2 (8.33%) and 3 (60%) were resistant to amikacin. There was a statistically significant difference among the effects of the antibiotics on the studied carbapenem resistant gram negative bacteria ($P < 0.05$).

Table 1
Antibiogram of 66 carbapenem-resistant Gram-negative bacteria (total number (%))

Isolated Carbapenem Resistant GN by BD phoenix	Total number (%)	Amikacin a	Gentamicin	Ertapenem	Imipenem	Meropenem	Ceftazolin	Ceftoxime	Ceftazidime	Ceftioxone	Cefepime	Ceftolozane-Tazobactam	Ampicillin	Amoxicillin-Clavulane	Piperacillin-Tazobactam	Trimethoprim-Sulfamethoxazole	Ciprofloxacin	Levofloxacin	Tigecycline
CR- <i>Pseudomonas aeruginosa</i>	24 (36.4)	2(8.3)	14(58.3)	21(87.5)	17(70.8)	14(58.3)	22(91.7)	22(91.7)	14(58.3)	22(91.7)	16(66.7)	12(50.0)	22(91.7)	22(91.7)	10(41.7)	22(91.7)	16(66.7)	15(62.5)	22(91.7)
CR- <i>Escherichia coli</i>	10 (15.2)	0	1(10)	1(10)	1(10)	1(10)	9(90)	9(90)	7(70)	7(70)	7(70)	3(30)	9(90)	3(30)	1(10)	7(70)	6(60)	5(50)	0
CR- <i>Enterobacter cloacae</i>	6 (9.1)	0	5(83.3)	0	1(16.7)	0	6(100)	6(100)	5(83.3)	5(83.3)	5(83.3)	0	6(100)	6(100)	0	4(66.7)	3(50)	2(33.3)	0
CR- <i>Klebsiella pneumoniae</i>	5 (7.6)	3(60)	4(80)	4(80)	3(60)	3(60)	4(80)	4(80)	4(80)	4(80)	4(80)	4(80)	5(100)	4(80)	4(80)	3(60)	4(80)	4(80)	1(20)
CR- <i>Acinetobacter baumannii</i>	4 (6.1)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	3(75)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)
CR- <i>Proteus mirabilis</i>	2 (3.0)	0	0	1(50)	1(50)	0	0	0	0	0	0	0	0	0	0	0	0	0	2(100)
CR- <i>Morganella morganii</i>	4 (6.1)	0	2(50)	0	3(75)	0	4(100)	4(100)	0	0	0	0	4(100)	4(100)	0	4(100)	3(75)	2(50)	4(100)
CR- <i>Citrobacter freundii</i>	2 (3.0)	0	1(50)	1(50)	1(50)	1(50)	2(100)	2(100)	1(50)	1(50)	1(50)	1(50)	2(100)	2(100)	1(50)	2(100)	2(100)	2(100)	2(100)
CR- <i>Acinetobacter calcoaceticus-baumannii</i> complex	1 (1.5)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0
CR- <i>Stenotrophomonas maltophilia</i>	1 (1.5)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0	0
CR- <i>Kluyvera ascorbata</i>	1 (1.5)	0	1(100)	1(100)	0	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0	1(100)
CR- <i>Escherichia vulneris</i>	1 (1.5)	0	1(100)	1(100)	0	0	1(100)	1(100)	1(100)	1(100)	1(100)	0	1(100)	1(100)	0	0	1(100)	0	1(100)
CR- <i>Pantoea agglomerans</i>	1 (1.5)	0	0	1(100)	1(100)	1(100)	1(100)	1(100)	0	1(100)	1(100)	0	1(100)	1(100)	1(100)	0	0	0	0
CR- <i>Pseudomonas fluorescens</i>	1 (1.5)	0	0	1(100)	0	0	1(100)	1(100)	0	1(100)	0	0	1(100)	1(100)	0	1(100)	0	0	1(100)
CR- <i>Serratia marcescens</i>	1 (1.5)	0	0	1(100)	1(100)	0	1(100)	1(100)	0	0	1(100)	0	1(100)	1(100)	0	0	0	0	1(100)
CR- <i>Citrobacter farmeri</i>	1 (1.5)	0	1(100)	0	0	0	1(100)	1(100)	0	1(100)	0	0	1(100)	0	0	0	1(100)	1(100)	0
CR- <i>Pseudomonas putida</i>	1 (1.5)	0	0	1(100)	0	1(100)	1(100)	1(100)	0	0	0	0	1(100)	1(100)	0	1(100)	0	0	0
Total (%)	66 (100.0)	11(16.7)	36(54.5)	40(60.6)	35(54.0)	28(42.4)	60(90.9)	60(90.9)	39(59.1)	48(72.7)	42(63.6)	27(40.9)	61(92.4)	54(81.8)	24(36.4)	51(77.3)	43(65.2)	36(54.5)	39(59.1)

The phenotypic patterns of carbapenem resistance were explored. Those isolates that exhibited resistance to at least three classes of antimicrobials were categorised as multidrug resistance (MDR). Those isolates that were resistant to two or less classes of antimicrobials were recognised as extensively drug resistant (XDR), while pan drug resistant (PDR) refers to isolates resistant to all classes of available antimicrobial agents.

Of the 66 carbapenem-resistant Gram-negative isolates obtained in this study, 41(62%) were defined as MDR, these were *Pseudomonas aeruginosa* (n = 14), *Escherichia coli* (n = 9), *Enterobacter cloacae* (n = 5) and *Morganella morganii* (n = 4). Single MDR isolates were: *Citrobacter farmeri*, *Citrobacter freundii*, *Escherichia vulneris*, *Klebsiella pneumoniae*, *Kluyvera ascorbata*, *Pantoea agglomerans*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Serratia marcescens*. Eighteen (27%) isolates were categorised as XDR, these were *Pseudomonas aeruginosa* (n = 10), *Klebsiella pneumoniae* (n = 4), and single specimens of *Acinetobacter baumannii*, *Acinetobacter calcoaceticus-baumannii* complex, *Citrobacter freundii* and *Stenotrophomonas maltophilia*. Finally, three (5%) isolates were found to be PDR, resistant to all the antimicrobials used in the study, these were all isolates of *Acinetobacter baumannii*. There was a statistically significant difference in the distribution of MDR, XDR and PDR isolates ($P < 0.05$, Fig. 2).

The fraction of *Z. spina-christi* leaves that was extracted using ethyl acetate inhibited all of the carbapenem-resistant isolates studied. The diameter of the ZoI was between 19 and 24 mm. Meanwhile, the diameter of the ZoI of black deer musk was 11–19 mm. There was a statistically significant difference between diameter of the ZoI of ethyl acetate extract of *Z. spina-christi* leaves and black musk on the studied carbapenem resistant gram negative bacteria ($P < 0.05$, Fig. 3).

To establish the MIC, the extent of a colour-changing reaction between Resazurin dye and the extracts in presence of bacteria was measured. When bacteria are absent, such as in the negative control, Resazurin is

blue. When bacteria are present, such as in the positive control, the colour changes from blue to pink.

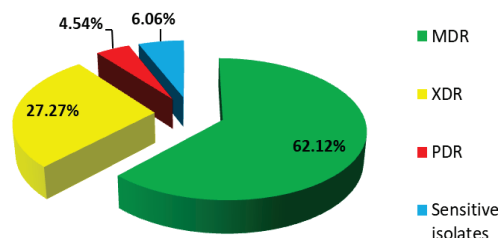


Fig. 2. The prevalence of MDR, XDR and PDR among the carbapenem-resistant Gram-negative bacteria

The MIC was determined to be the lowest concentration of plant extract able to prevent the dye from changing colour. The MIC for the ethyl acetate extract of *Z. spina-christi* leaves for *Acinetobacter baumannii* (PDR), CR-*Citrobacter farmeri*, CR-*Escherichia coli*, CR-*Escherichia vulneris*, CR-*Kluyvera ascorbata*, CR-*Pantoea agglomerans*, CR-*Pseudomonas putida*, CR-*Serratia marcescens*, CR-*Proteus mirabilis* and CR-*Pseudomonas fluorescens*, was 6.25 mg/mL. Meanwhile, the MIC for CR-*Acinetobacter calcoaceticus-baumannii* complex, CR-*Citrobacter freundii*, CR-*Enterobacter cloacae*, CR-*Klebsiella pneumoniae*, CR-*Morganella morganii*, CR-*Pseudomonas aeruginosa* and CR-*Stenotrophomonas maltophilia* was 12.5 mg/mL (Fig. 4, 5).

