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Article

Antibacterial, anti fungal and *E. coli* DNA cleavage of *Euphorbia prostrata* and *Pelargonium graveolens* extract and their combination with novel nanoparticles

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As there are a lot of antibacterial and anti-fungal resistant pathogens, researchers attempt to substitute antimicrobial drugs with various medical plants and novel nanoparticles. The present study was conducted to characterize antimicrobial activities of *Euphorbia prostrata* and *Pelargonium graveolens* extract alone and in combination with Mn-Ni@Fe3O4-NPs & Mn: Fe (OH)3-NPs on the DNA cleavage of *E. coli* and also *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Aspergillus oryzae,* and *Candida albicans*. The effects of antimicrobial activities on above scenarios were evaluated using disc diffusion, MIC, MBC, and *E. coli* DNA electrophoresis methods. The results showed that the effects of antibacterial assay values of *Euphorbia prostrata* & Mn: Fe(OH)3 was 21.00 mm for *E. coli* and while it was 19.5 mm for *Euphorbia prostrata* & Mn-Ni@Fe3O4 against *Pseudomonas aeruginosa* at a concentration of 100mg/mL. The highest level of DNA cleavage was seen in mixed of *Euphorbia prostrata* & Mn: Fe(OH)3 nanoparticles. In conclusion, the combination of *Euphorbia prostrata* and *Pelargonium graveolens* extracts with nanostructures showed synergic effects on eliminating the bacteria via DNA destruction and others mechanisms. Moreover, the synergistic effect of nanoparticles with plant extracts seems to bring about new choices for the treatment of infectious diseases.

Keywords: *Euphorbia prostrata*/extract/antimicrobial activities. *Pelargonium graveolens*/extract/antimicrobial activities. *E. coli*. *Candida albicans.* Nanoparticles.

INTRODUCTION

Despite producing a number of new synthetic antibiotics by pharmacological industries, resistance to these drugs has increased. Bacteria have the genetic ability to transmit resistance to drugs. In this connection, it is necessary to develop an herbal drug to treat such bacterial diseases. Plants have an ability to produce metabolites that can be the source of antimicrobial substances. Besides, a high percentage of the peoples still use herbal drugs along with or in preference to conventional medicines (Cowan, 1999). The gram-negative cell walls, which have a complex structure, are composed of thin outer membranes that confer resistance to hydrophobic compounds detergents. Also, this layer contains lipo-polysaccharides, which are responsible for increasing the negative charge of cell membranes and their viability (Ahmad *et al.,* 2001). Nanomaterials are among the nontraditional antibacterial treatments. Nanotechnology is a new approach to reduce the serious disease caused by bacteria and fungi and can be used as a weapon against microorganisms resistant to drugs (Ebrahiminezhad *et al.,* 2012; Luqman *et al.,* 2008). Iran has a large diversity of plant species containing many useful metabolites. *Euphorbia prostrata* and *Pelargonium graveolens* are two main herbs that seem to be good for microbial infections treatment. In Iran, these herbs are cultivated and collected in Fars and Chaharmahal and Bakhtiari provinces, respectively. *Pelargonium graveolens* has about 250 species. Essential oil of this plant contains diuretic antispasmodic properties. Medicinal values of these species show that this extract can be used for the treatment of skin diseases and as an anti-inflammatory and even an anticancer agent considering their antibacterial/antioxidant property

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(McGaw, Jäger, Van Staden, 2002; Lalli *et al.,* 2008; Safaepour *et al.,* 2009). Also, *Euphorbia prostrata* plant has shown anti-inflammatory and analgesic properties (Singla, Patahk, 1990). In some studies, it has been shown that combination of two antimicrobial agents (plant extract and nanomaterials) against bacterial populations, leads to enhancing their antimicrobial activity (Dorsthorst *et al.,* 2002). Moreover, it seems that the combination of these plants and nanoparticles would have synergic effects with lower toxicity. The purpose of present study is to assess the antibacterial (*Pseudomonas aeruginosa*, *Bacillus subtilis, E. coli* and *Staphylococcus aureus)* as well as the anti-fungal (*Candida albicans, Aspergillus oryzae)* and DNA cleavage (*E. coli)* effects of *Euphorbia prostrata* and *Pelargonium graveolens* with/without Mn-Ni@Fe_3O_4-NPs nanoparticles and Mn: $Fe(OH)_3$.

