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The antibacterial activity of nano-encapsulated basil and cinnamon essential oils against certain multidrug-resistant bacteria recovered from infected wounds

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Abstract

This study was conducted to evaluate the antibacterial activity of nano-encapsulated essential oils of basil (*Ocimum basilicum*) and cinnamon (*Cinnamomum verum*) against multidrug-resistant (MDR) bacteria. A total of 115 wound swab samples were collected from patients admitted to Naser Institute, Cairo, Egypt, suffering from wounds discharge; pain, swelling, foul-smelling, delayed and non-healing wound infections. Six genera of bacteria were isolated from the collected swab samples, and then identified using conventional biochemical methods and API 20 kits. *Staphylococcus aureus* was found to be the most prevalent isolate (26.1 %), following by *Pseudomonas aeruginosa* (25.2 %), *Klebsiella pneumonia* (23.5 %), *Acinetobacter baumannii* (12.2 %), *Proteus vulgaris* (7.8 %), and the less common isolate of *Escherichia coli* (5.2 %). Among 14 antibiotics tested *in vitro* for their susceptibility using the standard disk diffusion assay, results showed that imipenem was the most efficient antibiotic against most of the tested Gram (-) and Gram (+) isolates followed by meropenem. Currently, all the recovered bacterial isolates were MDR. The nano-encapsulated basil oil (NEBO) and nano-encapsulated cinnamon oil (NECO) showed potential antibacterial potentials against all the tested MDR bacteria. Results of testing the antibacterial potential of the NEBO and NECO demonstrated that the encapsulation process protected the oils from oxidation, and consequently enhanced their antibacterial potencies. It could be concluded that the nano-encapsulated essential oils act as promising antibacterial agents against the MDR bacteria.

Keywords: Essential oils, Nano-encapsulation, Multidrug-resistant bacteria, Antibacterial potency

1. Introduction

The multidrug resistant bacteria (MDR) are defined as those bacteria that cause diseases and withstand the action of the antibacterial drugs, leading to enormous economic and human losses. More than 700,000 people pass away every year all over the world due to the improper use of antibiotics, where the bacterial pathogens develop resistance to the traditional therapy (Willyard, 2017). For example, *Staphylococcus aureus* which is well known as a methicillin-resistant bacterium caused about 20,000 blood-borne infections, and more than 20,000 correlated deaths in the United States at 2017 (Kourtis *et al*., 2019). Additionally, Iovleva and Doi, (2017) reported that carbapenem-resistant Enterobacteriaceae was regarded as a public health threat, and requires fast and invasive measures. There are several issues related to the traditional use of antibacterial drugs including diminished stability; low water solubility, drug targeting complexity, minimum oral bioavailability and the compliance of patient depression, due to the frequent administration of drugs and their consequent potential toxicity (Parisi *et al*., 2017). Recently, the World Health Organization (WHO) reported the increased prevalence of the antibiotic-resistant bacteria, which represents the main human health challenge (Eleraky *et al*., 2020). The availability of novel antibacterial drugs seems a very complex process, due to the susceptibility of production of new safe and effective drugs, in addition to high costs of production and the long time required for approval of such new drugs (Parisi *et al*., 2017).

The long term administration of antibiotics generates unbearable toxicity and other adverse effects, and hence, the application of nano-materials may be a promising strategy to control infections of the MDR bacteria (Natan and Banin, 2017; Baptista *et al*., 2018; Muzammil *et al*., 2018). Nanoparticles (NPs) have a therapeutic promise due to their unique chemical and physical properties (Beyth *et al*., 2015; Hemeg, 2017), and are reported to exhibit antibacterial potentials and are able to eliminate growth of the MDR bacteria (Slavin *et al*., 2017). Nevertheless, the application of NPs to the clinical use needs comprehensive understanding of their physicochemical properties; their pharmacodynamics, pharmacokinetics and bio-distribution (Burdusel *et al*., 2018). During the past decade, several studies were conducted to discover new antimicrobial essential oils derived from medicinal plants (Rudramurthy *et al*., 2016), due to their high contents of chemical substances that belong to various families such as aldehydes; terpenes, esters, ethers, alcohol, ketones and phenolics (Akhtar *et al*., 2014). However, some chemical constitutes of these essential oils loss their activities, due to the changes in their physical and chemical properties caused by the different environmental factors (Abdel-Wahhab *et al*., 2018). The encapsulation of these essential oils is important to prevent undesirable changes in their components (Arpagaus *et al*., 2018). Thus, application of nanotechnology is a promising candidate to improve the stability and physicochemical properties of the essential oils, and allows their human use as potent antibacterials against the MDR bacteria (Patra *et al*., 2018). Therefore, the objectives of the current study were to formulate the encapsulated cinnamon and basil essential oils, and to evaluate their antibacterial efficacies against the MDR bacteria that cause wound infections.