The study found the MIC of black musk for CR-*Citrobacter farmeri*, CR-*Citrobacter freundii*, CR-*Enterobacter cloacae*, CR-*Escherichia vulneris*, CR-*Klebsiella pneumoniae*, CR-*Kluyvera ascorbata*, CR-*Morganella morganii*, CR-*Pantoea agglomerans*, CR-*Proteus mirabilis*, CR-*Pseudomonas fluorescens*, CR-*Pseudomonas putida* and CR-*Serratia marcescens* to be 50 mg/mL.

Table 2

Distribution of MDR, XDR and PDR among the 66 carbapenem-resistant Gram-negative bacteria isolated in this study

Isolated carbapenem resistance gram negative	Total No.	Kind of Resistance	name of antibiotics	No. of antibiotics / No. of classes
CR- <i>Pseudomonas aeruginosa</i>	24	9CR-XDR	gentamicin, ertapenem, imipenem, meropenem, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	17/7
		1CR-XDR	amikacin, gentamicin, ertapenem, imipenem, meropenem, ceftazolin, cefuroxime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	17/7
		1CR-MDR	amikacin, gentamicin, ertapenem, imipenem, meropenem, ceftazolin, cefuroxime, ceftriaxone, cefepime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	15/7
		1CR-MDR	ertapenem, imipenem, meropenem, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	15/6
		1CR-MDR	gentamicin, ertapenem, imipenem, meropenem, ceftazolin, cefuroxime, ceftriaxone, cefepime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	14/7
		1CR-MDR	ertapenem, imipenem, meropenem, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	14/6
		1CR-MDR	gentamicin, ertapenem, ceftazolin, cefuroxime, ceftriaxone, cefepime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin, tigecycline	11/7
		1CR-MDR	ertapenem, ceftazolin, cefuroxime, ceftriaxone, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	10/6
		1CR-MDR	gentamicin, ertapenem, ceftazolin, cefuroxime, ceftriaxone, cefepime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, tigecycline	10/6
		3CR-MDR	imipenem, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, tigecycline	9/5
		1CR-MDR	ertapenem, ceftazolin, cefuroxime, ceftriaxone, cefepime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, tigecycline	9/5
		3CR-MRD	ertapenem, ceftazolin, cefuroxime, ceftriaxone, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, tigecycline	8/5
–	Total	10 XDR 14 CR-MDR	–	–
CR- <i>Escherichia coli</i>	10	CR-MDR	ertapenem, imipenem, meropenem, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin	15/5
		2CR-MDR	cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ampicillin, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin	9/4
		1CR-MDR	cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, ciprofloxacin, levofloxacin	9/3
		1CR-MDR	gentamicin, ceftazolin, cefuroxime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin	8/4
		1CR-MDR	cefazolin, cefuroxime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin	7/4
		1CR-MDR	cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ampicillin	7/3
		2CR-MDR	cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ampicillin, trimethoprim-sulfamethoxazole	6/2
–	Total	9CR-MDR	–	–
CR- <i>Klebsiella pneumoniae</i>	5	1CR-XDR	amikacin, gentamicin, ertapenem, imipenem, meropenem, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin	17/6
		2CR-XDR	amikacin, gentamicin, ertapenem, imipenem, meropenem, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, ciprofloxacin, levofloxacin	16/5
		1CR-MDR	gentamicin, ertapenem, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	15/7
		4CR-XDR 1CR-MDR	–	–
CR- <i>Enterobacter cloacae</i>	6	1CR-MDR	gentamicin, imipenem, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin	12/6
		1CR-MDR	gentamicin, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin	11/5
		1CR-MDR	gentamicin, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ampicillin, amoxicillin-clavulanate, ciprofloxacin	10/4
		2CR-MDR	gentamicin, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole	10/4
–	Total	5CR-MDR	–	–
CR- <i>Acinetobacter baumannii</i>	4	3 CR-PDR	amikacin, gentamicin, ertapenem, imipenem, meropenem, ceftazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	18/7

Isolated carbapenem resistance gram negative	Total No.	Kind of Resistance	name of antibiotics	No. of antibiotics / No. of classes
		1CR-XDR	amikacin,gentamicin, ertapenem, imipenem, meropenem, cefazolin, cefuroxime, ceftazidime, ceftriaxone, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	17/7
–	Total	3CR-PDR 1CR-XDR	–	–
CR- <i>Morganella morganii</i>	4	1CR-MDR	gentamicin, imipenem, cefazolin, cefuroxime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	10/7
		1CR-MDR	imipenem, cefazolin, cefuroxime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole,, ciprofloxacin, levofloxacin, tigecycline	9/6
		1CR-MDR	gentamicin, cefazolin, cefuroxime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ciprofloxacin, tigecycline	8/6
		1CR-MDR	imipenem, cefazolin, cefuroxime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, tigecycline	7/5
		1CR-MDR	imipenem, cefazolin, cefuroxime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, tigecycline	7/5
–	Total	4CR-MRD	–	–
CR- <i>Citrobacter freundii</i>	2	1CR-XDR	gentamicin, ertapenem, imipenem, meropenem, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	17/7
		1CR-MDR	cefazolin, cefuroxime, ampicillin, amoxicillin-clavulanate,, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin, tigecycline	8/5
–	Total	1 CR-XDR 1 CR-MDR	–	–
CR- <i>Acinetobacter calcoaceticus–baumannii complex</i>	1	CR-XDR	amikacin,gentamicin, ertapenem, imipenem, meropenem, cefazolin, cefuroxime, ceftazidime, ceftriaxone, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, ciprofloxacin, levofloxacin.	16/6
CR- <i>Stenotrophomonas maltophilia</i>	1	CR-XDR	amikacin,gentamicin, ertapenem, imipenem, meropenem, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, ciprofloxacin	16/6
CR- <i>Kluyvera ascorbata</i>	1	CR-MDR	gentamicin, ertapenem, meropenem, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, trimethoprim-sulfamethoxazole	13/5
CR- <i>Escherichia vulneris</i>	1	CR-MDR	gentamicin, ertapenem, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime,, ampicillin, amoxicillin-clavulanate, ciprofloxacin, tigecycline	11/6
CR- <i>Pantoea agglomerans</i>	1	CR-MDR	ertapenem, imipenem,meropenem, cefazolin, cefuroxime, ceftriaxone, cefepime, ceftolozane-tazobactam, ampicillin, amoxicillin-clavulanate	10/3
CR- <i>Pseudomonas fluorescens</i>	1	CR-MDR	ertapenem, cefazolin, cefuroxime, ceftriaxone, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, tigecycline.	8/5
CR- <i>Serratia marcescens</i>	1	CR-MDR	ertapenem, imipenem, cefazolin, cefuroxime, ceftriaxone, ampicillin, amoxicillin-clavulanate, tigecycline.	8/4
CR- <i>Pseudomonas putida</i>	1	CR-MDR	ertapenem, meropenem, cefazolin, cefuroxime, ampicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole.	7/4
CR- <i>Citrobacter farmeri</i>	1	CR-MDR	gentamicin, cefazolin, cefuroxime, ceftriaxone, ampicillin, ciprofloxacin, levofloxacin.	7/4
Total of all 66 isolates (%)	–	41 MDR (62.1%) 18 XDR (27.3%) 3 PDR (4.5%) 4 sensitive isolates (6.1%)	–	–

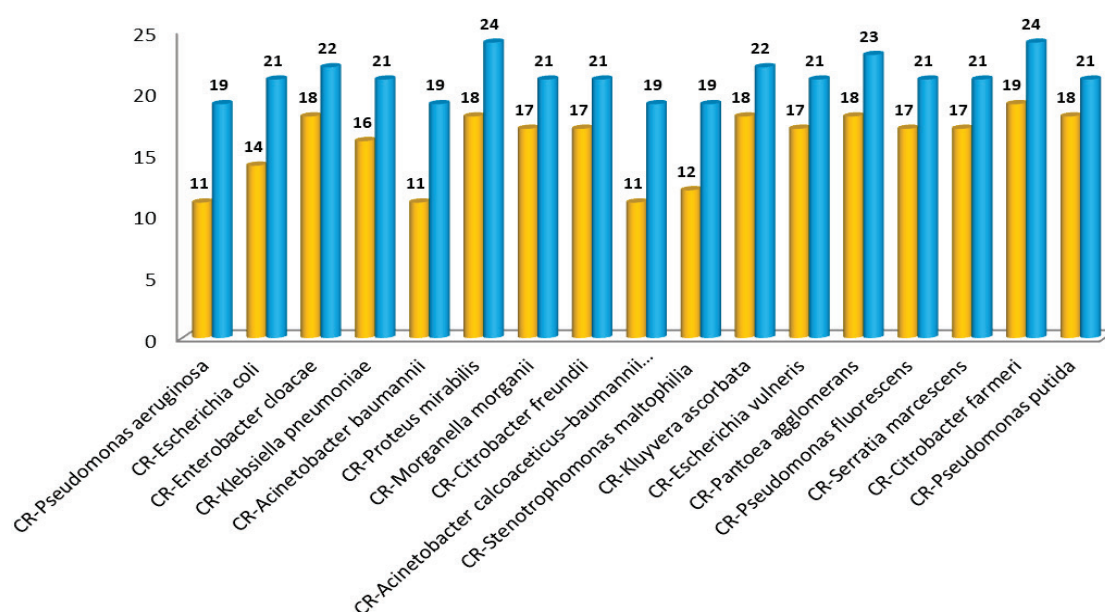


Fig. 3. The diameters of the zones of inhibition (mm) of the studied carbapenem-resistant Gram-negative isolates when incubated with *Ziziphus spina-christi* leaves extracted using ethyl acetate and black deer musk: orange – black deer musk, blue – ethyl acetate sidr leaves extract

In contrast, the MIC of black musk was 100 mg/mL for *Acinetobacter baumannii* (PDR), *Acinetobacter CR-calcoaceticus-baumannii* complex, CR-*Escherichia coli*, CR-*Pseudomonas aeruginosa* and CR-*Stenotrophomonas maltophilia*. There was a statistically significant difference between MIC of ethyl acetate extract of *Z. spina-christi* leaves and

black musk on studied carbapenem resistant gram negative bacteria ($P < 0.05$, Fig. 4, 5). HPLC analysis of the components of the *Z. spina-christi* leaf extract obtained using ethyl acetate identified gallic acid (760.3 ppm/mL), caffeine (84.0 ppm/mL) and quercetin (2.2 ppm/mL); the total phenolic content was 846.5 ppm/mL (Fig. 7, 8).

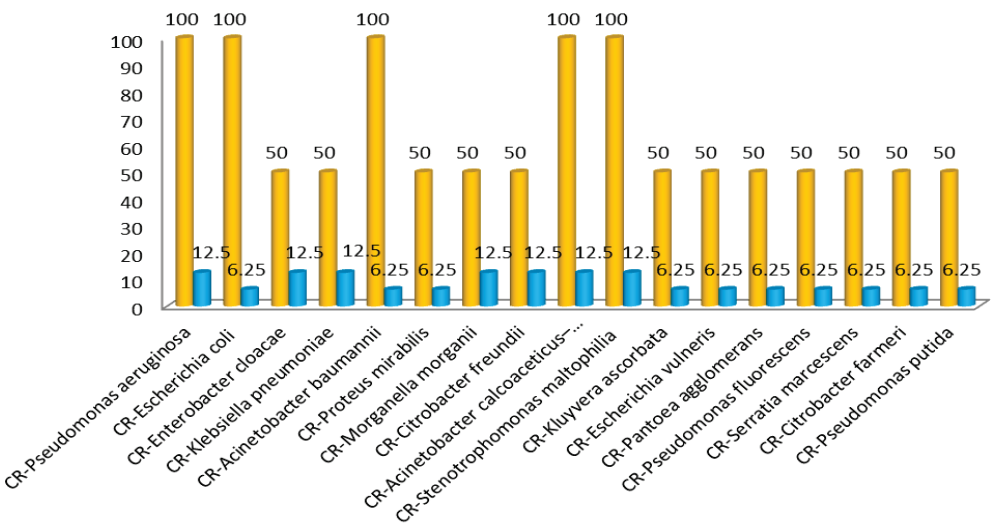


Fig. 4. The MIC of *Z. spina-christi* leaves extracted using ethyl acetate and black deer musk against the studied carbapenem-resistant Gram-negative isolates: orange – black deer musk, blue – ethyl acetate sidr leaves extract

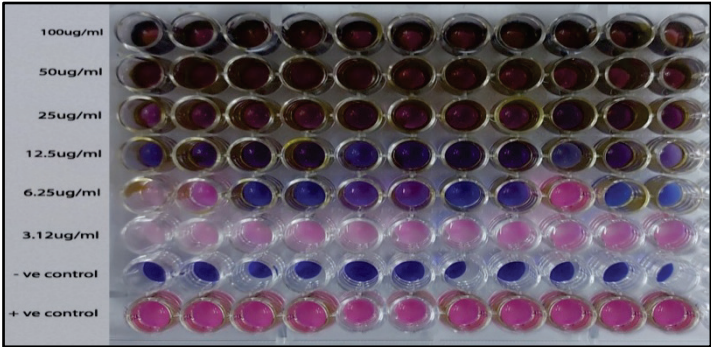


Fig. 5. Micro-titre plates showing resazurin method for determining Minimum Inhibitory Concentration of *Z. spina-christi* leaves ethyl acetate extract against the tested carbapenem resistant bacteria

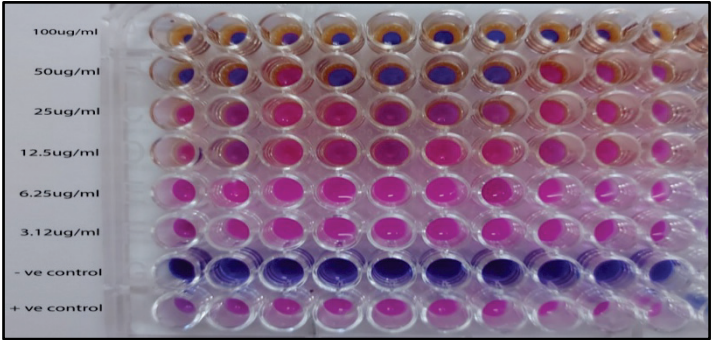


Fig. 6. Micro-titre plates showing resazurin method for determining Minimum Inhibitory Concentration of black musk against the tested carbapenem resistant bacteria

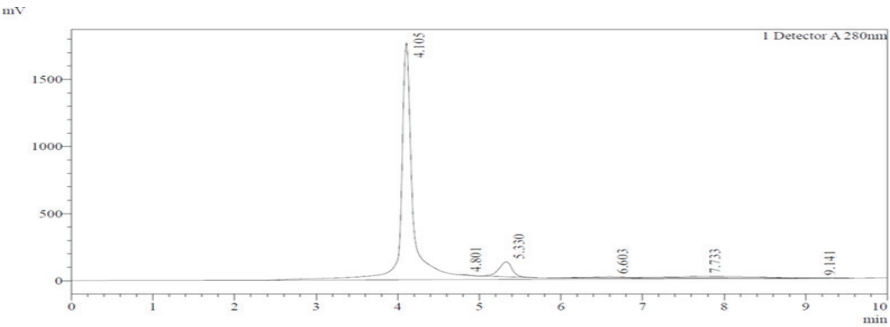


Fig. 7. HPLC chromatogram of ethyl acetate extract of *Z. spina-christi* leaves at 280 nm