2. Materials and methods

2.1. Essential oils

The essential oils used in this study were provided by Prof. Mosaad A. Abdel-Wahhab, Food Toxicology and Contaminants Department, National Research Centre, Dokki, Giza, Egypt. According to the supplier, Gas chromatography–mass spectrometry (GC-MS) analysis reported that the basil essential oil is composed of 48 compounds mainly; Linalool, 1,8-Cineole, (z)-isoeugeno, α-trans-bergamotene, 1-epi-cubenol, (Z)-Anethole, trans-muurola-4-(14),5-diene

and €-Caryophyllene (El-Nekeety *et al*., 2021). On the other hand, Mohammed *et al*., (2020) reported that cinnamon essential oil contained 16 compounds including; cinnamaldehyde; 1,8Cineole, acetic acid, 1,7,7 trimethylbicyclo [2.2.1] hept2yl ester, α-Pinene and α- Terpineol.

2.2. Encapsulation and characterization of the essential oils

Whey protein (WP) recovered from Whey was used at a concentration of 10 % in dist. water as a wall material. The solution was stirred for 1 h, incubated overnight at room temperature, and then Tween 80 was added to the polymer mixture as an emulsifier. The essential oils (cinnamon and basil) were successively added to the polymer solution with homogeneous shaking at 20000 rpm for 10 min. to form an emulsion. The concentration of the polymer was 20 %, and the used amount of the essential oils was 10 % of the mass of the polymer concentration, according to Jinapong *et al*., (2008). The emulsion solution was encapsulated using the spray drying method in reference to Abdel-Wahhab *et al*., (2018). The prepared emulsion was spray dried using laboratory Mini spray dryer (BÜCHI, Labortechnik, Switzerland) with a 1.5-mm nozzle diameter and main spray chamber of 500 × 215 mm. The emulsion was then pumped to the dryer by a peristaltic pump with a flow rate of 5 cm^3/ min. The flow rate of the drying air was adjusted to 2.5 m^3/ min. and at a pressure of 0.06 MPa. The inlet and outlet air temperatures were 180-195 and 71-75 °C, respectively. The powdered microcapsules were stored for further characterization in air-tight desiccators. Morphological characterization of the produced nano-encapsulated cinnamon (NECO) and basil (NEBO) oils was analyzed using the Transmission electron microscope (TEM) (JEOL JSM6300 SEM, Tokyo, Japan), as described by Mohammed *et al*., (2017). In brief, the fresh samples of NECO and NEBO were placed individually onto a carbon-coated copper grid to form a thin liquid film, and then were negatively stained by adding one drop of uranyl acetate. The excess of staining solution was dragged using a filter paper, and the film was air-dried before observation. An Orius 1000 CCD camera (GATAN, Warrendale, PA, USA) was used for the image acquisition. The average diameter, zeta potential (ZP) and size distribution of the NECO and NEBO were measured using a particle size analyzer (Nano-ZS, Malvern Instruments Ltd., UK). For measuring zeta potential, each sample was sonicated for 30-60 min. before assessment, in reference to Abdel-Wahhab *et al*., (2018).

2.3. Collection of swab samples

About 115 wound swab samples were collected from patients admitted to the Naser Institute, Cairo, Egypt, from both sexes with ages that ranged from 14-60 years with complaints of discharge; pain, swelling, foul-smelling, delayed and non-healing wound infections. Sterile cotton swabs were used for samples collection by gently swabbing the interior surface of the infected area, and then the swabs were transported immediately to the microbiology laboratory for bacterial isolation.