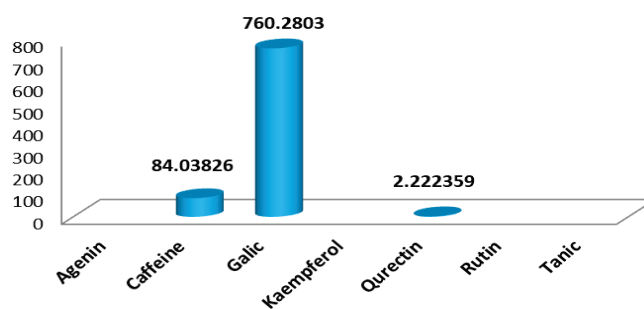


Fig. 8. HPLC analysis of the phenolic contents of the ethyl acetate extract (ppm/mL) of *Z. spina-christi* leaves compared to the standards

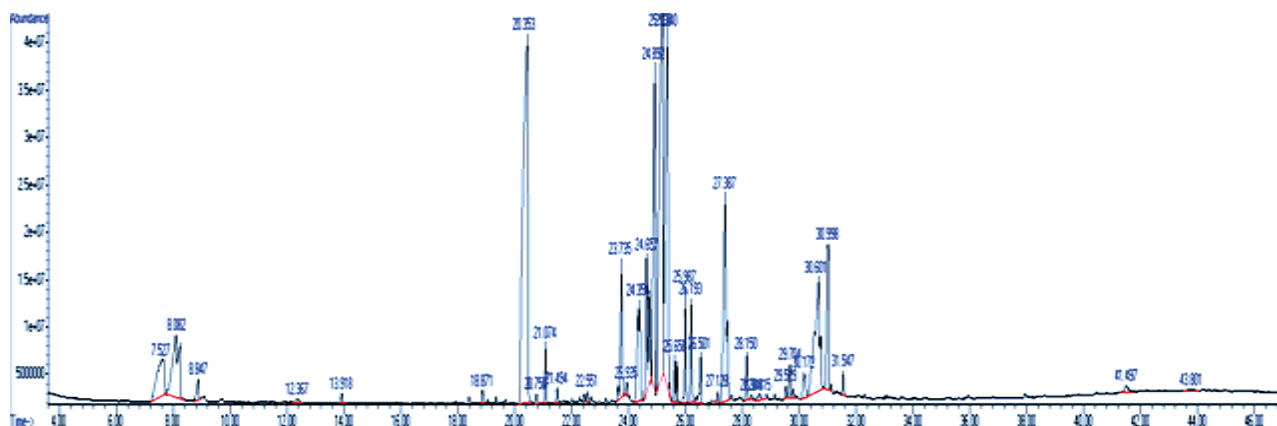


Fig. 9. GC-MS curve depicting the compounds identified in musk oil: major constituents (diethyl phthalate, 7-acetyl-6-ethyl-1,1,4,4-tetramethyltetralin, tonalid) and minor components vary in their percentages

Table 3

A comprehensive guide to the parameters and identifiers employed during the GC-MS analysis, providing a detailed overview of the compounds identified in black musk oil constituents

PK#	RT	AREA%	LIBRARY/ID	REF#	CAS#	QUAL
1	7.525	3.07	2-Propanol, 1,1'-oxybis-	15094	000110-98-5	91
2	8.079	4.58	1-Propanol, 2-(2-hydroxypropoxy)-	15107	000106-62-7	90
3	8.849	0.45	2-Butanol, 3,3'-oxybis-	32646	054305-61-2	90
4	12.363	0.14	Octadecanoic acid, 2-[(trimethylsilyloxy)-, methyl ester	205661	056196-58-8	9
5	13.921	0.16	1H-Indene, 2,3-dihydro-1,1,2,3,3-pentamethyl-	51785	001203-17-4	96
6	18.872	0.22	1,2-Benzenedicarboxylic acid, ethyl methyl ester	67333	034006-77-4	35
7	20.352	19.58	Diethyl phthalate	78783	000084-66-2	90
8	20.759	0.17	Cedrane, 8-propoxy-	114269	019870-75-8	76
9	21.071	0.78	Quinoline-2-carboxylic acid, 4-methoxy-, (1-methyl-2-oxo-2-piperidin-1-ylethyl)amide	177582	1000302-42-5	16
10	21.495	0.21	Triethyl citrate	123853	000077-93-0	50
11	22.551	0.16	1H-Indene-1-carboxylic acid, 1-ethyl-2,3-dihydro-3,3-dimethyl-, methyl ester	87716	062185-65-3	35
12	23.737	2.96	Benzyl benzoate	71178	000120-51-4	87
13	23.936	0.18	Ethanone, 1-[2,3-dihydro-1,1,2,6-tetramethyl-3-(1-methylethyl)-1H-inden-5-yl]-	109341	068140-48-7	91
14	24.351	3.46	1H-Benzimidazole, 2-(2-furanyl)-5-nitro-	85041	027146-12-9	45
15	24.654	4.19	Z-4-Tridecen-1-yl acetate	94129	065954-19-0	87
16	24.888	6.80	Oxacyclotetradecane-2,11-dione, 13-methyl-	93925	074685-36-2	87
17	25.139	15.45	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	109337	000088-29-9	87
18	25.338	12.24	Tonalid	109329	021145-77-7	99
19	25.658	0.95	Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	109339	001222-05-5	58
20	26.498	0.87	Hexadecanoic acid, methyl ester	119400	000112-39-0	97
21	27.129	0.20	2-Propenoic acid, 2-cyano-3-[4-(diethylamino)phenyl]-	97303	1000115-72-8	53
22	27.389	6.73	Musk ketone	139119	000081-14-1	64
23	28.151	0.61	Ethylene brassylate	119000	000105-95-3	91
24	28.341	0.12	Ethanone, 1-[2,3-dihydro-1,1,2,6-tetramethyl-3-(1-methylethyl)-1H-inden-5-yl]-	109341	068140-48-7	38
25	28.618	0.21	As-Indacen-1(2H)-one, 3,6,7,8-tetrahydro-3,3,6,6-tetramethyl-	84631	055591-18-9	95
26	29.527	0.24	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	139726	000112-63-0	83
27	29.700	0.71	9-Octadecenoic acid, methyl ester, (E)-	141310	001937-62-8	99
28	30.600	6.64	9-Octadecenoic acid, (E)-	129353	000112-79-8	99
29	30.998	3.56	3-Carboxy-10-methylphenothiazine	108135	107622-45-7	38
30	31.544	0.36	Propanoic acid, 3-(diphenylphosphino)-, methyl ester	121049	076734-29-7	42
31	41.497	0.30	Ethyl iso-allocholate	223770	1000043-05-3	43
32	43.800	0.13	1-Bromo-11-iodoundecane	190167	139123-69-6	15

The major constituents in black musk were diethyl phthalate, 7-acetyl-6-ethyl-1,1,4,4-tetramethyltetralin and tonalid, which ranged in their abundance from 12.2–19.6% as mentioned in Table 3. Oxacyclotetradecane-2,11-dione, musk ketone, benzyl benzoate, z-4-tridecen-1-yl acetate,

3-carboxy-10-methylphenothiazine, 1h-benzimidazole, 2-(2-furanyl)-5-nitro-, 2-propanol, 1,1'-oxybis-, 1-propanol, 2-(2-hydroxypropoxy)-, 9-octadecenoic acid, methyl ester,(E)- were moderately present constituents, ranging between 2.96% and 6.80%. Among the minor constituents were

3-carboxy-10-methylphenothiazine, propanoic acid, 3-(diphenylphosphino)-, methyl ester and ethyl iso-allocholate, all of which were present at concentrations of < 1%. At just 0.13%, the least abundant compound was 1-bromo-11-iodoundecane, categorising it as a trace constituent.

Discussion

The presence of carbapenemases in Enterobacteriaceae has been reported globally (Mahrach et al., 2019; Hasan et al., 2022). Yet few studies examining carbapenem-resistant Gram-negative bacteria have been undertaken in Iraq. The aim of this study was to determine the prevalence of carbapenem-resistant Gram-negative bacteria in the country and to use that information to promote patient outcomes in a manner that benefits the community. Using the accurate and efficient Rapidec Carba NP test, we established that 61 of the 66 carbapenem-resistant isolates synthesise carbapenemase. This figure is alarmingly high and it points to carbapenemase production being the primary carbapenem resistance mechanism used by pathogenic bacteria in Iraq. Globally, carbapenemase-producing pathogens present a significant threat to health. This finding is consistent with other research undertaken in Spain, Iraq and Italy (Suay-García & Pérez-Gracia, 2019; Haji et al., 2021; Aurilio et al., 2022). Two isolates of *P. aeruginosa* and one isolate each of *Acinetobacter baumannii*, *Khyvera ascorbata* and *Stenotrophomonas maltophilia* tested negative with the Rapidec Carba NP test. As the antibiotic sensitivity test results revealed these isolates to be resistant to carbapenem, it can be concluded that their resistance mechanism is not due to carbapenemase production, rather the resistance exhibited by these isolates arises from porin-mediated mechanisms that limit the uptake of carbapenems and/or efflux pumps that return carbapenem to the outside of the cell (Elshamy & Aboshanab, 2019; Haji et al., 2021; Aurilio et al., 2022).

This study used the BD Phoenix™ M50 system to determine the antibiotic susceptibility of carbapenem-resistant Gram-negative bacteria to eighteen different antibiotics. The number of isolates recovered from burn and wound patients that exhibited resistance to the carbapenem antibiotics, ertapenem, imipenem and meropenem were 40 (61%), 35 (54%) and 28 (42%) respectively. There are three key mechanisms considered responsible for carbapenem resistance, these are porin-mediated resistance, which limit the cell's uptake of carbapenems; efflux pumps, which return imported carbapenem to the extracellular environment; and most commonly, the biosynthesis of hydrolysing enzymes, β -lactamases, which deactivate carbapenems and other β -lactam antimicrobials. The latter mechanism is considered the most serious, as carbapenemases able to deactivate the resistance mechanism pose the greatest threat, as these enzymes can inactivate most β -lactams. Furthermore, the genes that code for these enzymes are carried on mobile genetic elements, such as transposons and plasmids, meaning the genes can be transferred horizontally to other species of bacteria (Elshamy & Aboshanab, 2020).

While the high level of resistance to β -lactam antibiotics (penicillins and cephalosporins) is attributed to the biosynthesis of β -lactamase, there are also changes in the penicillin-binding proteins targeted by antimicrobial drugs and changes in the permeability of the bacterial outer membrane (Hasan & Abass, 2019).

The resistance shown by 51 isolates (77%) to trimethoprim-sulfamethoxazole could be due to the loss of bacterial drug-binding ability, altered cell permeability and changes in the structure or overproduction of dihydrofolate reductase. The synthesis of plasmid-mediated dihydrofolate reductases, which have a low affinity for trimethoprim, is the most important inherent resistance mechanism (Perelomov et al., 2023).

Thirty-nine isolates (59%) showed resistance to tigecycline, a tetracycline. There are several mechanisms considered responsible for tetracycline resistance, and multiple mechanisms may be deployed. These include acquiring mobile genetic elements that bear genes that specifically confer resistance to tetracycline, chromosomal mutations and/or mutations of the ribosomal binding site, which results in the increased expression of intrinsic resistance mechanisms. Three general class-specific mechanisms that confer resistance to tetracyclines have been studied extensively: efflux, enzymatic deactivation and ribosomal protection (Grossman, 2016).

This study counted 43 isolates (65%) that were resistant to ciprofloxacin and 36 isolates (55%) were resistant to levofloxacin, both of which

are quinolone antibiotics. The mechanisms deemed responsible for the majority of ciprofloxacin-resistant bacterial infections are chromosomal mutations of DNA gyrase and topoisomerase IV, the horizontal transfer of resistance genes by plasmids, changes to the number or types of porins and the increased expression of efflux pumps (Kim & Hooper, 2014; Redgrave et al., 2014).

Finally, isolates were identified which were resistant to the aminoglycosides antibiotics, amikacin (n = 11; 17%) and gentamycin (n = 36; 55%). Serio et al. (2018) report that the resistance mechanisms responsible for aminoglycoside resistance are decreased cell permeability, changes in the ribosomal binding sites or the biosynthesis of enzymes that modify aminoglycosides.

Amikacin was the most active antibiotic in this study. However, CR-*Acinetobacter baumannii*, CR-*Acinetobacter calcoaceticus-baumannii* complex and CR-*Stenotrophomonas maltophilia* were completely resistant to amikacin. Two (8%) isolates of CR-*Pseudomonas aeruginosa* and three (60%) isolates of CR-*Klebsiella pneumonia* were 2(8.3%) and 3(60%) were resistant to amikacin.

Divergence in the prevalence rate of bacterial resistance between different studies can be accounted for by differences in the type of clinical specimens investigated, population level of exposure to antimicrobial drugs and the culture of hygiene in the population (Ahmed Hasan et al., 2021; Ahmed Hasan & Mohammed Bakr, 2022; Fakhraddin Raheem & Ahmed Hasan Ali, 2022).

Due to their increased occurrence, MDR profile, and presence of transmissible genetic elements (e.g. insertion sequences, transposons and plasmids), the risks associated with clinically important carbapenemase-producing pathogens are three-fold. The latter capacity to transfer genetic elements occurs rapidly and the elements can be passed between species, as well as between different genera of Gram-negative bacteria). Furthermore, other antimicrobial resistance genes can also be passed on via these transmissible genetic elements, giving rise to MDR or XDR characteristics. This significantly curtails the scope of antibiotic treatments (Workneh et al., 2019; Hasan, 2022).

It is concerning that there was a high incidence of MDR, XDR and PDR profiles in the isolates recovered in this study. MDR was the most common resistance pattern observed. Indeed, CR-*Klebsiella pneumoniae* and CR-*Pseudomonas aeruginosa* isolates were resistant to 15 different antibiotics from seven classes of antibiotics. Furthermore, one isolate each of CR-*Acinetobacter baumannii* and CR-*Citrobacter freundii* exhibited an XDR resistance pattern, being resistant to 17 antibiotics from seven different classes. Disturbingly, three *Acinetobacter baumannii* isolates were resistant to all of the antibiotics used in the study, classifying these isolates as PDR.

There is a growing trend of reports of MDR, XDR and PDR isolates occurring globally. In particular, greater numbers of Gram-negative bacteria carrying the carbapenemase gene are being reported. Those isolated in this study might reflect an increase in selective pressure arising from self-medication, as well as the overapplication and empirical use of carbapenems and third generation cephalosporins. The issue might be compounded by inadequate monitoring of MDR isolates present in hospitals, especially in burns units, ICUs and surgical wards. Sadeghi et al. (2016) argue that for these reasons, it is imperative that greater numbers of effective monitoring programmes be implemented. Furthermore, there is urgent need to identify and devise novel compounds and pharmaceuticals capable of minimising the morbidity and mortality caused by carbapenem-resistant MDR, XDR and PDR bacteria. To this end, this study evaluated the antibiotic effects of *Z. spina-christi* leaves and black deer musk upon the isolates obtained from the patients. The study also endeavoured to determine their antibiotic mechanisms of action against the carbapenem-resistant Gram-negative isolates.