2.4. Isolation and identification of the bacteria

Each swab sample was streaked individually onto the surface of 3 Petri plates of McConkey agar; blood agar and nutrient agar (NA) (Oxoid Ltd. Co, Nepean, ON, Canada), and then all plates were incubated at 37°C for 24 h. After incubation, the growing bacterial colonies were observed through the formation of macroscopic colonies. The isolated bacteria were purified, and then identified phenotypically using cell morphology and Gram staining. Biochemical identification of the isolates was carried out using several biochemical assays, according to Forbes *et al*., (1998); Federal Drug Administration. (2001). Furthermore, characterization of the bacterial isolates was confirmed using API 20 kits, as recommended by the manufacturer's instructions (Biomereux, France).

2.5. Antibiotic sensitivity of the bacterial isolates

The antibacterial susceptibility was performed on Mueller-Hinton Agar (MHA) medium using the standard disk diffusion method (Mueller and Hinton,

1941; Raja and Singh, 2007). The 14 used antibiotics that were recommended by NCCLS. (1979) and the concentration of each antibiotic per disc (µg/ disc) included; amikacin (30 µg), ampicilin (10 µg), amoxicillin (10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), efotaxime (10 µg), cefaclor (30 µg), gentamicin (10 µg), meropenem (10 µg), imipenem (10 µg), ofloxacin (5 µg), tetracycline (30 µg) and tobramycin (10µg). After incubation, the zone of inhibition around each antibiotic disc was measured using a calibrated ruler.

2.6. Antibacterial potential of the essential oils

Using MHA medium, about 20 ml of this medium was dispensed into sterile Petri plates, seeded with 100 µl of 10^8 cells/ ml of broth culture of each tested MDR bacteria, and then spread on the plate surface using a sterile glass spreader (El-Mahmood and Doughari, 2009). Filter paper discs (6 mm) were impregnated with different concentrations (10-50 µl) of 40 % encapsulated essential oils (NECO and NEBO), and then placed aseptically on the surface of the seeded plates. After incubation of the plates for 24 h at 37°C, the diameters of inhibition zones surrounding the discs were measured (mm) using a calibrated ruler. Three replicate plates were used for each tested bacterial strain, and the assay was repeated 3 times.

2.7. Statistical analysis

Three replicates plates were used for each tested concentration of the essential oils, and the assay was replicated 3 times. The significances of the tested essential oils based on the zones of inhibition were determined using analysis of variance (ANOVA) at the 5 % level. Means were compared with Duncan's multiple range test of SAS statistical analysis system.

3. Results and Discussion

3.1. Composition of the essential oils

Essential oils (EO) are complex aromatic substances derived from plants, which are composed mainly of complex mixture of polar and non-polar compounds including terpenes (Dhifi *et al*., 2016). According to El-Nekeety *et al*. (2021), GC-MS analysis of the basil essential oil used in the current study revealed the presence of 48 compounds. In accordance, other studies also revealed that Linalool was the major compound that exists in basil essential oil (Benedec *et al*., 2013; Amor *et al*., 2021). However, a previous study conducted by Olugbade *et al*., (2017) stated that the major components of basil oil were methyl eugenol followed by methyl chavicol, whereas another study of Ghasemi Pirbalouti *et al*., (2017) reported that methyl chavicol was the major compound followed by linalool. On the other hand, the GC-MS analysis of cinnamon essential oil used in this study was carried out by Mohammed *et al*., (2020), and notified the presence of 16 compounds. Similar results were previously obtained by Wang *et al*., (2009); Abdel-Wahhab *et al*., (2018), who reported that trans-cinnamaldehyde was the major compound present in cinnamon essential oil. Conversely, Raina *et al*., (2001) found that eugenol, linalool and pipertone were the major 3 components in ECO, and the recorded concentrations of these compounds were 76.6 %, 8.5 % and 3.3 %, respectively. The variation between these results may be attributed to several factors such as; the existence of numerous botanical varieties, cultivars and chemotypes (Filip, 2017), climatic factors (Milenkovic *et al*., 2019), and the part of the plant used during extraction of the oil (Kaul *et al*., 2003).