The results indicate that the fraction of *Z. spina-christi* leaf extracted using ethyl acetate exerted excellent antibacterial activity against all of the carbapenem-resistant Gram-negative isolates. It inhibited the growth of MDR, XDR and PDR isolates. This was attributed to the high gallic acid content, which indicates its high solubility in ethyl acetate. It is believed that gallic acid penetrates the cell membranes and chelates calcium ions within the cell, leading to the death of the bacterium (Sarjit et al., 2015). Rajamanickam et al. (2019) and Selvaraj et al. (2022) describe gallic acid

as a potent antimicrobial product. The ethyl acetate extract also had a significant effect upon the content of caffeine, which might also have potential as an effective antimicrobial agent. Caffeine is an attractive compound and worthy of more research because it is ubiquitous, cheap and is generally only harmful to humans in high doses (Gaul & Kelly, 2015). Caffeine alters the morphology of some bacteria and stimulates cell lysis in other types of bacteria (Dash & Gummadi, 2008).

Quercetin was also present in the leaf treatment extracted with ethyl acetate solvent. Quercetin is recognised as being able to inhibit the growth of fungi, viruses and various Gram-positive and Gram-negative bacteria. Quercetin's antimicrobial action comes from changing the permeability of the bacterial cell membrane, disrupting the membrane and causing dysfunction of mitochondria, inhibiting nucleic acid and protein synthesis, reducing the expression of virulence factors and preventing formation of biofilms to form. It can also inhibit the growth of various drug-resistant microorganisms so there is the possibility that it could be useful to treat drug-resistant strains (Nguyen & Bhattacharya, 2022).

This study found the total phenolic content of *Z. spina-christi* leaf treatment extracted with ethyl acetate to be high. Phenolic compounds from natural sources are recognised as possessing potent antimicrobial capabilities, effective against diverse clinically relevant pathogens. Furthermore, such compounds can increase the sensitivity of MDR strains to antibiotics with bactericidal or bacteriostatic activity (Ecevit et al., 2022). The diversity and wealth of variously structured phenolic compounds is tremendous, meaning there is considerable potential to identify phenols suitable for inhibiting the growth of bacteria. The functional groups present on phenols can work against bacteria in different ways, such as damaging the cell membrane, inhibiting enzymes and intercalating DNA (Lobiuc et al., 2023).

The result of this study revealed that the leaves of the sidr plant contain some major bioactive compounds that inhibit the growth of all tested carbapenem resistant gram negative bacteria, thereby proving very effective as a natural product source of antibiotics, as shown by many other studies (Temerk et al., 2017; Khaleel et al., 2019; Sarjito et al., 2021).

Natural musk is revealed to have numerous beneficial physiological properties such as anti-inflammatory, anti-tumour and cardiac effects, it prevents ischaemic cardiac disorder and exerts bidirectional effects upon the central nervous system (Fan et al., 2017; Ali et al., 2018). It is included in the Chinese Pharmacopoeia. Wang et al. (2022) state that natural musk is a common component of many drugs. There are a number of compounds present in musk. As well as being responsible for its characteristic scent, musk's primary compound, muscone (3-methylcyclopentadecanone-1), is the active compound with medicinal properties (Lai, 1976). However, in this study which used Abdulsamad Al Qurashi Black Deer musk, no muscone was detected by GS-MS. Nonetheless, the musk used in this study did exert antibacterial action against 17 different species and genera of carbapenem-resistant Gram-negative bacteria. The MIC of the Musk ranged from 50 to 100 mg/mL, which echoes the results published by others (Saddiq, 2014; Yosef Shami & Almasri, 2018; Aljuriss et al., 2020) who report that deer musk exhibits bactericidal and bacteriostatic effects upon fungal growth and MDR bacteria. Based upon the absence of muscone in this study, it is concluded that there are other active compounds present in musk which give it curative effects.

The Chinese Pharmacopoeia (2015) suggests that the standard that determines the quality of musk is exclusively the concentration of muscone, which should exceed 2%. However, Jiang et al. (2018) argue that determining the quality of musk by the single parameter of muscone concentration is inadequate, they suggest that the quality is a product of various chemical components, including esters, fatty acids, macrocyclic ketones, polypeptides, pyridine and steroids; thus, they recommend that comprehensive evaluations and chemical fingerprint chromatography be performed to establish quality. Wang (2011) concurs, stating that muscone concentration alone is not adequate to determine the quality of musk. Wang suggests the quality of musk be evaluated from a combined index of muscone, androstanols and cholesterol, which should all exceed 2% (Wang, 2011). Consequently, assessing the quality of musk simply upon its concentration of muscone as stated by the Chinese Pharmacopoeia (2015) is inadequate. In Li et al.'s study (2016), the researchers evaluated the constituents of musk in different deer animals and reported that the

concentrations of androstanols and cholesterol could be better markers for determining the quality of deer musk, rather than focusing only on the muscone concentration (Wang et al., 2022).

Depending on the results of GC-Mass of black musk, diethyl phthalate was the highest compound in Abdul Samad Al Qurashi Black Deer Musk, which is a synthetic substance (clear and colorless liquid without significant odor), used in perfumes manufacturing, cosmetics, aspirin and many other products. In the last few years, using of phthalates in perfumes gained high attention because these chemicals intentionally are sometimes added as a solvent and a fixative (Al-Saleh & Elkhatib., 2016). Synthetic fragrance musk 7-acetyl-6-ethyl-1,1,4,4-tetramethyltetralin (AETT) is present in fragrances (Wisneski et al., 1982) while the tonalid, considered as an important fragrance ingredient with widespread use (Han et al 2022), a kind of synthetic polycyclic musk with the chemical name of 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (AHMT), is considered as an outstanding substitute to nitro musk due to its excellent fragrance, stable chemical properties and safety (Xu & Cao et al., 2016). It is considered as the most important commercial synthetic polycyclic musk with global consumption of 5000–10000 tons per year (Homem et al., 2015).

This study found artificial black musk to exert wide spectrum antibacterial activity and have a pleasant scent, the aim of this study was to identify novel antibacterial agents from natural sources that are capable of inhibiting drug-resistant bacteria. We found artificial musk to be effective against diverse bacteria, particularly carbapenem-resistant Gram-negative species.

Conclusion

Several of the isolates of carbapenemase-producing Gram-negative bacteria obtained were categorised as being MDR, XDR or PDR for multiple classes of antimicrobials. This can be considered to signal the use of multiple therapies to treat the diseases caused by drug-resistant pathogens.

The recommendation that arises from the results of this study is for vigilant antimicrobial susceptibility monitoring to identify promptly the presence of multidrug-resistant isolates. Also, efforts should be increased to limit the use of antibiotics in hygienic practices. To that end, Iraq's Ministry of Health should consider implementing monitoring units to ensure that pharmacies do not dispense antibiotics without a doctor's prescription and ensure that doctors routinely perform susceptibility tests and carbapenemase detection tests before prescribing antibiotics to patients. The ministry could consider imposing legal penalties on doctors and pharmacists who violate safe practices. The threats posed by the rapid increase in antimicrobial resistance, resulting in MDR, XDR or PDR carbapenemase-producing Gram-negative bacteria, cannot be underestimated.

The leaves of the *Z. spina-christi* plant are an effective, natural product that can be used to treat infections caused by carbapenem-resistant Gram-negative bacteria. The leaves warrant further study as a potential source of new antibiotic drugs. Black musk exhibited a wide spectrum of antibacterial activity, able to inhibit 17 different species of carbapenem-resistant Gram-negative bacteria. Artificial black musk, which has a pleasant smell, is useful as a detergent.

References

- Abd El Wahab, M. G., Ali, S. S., & Ayuob, N. N. (2018). The role of musk in relieving the neurodegenerative changes induced after exposure to chronic stress. *American Journal of Alzheimer's Disease and Other Dementias*, 33(4), 221–231.
- Ahmed Hasan, S., & Mohammed Bakr, M. (2022). Bacteriological and molecular detection of *Klebsiella oxytoca* and its resistance to antibiotics among clinical specimens from Kirkuk, Iraq. *Archives of Razi Institute*, 77(5), 1521–1525.
- Ahmed Hasan, S., Fakhraddin Raheem, T., & Mohammed Abdulla, H. (2021). Phenotypic, antibiotyping, and molecular detection of *Klebsiella pneumoniae* isolates from clinical specimens in Kirkuk, Iraq. *Archives of Razi Institute*, 76(4), 1061–1067.
- Alhassan, K. A., Indabawa, A. S., & Shah, M. M. (2019). Phytochemical analysis, proximate composition and antibacterial activities of *Ziziphus* species (*Z. jujube* and *Z. spina christi*). *Journal of Applied and Advanced Research*, 4(1), 42–46.
- Al-Jobori, K. M., Al-Ameed, A. I., & Witwit, N. M. (2016). *In vitro* antifungal activity of musk. *Didactic Journal of Medicinal Plant Research*, 1(1), 1–6.

- Aljuriss, J. A., Alnajem, N. S., Alghofaili, F., Al-Arifi, T. N., & AL-Khattaf, F. S. (2020). Antagonistic effect of musk on organisms different types. *International Journal of Current Pharmaceutical Research*, 12(5), 90–94.
- Al-Mutairi, M. H., Ali, S., Aly, S. M., & Aldebasi, Y. (2016). Antibacterial activity of sidr (*Ziziphus spina-christi*), leaves extract against selected pathogenic bacteria. *European Journal of Pharmaceutical and Medical Research*, 3(5), 138–144.
- Al-Saleh, I., & Elkhatib, R. (2016). Screening of phthalate esters in 47 branded perfumes. *Environmental Science and Pollution Research International*, 23(1), 455–468.
- Asgarpanah, J., & Haghighat, E. (2012). Phytochemistry and pharmacologic properties of *Ziziphus spina christi* (L.) Willd. *African Journal of Pharmacy and Pharmacology*, 6(31), 2332–2339.
- Aurilio, C., Sansone, P., Barbarisi, M., Pota, V., Giaccari, L. G., Coppolino, F., Barbarisi, A., Passavanti, M. B., & Pace, M. C. (2022). Mechanisms of action of carbapenem resistance. *Antibiotics*, 11(3), 421.
- Badawy, A. F., Elleboudy, N. A., & Hussein, H. M. (2014). Assessment of *in vitro* anti-*Trichomonas vaginalis* activity of deer musk. *International Journal of Advanced Research*, 2(8), 668–678.
- Bush, K., Pannell, M., Lock, J. L., Queenan, A. M., Jorgensen, J. H., Lee, R. M., Lewis, J. S., & Jarrett, D. (2013). Detection systems for carbapenemase gene identification should include the SME serine carbapenemase. *International Journal of Antimicrobial Agents*, 41(1), 1–4.
- Dash, S. S., & Gummadi, S. N. (2008). Inhibitory effect of caffeine on growth of various bacterial strains. *Research Journal of Microbiology*, 3, 457–465.
- Ecevit, K., Barros, A. A., Silva, J. M., & Reis, R. L. (2022). Preventing microbial infections with natural phenolic compounds. *Future Pharmacology*, 2(4), 460–498.
- Elshamy, A. A., & Aboshanab, K. M. (2020). A review on bacterial resistance to carbapenems: Epidemiology, detection and treatment options. *Future Science OA*, 6(3), fso438.
- Fakhreddin Raheem, T., & Ahmed Hasan Ali, S. (2022). Prevalence and multi-drug resistance patterns of uropathogenic *E. coli* isolated from women patients in Kirkuk City, Iraq. *Iranian Journal of Medical Microbiology*, 16(6), 609–614.
- Fan, D. C., Qi, J. Y., & Zhang, M. Z. (2017). Insights of Chinese medicine on ventricular remodeling: Multiple-targets, individualized-treatment. *Chinese Journal of Integrative Medicine*, 23(9), 643–647.
- Gaula, J., & Kelly, D. (2015). Caffeine and its effect on bacteria growth. *The Journal of Biological Sciences*, 1, 4–8.
- Grossman, T. H. (2016). Tetracycline antibiotics and resistance. *Cold Spring Harbor Perspectives in Medicine*, 6(4), a025387.
- Haji, S. H., Aka, S. T. H., & Ali, F. A. (2021). Prevalence and characterisation of carbapenemase encoding genes in multidrug-resistant Gram-negative bacilli. *PLoS One*, 16(11), e0259005.
- Hamed, S. L., & Hasoon, N. (2019). Molecular characterization of carbapenemase-producing Gram-negative bacteria isolated from clinical specimens in Baghdad, Iraq. *Journal of Pure and Applied Microbiology*, 13(2), 647–652.
- Han, Y., Liu, Y., Wang, S., Ge, X., Wang, X., & Qiu, T. (2022). High-efficiency and safe synthesis of tonalid via two Friedel-Crafts reactions in continuous-flow microreactors. *Chinese Journal of Chemical Engineering*, 52, 126–135.
- Hasan, S. A. (2022). *Pseudomonas aeruginosa* and the multifactorial antibiotic resistance. *Eurasian Medical Research Periodical*, 11, 85–94.
- Hasan, S. A., & Abass, K. S. (2019). Prevalence of gram negative bacteria isolated from patients with burn infection and their antimicrobial susceptibility patterns in Kirkuk City, Iraq. *Indian Journal of Public Health Research and Development*, 10(8), 2197–2201.
- Hasan, S. A., Raoof, W. M., & Kamal Rachid, S. (2022). A systematic review: The current status of carbapenem resistance in Iraq. *World Bulletin of Public Health*, 13, 88–94.
- Homem, V., Silva, J. A., Ratola, N., Santos, L., & Alves, A. (2015). Long lasting perfume – a review of synthetic musks in WWTPs. *Journal of Environmental Management*, 149, 168–192.
- Iraz, M., Özad Düzgün, A., Sandalli, C., Doymaz, M. Z., Akkoyunlu, Y., Saral, A., Peleg, A. Y., Özgülmüş, O. B., Beriş, F. Ş., Karaoğlu, H., & Çopur Çiçek, A. (2015). Distribution of β -lactamase genes among carbapenem-resistant *Klebsiella pneumoniae* strains isolated from patients in Turkey. *Annals of Laboratory Medicine*, 35(6), 595–601.
- Jiang, Q. Y., Luo, Y., Tan, T., Yang, M., & Liao, Z. G. (2018). Comparison of volatile chemical compositions of muscus from different species and producing areas by GC-MS and chemometric analysis. *Chinese Journal of Experimental Traditional Medical Formulae*, 24(3), 49–55.
- Khaleel, S. M., Jaran, A. S., & Al-Deeb, T. M. F. (2019). Antimicrobial and lipid peroxidation inhibition potential of *Ziziphus spina-christi* (Sedr), a Jordanian medicinal plant. *Journal of Biological Sciences*, 19(2), 131–136.
- Kim, E. S., & Hooper, D. C. (2014). Clinical importance and epidemiology of quinolone resistance. *Infection and Chemotherapy*, 46(4), 226–238.
- Lai, J. H. (1976). *Pharmacognosy*. Chuang-I Press, Taipei.
- Li, D., Chen, B., Zhang, L., Gaur, U., Ma, T., Jie, H., Zhao, G., Wu, N., Xu, Z., Xu, H., Yao, Y., Lian, T., Fan, X., Yang, D., Yang, M., Zhu, Q., & Satkoski Trask, J. (2016). The musk chemical composition and microbiota of Chinese forest musk deer males. *Scientific Reports*, 6, 18975.
- Lin, D. L., Chang, H. C., & Huang, S. H. (2004). Characterization of allegedly musk-containing medicinal products in Taiwan. *Journal of Forensic Sciences*, 49(6), 1187–1193.
- Lobiuc, A., Pavl, N. E., Mangalagiu, I. I., Gheorghita, R., Teliban, G. C., Amăriucă-Mantu, D., & Stoleru, V. (2023). Future antimicrobials: Natural and functionalized phenolics. *Molecules*, 28(3), 1114.
- Nasim, N., Sandeep, I. S., & Mohanty, S. (2022). Plant-derived natural products for drug discovery: Current approaches and prospects. *The Nucleus*, 65(3), 399–411.
- Nguyen, T. L. A., & Bhattacharya, D. (2022). Antimicrobial activity of quercetin: An approach to its mechanistic principle. *Molecules*, 27(8), 2494.
- Nordmann, P. (2014). Carbapenemase-producing Enterobacteriaceae: Overview of a major public health challenge. *Medecine et Maladies Infectieuses*, 44(2), 51–56.
- Patel, G., & Bonomo, R. A. (2011). Status report on carbapenemases: Challenges and prospects. *Expert Review of Anti-Infective Therapy*, 9(5), 555–570.
- Perelomov, L., Sizova, O., Gertsen, M., Perelomova, I., Arlyapov, V., & Atroshchenko, Y. (2023). Antibiotic resistance in metal-tolerant microorganisms from treatment facilities. *Antibiotics*, 12(12), 1678.
- Pinheiro, P. F., Menini, L. A. P., Bernardes, P. C., Saraiva, S. H., Carneiro, J. W. M., Costa, A. V., Arruda, T. R., Lage, M. R., Gonçalves, P. M., Bernardes, C. O., Alvarenga, E. S., & Menini, L. (2018). Semisynthetic phenol derivatives obtained from natural phenols: Antimicrobial activity and molecular properties. *Journal of Agricultural and Food Chemistry*, 66(1), 323–330.
- Rajamanickam, K., Yang, J., & Sakharkar, M. K. (2019). Gallic acid potentiates the antimicrobial activity of tulathromycin against two key bovine respiratory disease (BRD) causing pathogens. *Frontiers in Pharmacology*, 9, 1486.
- Redgrave, L. S., Sutton, S. B., Webber, M. A., & Piddock, L. J. (2014). Fluoroquinolone resistance: Mechanisms, impact on bacteria, and role in evolutionary success. *Trends in Microbiology*, 22(8), 438–445.
- Saddiq, A. A. (2011). Impact of fungal content of some Arabic nuts to induce kidney toxicity and agonistic action of natural resources. *African Journal of Microbiology Research*, 5(9), 1046–1056.
- Saddiq, A. A. N. (2014). Antiagnostic effect of musk and sidr leaves on some of the opportunistic fungi that cause lung toxicity. *Life Science Journal*, 11(2), 99–108.
- Sadeghi, M. R., Ghotaslou, R., Akhi, M. T., Asgharzadeh, M., & Hasani, A. (2016). Molecular characterization of extended-spectrum β -lactamase, plasmid-mediated AmpC cephalosporinase and carbapenemase genes among Enterobacteriaceae isolates in five medical centres of East and West Azerbaijan, Iran. *Journal of Medical Microbiology*, 65(11), 1322–1331.
- Sarjit, A., Wang, Y., & Dykes, G. A. (2015). Antimicrobial activity of gallic acid against thermophilic *Campylobacter* is strain specific and associated with a loss of calcium ions. *Food Microbiology*, 46, 227–233.
- Sarjito, P., Purnamayati, L., Riyadi, P. H., Desrina, & Prayitno, S. B. (2021). Phytochemical analysis and antibacterial activities of sidr leaf extract (*Ziziphus spina-christi*) against pathogenic bacteria in aquaculture. *Pertanika Journal of Tropical Agricultural Science*, 44(4), 845–864.
- Selvaraj, S., Amaral, J. M., & Murty, V. R. (2022). Kinetics and antimicrobial activity of gallic acid by novel bacterial co-culture system using Taguchi's method and submerged fermentation. *Archives of Microbiology*, 204(9), 584.
- Serio, A. W., Keepers, T., Andrews, L., & Krause, K. M. (2018). Aminoglycoside revival: Review of a historically important class of antimicrobials undergoing rejuvenation. *EcoSal Plus*, 8(1), 2.
- Shahzad, M., Millhouse, E., Culshaw, S., Edwards, C. A., Ramage, G., & Combet, E. (2015). Selected dietary (poly)phenols inhibit periodontal pathogen growth and biofilm formation. *Food and Function*, 6(3), 719–729.
- State Pharmacopoeia Commission of the PRC (2015). *Chinese pharmacopoeia*. The Pharmacopoeia Committee of the People's Republic of China. People's Medical Publishing House. Vol. 1. Pp. 384–385.
- Suay-García, B., & Pérez-Gracia, M. T. (2019). Present and future of carbapenem-resistant Enterobacteriaceae (CRE) infections. *Antibiotics*, 8(3), 122.
- Sytniczuk, A., Leszczyńska, A., Kajetanowicz, A., & Grela, K. (2018). Preparation of musk-smelling macrocyclic lactones from biomass: Looking for the optimal substrate combination. *ChemSusChem*, 11(18), 3157–3166.
- Tarashi, S., Goudarzi, H., Erfanimesh, S., Pormohammad, A., & Hashemi, A. (2016). Phenotypic and molecular detection of metallo-beta-lactamase genes among imipenem resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains isolated from patients with burn injuries. *Archives of Clinical Infectious Diseases*, 11(4), e39036.
- Temerk, H. A., Salem, W. M., Sayed, W. F., & Hassan, F. S. (2017). Antibacterial effect of phytochemical extracts from *Ziziphus spina-christi* against some pathogenic bacteria. *Egyptian Journal of Botany*, 57(3), 595–604.
- Thevis, M., Schänzer, W., Geyer, H., Thieme, D., Grosse, J., Rautenberg, C., Flenker, U., Beuck, S., Thomas, A., Holland, R., & Dvorak, J. (2013). Traditional Chinese medicine and sports drug testing: Identification of natural steroid administration in doping control urine samples resulting from musk (pod) extracts. *British Journal of Sports Medicine*, 47(2), 109–114.
- Thomson, K. S. (2010). Extended-spectrum-beta-lactamase, AmpC, and carbapenemase issues. *Journal of Clinical Microbiology*, 48(4), 1019–1025.