3.2. Characterization of the NECO and NEBO

The TEM analysis demonstrated that NEBO (Fig. 1A) and NECO (Fig. 1B) have a smooth rounded shape, whereas the size analyses revealed that the average particle size of NEBO was 120 nm, while the average size of NECO was 130 nm. The recorded ZP for NEBO and NECO were -4.73 and -17.85 mV, respectively. These current results expressed that the use of WP enhanced the droplet coalescence, in agreement with Goula and Adamopoulos, (2012). Moreover, the uniform size distribution of the oil droplets and their smooth round shape indicated the formation of a droplet wall material by WP (Xu *et al*.,

2013; Eratte *et al*., 2014). A previous study conducted by Roger *et al*., (2010) reported that the shape, surface property and size of the particles have critical roles in the uptake of nanosized particles by the cells, and added that the size between 50-300 nm has a favorable uptake than the other counterparts. Besides, Sadat *et al*., (2016) revealed that the size of NPs affects the distribution, clearance and the pharmacokinetics of these NPs. Meanwhile, ZP plays an important role in the distribution of the oil droplets and increases their stability (McClements and Rao, 2011). The negative charged ZP reported in this study for the NEBO and NECO might be attributed to the negative charge of the carboxylate group in WP, which is the only functional charge in the globulars of WP, in agreement with Eratte *et al*., (2014).

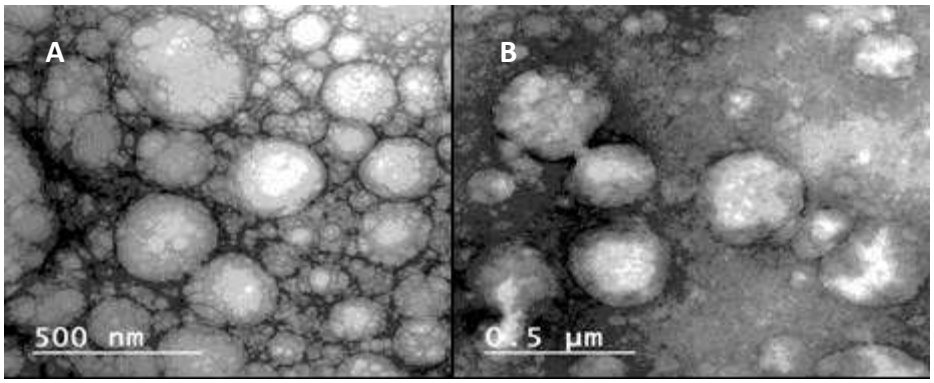

Fig. 1. TEM images of NEBO (A) and NECO (B), showing their smooth rounded shape and the average particle size of NEBO was 120 nm, while the average size of NECO was 130 nm

3.3. Identification of the isolated bacteria

In this study, a total of 115 pure bacterial isolates were recovered from the collected swab samples. Results of the phenotypic characterization and the characteristics obtained by API 20 kits indicated that these 115 isolates belong to 6 different genera of bacteria including; *E. coli*, *Staphylococcus aureus*, *A. baumannii*, *P. aeruginosa*, *K. pneumonia* and *Proteus vulgaris* (Table 1). The frequency of these bacterial strains are arranged in descending order according to their prevalence as *Staphylococcus aureus* (30 isolates, 26.1 %) > *P. aeruginosa* (29 isolates, 25.2 %) > *K. pneumoniae* (27 isolates, 23.5 %) > *A. baumannii* (14 isolates, 12.2 %) > *Proteus vulgaris* (9 isolates, 7.8 %) > *E. coli* (6 isolates, 5.2 %), as shown in Table (2). These strains are common contaminants of wounds in agreement with the previous results reported by Otokunefo and Brown, (1990); Adebayor *et al*., (2003); Mohammed *et al*., (2017).

3.4. Antibiotics susceptibility assay

The antibiotic resistance pattern of all the identified strains is given in Table (3). Results showed that all *Staphylococcus aureus* strains were resistant to amoxicillin and cefaclor, while most of these strains were resistant to amikacin (93.3 %), ampicillin (96.7 %), cefotaxime (96.7 %), ceftazidime (93.3 %), ceftriaxone (93.3 %), ciprofloxacin (90 %), gentamicin (80 %), ofloxacin (76.7 %), tetracycline (93.3 %) and tobramycin (80 %).

Table 1: Biochemical characteristics of the recovered bacterial isolates

Isolates identification	Biochemical characteristics																				
	Catalase	Coagulase	Indole	Methylered	Oxidase	Vogus proskauer	Citrate utilization	H_2S production	Urease	Motility	Lysine decarboxylase	Gelatin liquefaction test	Glucose	Lactose	Maltose	Mannitol	Sucrose	Salicin	L-arbinose	D-Sorbitol	Blood hemolysis
A. baumannii	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-
E. coli	+	-	+	+	-	-	-	-	-	+	-	-	+	+	+	+	-	-	+	+	-
K. pneumoniae	+	-	-	-	-	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	-
Proteus vulgaris	+	-	+	+	-	-	-	+	+	+	-	+	+	-	+	-	+	+	-	-	-
P. aeruginosa	+	-	-	-	+	-	+	-	-	+	-	+	-	-	-	+	-	-	-	-	-
Staphylococcus aureus	+	+	-	+	-	+	+	-	+	-	-	+	+	+	+	+	+	-	-	-	+

Where; "+" indicates positive result; "-" indicates negative result

Table 2: Frequency of the bacterial isolates recovered from wound samples

Bacterial isolates	Frequency	%
A. baumannii	14	12.2
E. coli	6	5.2
K. pneumoniae	27	23.5
Proteus vulgaris	9	7.8
P. aeruginosa	29	25.2
Staphylococcus aureus	30	26.1
Total	115	100

However about 73.3 % and 76.7 % of *Staphylococcus aureus* strains were sensitive to meropenem and imipenem, respectively. Similarly, resistance of *Staphylococcus aureus* bacteria that was isolated from infected wounds and cases of bacteraemia was reported previously by Takeda *et al*., (2000). The mechanism of this resistance may be attributed to several genetic factors including; mutations, ticking of the cell wall, production of ß-lactamase and modification of the receptor site(s) specific for antibiotics (Hiramatsu *et al*., 2014). The results presented in Table (3) also showed the antibiotic profile of *P. aeruginosa* strains, which expressed resistance to amikacin; ampicillin; cefotaxime; ceftazidime; ceftriaxone; ciprofloxacin; gentamicin; ofloxacin; tetracycline and tobramycin The resistance of these strains was reported previously by Harvey *et al*., (2010), who suggested that *P. aeruginosa* is able to make biofilms in the patients, and this bacterium could also be transmitted from the wounds to the blood circulation; leading to either gastrointestinal or urogenital infections as recorded in the AIDS patients. The antibiotic sensitivity profiles of *K. pneumonia*, *A. baumannii*, *Proteus vulgaris* and *E. coli* (Table 3) revealed that *K. pneumoniae* strains showed susceptibility to imipenem (7.4 %) and meropenem (11.1 %). On the other hand, about 100 % of *A. baumannii* strains can resist amikacin; amoxicillin, ampicillin and cefaclor; however, *Proteus vulgaris* strains were susceptible to imipenem and meropenem by 100 % and 88.9 %, respectively. Furthermore, all *E. coli* strains showed sensitivity to meropenem and imipenem. Although 50 % of these *E. coli* strains were susceptible to ceftazidime and ceftriaxone; however, 100 % of them expressed resistance to tetracycline. The remaining antibiotics showed variability in their effects against *E. coli* strains. The antibiotic resistance pattern of *K. pneumoniae*, *Proteus vulgaris* and *E. coli* was reported previously by several authors (Todar, 2007; Li *et al*., 2015; Fodor *et al*., 2020), and the mechanisms of this resistance may be probably attributed to genetic reasons or due to modifications of the cell source proteins, as reported by previous studies conducted by Enan, (2006a, b); Ouda *et al*., (2014); Abdel-Shafi *et al*., (2014). The antibiotic resistance of all the tested bacterial strains that were isolated from wounds confirmed the relationship between these bacteria, wound infections and wounds associated bacteremia.

3.5. Antibacterial potency of NEBO and NECO

In the present study, NEBO and NECO were applied as antibacterial agents. The data presented in Table (4), Fig. (2, 3) represent the antibacterial efficacy of various concentrations of NEBO and NECO (10 µl, 20 µl, 30 µl, 40 µl and 50 µl) against the currently identified six MDR bacterial strains. Results indicated NEBO and NECO have antibacterial efficacy against either all or some of the tested strains. NEBO showed a strong inhibitory effect against *Staphylococcus aureus* strains and a weak inhibitory effect on *Proteus vulgaris* and *E. coli* strains, but it did not show any inhibitory activity against *P. aeruginosa*, *K. pneumonia* and *A. baumannii*. However, NECO exhibited a strong antibacterial potency against *Staphylococcus aureus*, *A. baumannii*, *Proteus vulgaris* and *E. coli*; however, a good inhibitory effect was expressed against *P. aeruginosa* and *K. pneumoniae*.