- Villalobos, M. del C., Serradilla, M. J., Martín, A., Ordiales, E., Ruiz-Moyano, S., & Córdoba, M. de G. (2016). Antioxidant and antimicrobial activity of natural phenolic extract from defatted soybean flour by-product for stone fruit postharvest application. *Journal of the Science of Food and Agriculture*, 96(6), 2116–2124.
- Wang, H. (2011). Study on the quality control of moschus. East China University of Science and Technology, Shanghai.
- Wang, Y., Sun, M., Chang, F., Wang, J., Wang, Y., Tang, J., Zhang, K., Gao, L., Xue, X., & Wan, Y. (2022). The essential differences in microbial and chemical components of musk of different qualities secreted by captive male forest musk deer (*Moschus berezovskii*). *Microbial Biotechnology*, 15(6), 1783–1794.
- Wisneski, H. H., Yates, R. L., & Davis, H. M. (1982). Gas chromatographic determination of synthetic musk (7-acetyl-6-ethyl-1,1,4,4-tetramethyltetralin) in fragrances. *Journal of the Association of Official Analytical Chemists*, 65(3), 598–601.
- Workneh, M., Yee, R., & Simner, P. J. (2019). Phenotypic methods for detection of carbapenemase production in carbapenem-resistant organisms: What method should your laboratory choose? *Clinical Microbiology Newsletter*, 41(2), 11–22.
- Xu, L., & Cao, Y. (2016). Native musk and synthetic musk ketone strongly induced the growth repression and the apoptosis of cancer cells. *BMC Complementary and Alternative Medicine*, 16(1), 511.
- Yosef Shami, A., & Almasri, A. (2018). Bacteriostatic and bactericidal activity of deer musk on multidrug resistance bacteria. *Pakistan Journal of Biological Sciences*, 21(7), 331–339.