Previous reports showed that NEBO exhibited significant antibacterial potential against both Gram (-) and Gram (+) bacteria, where the Gram (+) bacterial strains expressed more sensitivity to this oil (Suppakul *et al*., 2003; Hussain *et al*., 2008; Vanin *et al*., 2014; Dhifi *et al*., 2016; Amor *et al*., 2021). This effect may be attributed to the high contents of Linalool, which was reported to have a strong antibacterial activity against Gram (+) than Gram (-) bacterial strains (Ebrahim Sajjadi, 2006; Sokovic and Van Griensven, 2006). This compound may cause disruption of the bacterial cell membrane leading to the cell death.

Table 3: The antibiotic susceptibility/ resistance pattern (%) of the recovered bacterial isolates

Antibiotic discs	*Staphylococcus aureus*		*P. aeruginosa*		*K. pneumoniae*		*A. baumannii*		*Proteus. vulgaris*		*E. coli*	
	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
Amikacin (30 µg)	93.3	6.7	82.8	17.2	85.2	14.8	100	0	77.8	22.2	66.7	33.3
Amoxicillin (10 µg)	100	0	100	0	92.6	7.4	100	0	77.8	22.2	66.7	33.3
Ampicillin (10 µg)	96.7	3.3	100	0	100	0	100	0	88.9	11.1	100	0
Cefaclor (30 µg)	100	0	96.6	3.4	96.3	3.7	100	0	66.7	33.3	66.7	33.3
Cefotaxime (10 µg)	96.7	3.3	96.6	3.4	85.2	14.8	85.7	14.3	66.7	33.3	66.7	33.3
Ceftazidime (30 µg)	93.3	6.7	93.1	6.9	92.6	7.4	100	0	77.8	22.2	50	50
Ceftriaxone (30 µg)	93.3	6.7	82.8	17.2	88.9	11.1	92.9	7.1	55.5	44.5	50	50
Ciprofloxacin (5 µg)	90	10	89.7	10.3	81.5	18.5	71.4	28.6	66.7	33.3	66.7	33.3
Gentamicin (10 µg)	80	20	82.8	17.2	85.2	14.8	85.7	14.3	77.8	22.2	66.7	33.3
Imipenem (10 µg)	26.7	73.3	6.9	93.1	7.4	92.6	14.3	85.7	0	100	0	100
Meropenem (10 µg)	23.3	76.7	10.3	89.7	11.1	88.9	21.4	78.6	11.1	88.9	0	100
Ofloxacin (5 µg)	76.7	23.3	75.9	24.1	85.2	14.8	85.7	14.3	55.5	44.5	33.3	66.7
Tetracycline (30 µg)	93.3	6.7	75.9	24.1	85.2	14.8	85.7	14.3	88.9	11.1	100	0
Tobramycin (10µg)	80	20	72.4	27.6	92.6	7.4	92.9	7.1	77.8	22.2	50	50

Where; “R” indicates resistance % of isolates; “S” indicates sensitivity % of isolates

Table 4: Antibacterial potential of NEBO and NECO against the tested bacterial isolates

Tested bacteria	Concentration of the oil (µl)									
	Inhibition activity of NEBO (mean ± SD)					Inhibition activity of NECO (mean ± SD)				
	10	20	30	40	50	10	20	30	40	50
A. baumannii	–	–	–	–	–	10.7± 0.47	13.3± 0.94	18.3± 1.25	19.0± 1.41	23.3± 0.47
E. coli	–	–	–	7.7± 0.47	9.7± 0.47	11.7± 0.47	13.3± 0.94	18.3± 1.25	19.0± 1.41	23.3± 0.47
K. pneumoniae	–	–	–	–	–	–	7.0± 0.00	7.7± 0.47	10.7± 0.47	11.7± 0.47
Proteus vulgaris	–	–	–	7.3± 0.47	8.7± 0.47	10.7± 0.47	13.3± 1.7	18.7± 0.94	20.7± 0.47	24.3± 0.47
P. aeruginosa	–	–	–	–	–	–	6.7± 0.47	7.3± 0.47	10.7± 0.47	11.7± 0.47
Staphylococcus aureus	–	10.0 ± 0.82	11.0± 0.82	15.7± 0.47	17.7± 0.47	–	7.3± 0.47	11.3± 0.94	16.7± 0.47	19.3± 0.94

Where; (–): No inhibition. Data are expressed as mean ± Standard deviation (SD) of the 3 replicates

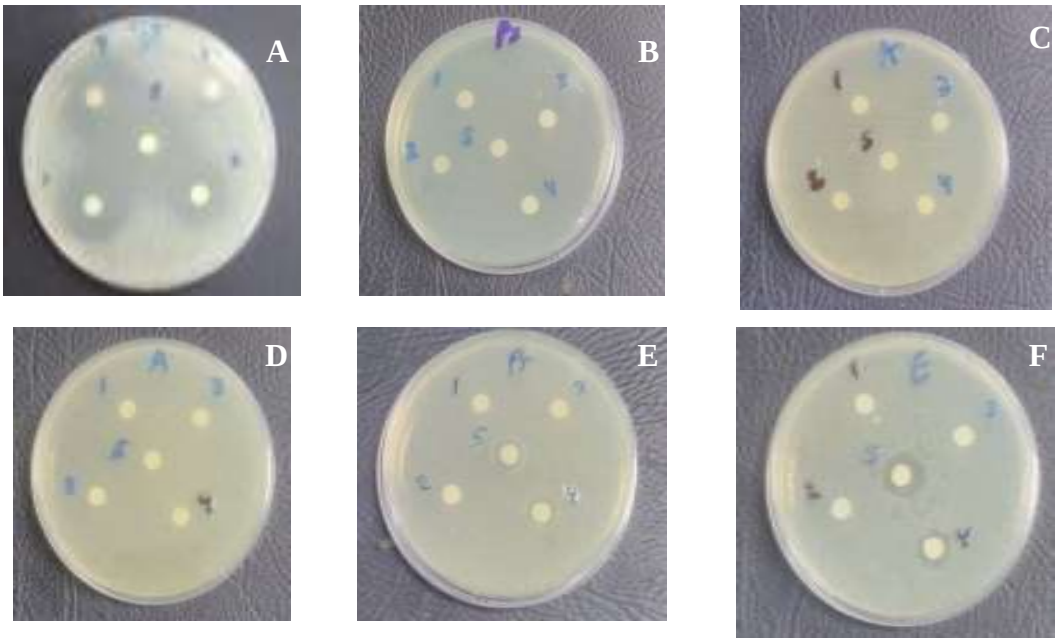

Fig. 2. Antibacterial potential of different concentrations of NEBO (40 %) on growth of each tested MDR bacteria. The dilutions used included; (1) 10 µl, (2) 20 µl, (3) 30 µl, (4) 40 µl and (5) 50 µl. Where; **A**: *Staphylococcus aureus*, **B**: *P. aeruginosa*, **C**: *K. pneumonia*, **D**: *A. baumannii*, **E**: *Proteus vulgaris*, **F**: *E. coli*

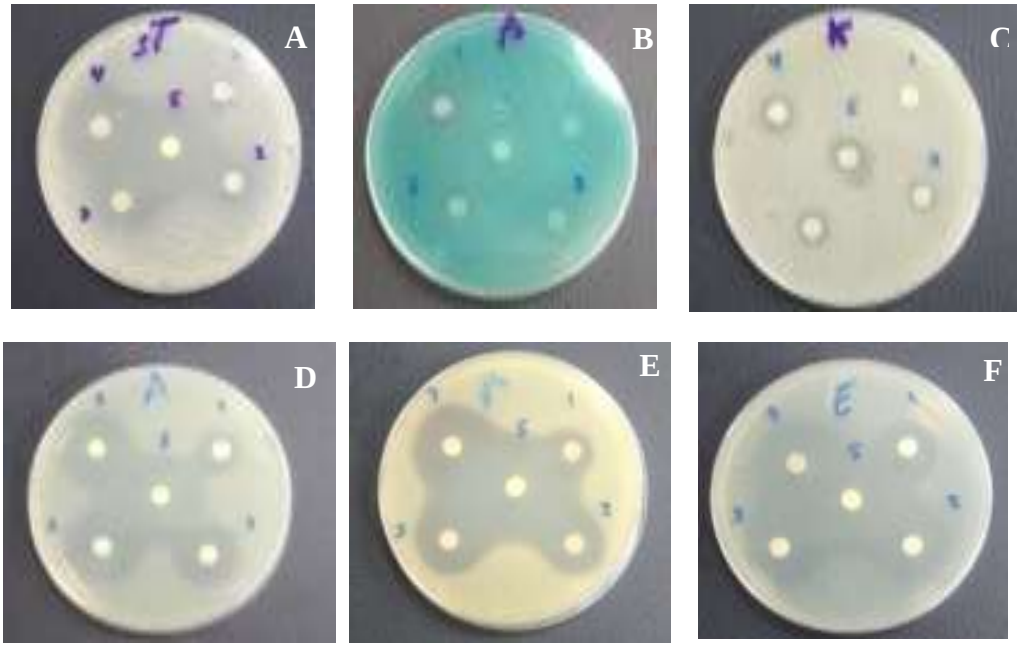

Fig. 3. Antibacterial potential of different concentrations of NECO (40 %) on growth of each tested MDR bacteria. The dilutions used included; (1)10 µl, (2) 20 µl, (3) 30 µl, (4) 40 µl and (5) 50 µl. Where; **A**: *Staphylococcus aureus*, **B**: *P. aeruginosa*, **C**: *K. pneumonia*, **D**: *A. baumannii*, **E**: *Proteus vulgaris*, **F**: *E. coli*

The antibacterial efficacy of NECO may be due to its high content of cinnamaldehyde, which previously demonstrated moderate antibacterial potency; however, this effect was more pronounced against *Staphylococcus aureus* than *E. coli* (Raeisi *et al*., 2015; Alizadeh Behbahani *et al*., 2020). These results indicated that the Gram (+) bacteria are more antibiotic resistant than Gram (-), due to the difference in their membranes structures.

The encapsulation process protected the oils against any oxidation or evaporation (Suppakul *et al*., 2003), and increased their stability (Liang *et al*., 2012; Terjung *et al*., 2012). Moreover, encapsulation increased the bioavailability, and provided control release of the bioactive compounds in the oils (Majeed *et al*., 2015). Similar to the current results, Dev Kumar *et al*., (2020) reported that encapsulation of the basil essential oil increased its potential antimicrobial and antioxidant efficacies.

In the current study, another mechanism for enhancement of the antibacterial activity of the encapsulated oils may be attributed to the antimicrobial activity of WP used during the encapsulation process. Previous reports of Perez-Gago and Krochta, (2002) suggested that the antimicrobial films made by WP were excellent barriers, flavorless; transparent and flexible. For these properties, WP films were widely used to carry the antimicrobial agents targeted against *Staphylococcus aureus* and *Listeria monocytogenes* (Oussalah *et al*., 2004, Royo *et al*., 2010). The antimicrobial efficacy of WP is mainly due the presence of some minor proteins such as immunoglobulins (Igs); lysozyme (Lyz), lactoperoxidase (LP) and lactoferrin (Lf) (Yamauchi *et al*., 2006). Lf and Lyz work synergistically as

antibacterials against Gram (+) bacteria, and inactivate Gram (-) bacteria such as *Salmonella enteritidis* (Uniacke-Lowe *et al*., 2010; Detha *et al*., 2018). Consequently, encapsulation of the essential oils using WP may be promising for enhancement of the antibacterial activities of EBO and ECO against the MDR Gram (+) and Gram (-) bacteria.

Conclusion

The current study revealed that 6 genera of bacteria including *E. coli*; *Staphylococcus aureus, A. baumannii*, *P. aeruginosa*, *K. pneumonia*, and *Proteus vulgaris*, were isolated from the wound samples collected from patients suffering from pus discharge; pain, swelling, foul-smelling and non-healing wound infections that are mainly of bacterial origin. Most of the isolated strains were resistant to amikacin; ampicillin, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, ofloxacin, tetracycline and tobramycin. The nano-encapsulated essential oils of basil and cinnamon (NEBO and NECO) showed potential antibacterial activities against the MDR bacteria. The encapsulation process may be effective to enhance the antibacterial potentials of the essential oils, since it protects the components of the oils from oxidation. Therefore, these encapsulated oils may be promising agents for possible application in treatment of the MDR bacteria.

Conflict of interest

The authors declare that there is no conflict of interests.

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Ethical approval

The patient's consents and statement of protection of the patient's privacy are provided.

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