MATERIAL AND METHODS

Extraction

The aerial parts of *Euphorbia prostrata* and cultivated *Pelargonium graveolens* were collected at the flowering stage during June 2014 respectively from Fars and Chaharmahal and Bakhtiari provinces in the southwest of Iran. The collected materials were dried in shadow and ground to coarse powder. Then, 1000 ml of ethanol (70%) was added to 50 gr of each plant and centrifuged at 10509 g for 2 h at room temperature. The supernatants were filtered through Whatman paper (NO. 2) and allowed to evaporate in vacuum at 45 °C for 24 h using a rotary evaporator until 1ml. The condensed extracts were incubated at 50 °C for 12 h and dried matter's weight was measured to be 6.55 g (Hammer, Carson, Riley, 2004).

GC-MS

The Gas chromatography-Mass spectrometry (GC-MS) analyses were performed using an Agilent-7890A chromatograph interfaced to an Agilent-5975C mass spectrometer (ionization voltage 70 eV, scan time 0.5 s, scan range (40-400 Da) and equipped with a capillary column HP-5 (30 m × 0.25 mm i.d., with film thickness: 0.25 μm). The oven temperature was held at 60 °C for 5 min, then programmed from 60 to 260 °C at a rate of 5 °C/min and finally held for 3 min at 260 °C using Helium as a carrier gas (1.0 mL/min), split 1: 50. Injector and detector temperatures were 250 °C. The relative percentage of the each extract constituents was expressed as percentage with peak area normalization (Gopinath *et al.,* 2012).

Synthesis of nanoparticles

The reaction solution for loading Mn and Ni doped Fe_3O_4 nanoparticles (Mn-Ni@Fe_3O_4-NPs) on activated carbon (Ac) was prepared as follows: 5.0 g $MnSO_4$, 5.0 g $NiSO_4$,$6H_2O$, 5.0 g $FeSO_4$,$7H_2O$ and 10 g of $NH_4Fe(SO_4)_2$ was dissolved in 20 ml deionized water using 7.0 ml conc. Then 240 ml of 2.0 M sodium hydroxide was added to the prepared mixed solution drop-by-drop along with strong stirring at room temperature in an Erlenmeyer flask. After stirring for 27 hours, washed several times and pre-dried at 70 °C for 2.5 hour. For Synthesis of Mn doped Ferric Hydroxide $Fe(OH)_3$. The obtained mixed solution was stirred for 21 hours at 70 °C and finally used for antimicrobial experiments (Emamifar *et al.,* 2010).

Apparatus

X- ray diffraction (XRD, Philips PW 1800) was preformed to characterize the phase and structure of the prepared nanoparticles using cuka radiation (40 KV and 40 mA) at angles ranging from 10 to 80°. The morphology of the nanoparticles were observed by field emission scanning electron microscopy (FE- SEM: Hitachi S- 4160) under an acceleration voltage of 15 KV.

Biological activity

Bacterial Strains

Various strains of the following bacteria were prepared from the Institute of Standard and Industrial Research of Iran. Overall, two gram-positive bacterium *S. aureus* (ATCC 2523), *B. subtilis* (ATCC 6633) and two Gram-negative bacteria, *E. coli* (ATCC 2592), *P. aeruginosa* (ATCC 9027) and fungi*, A. oryzae* (ATCC 2023), *C. albicans* (ATCC 1021) were studied. Bacterial strains were prepared in accordance with the manufacturer's instructions, cultured on Mueller-Hinton agar (Merck, Germany) plates, and incubated at 37 °C for 24 h before use.

Antibacterial activity

Disc diffusion method

The disc diffusion method was applied to study the antibacterial activity (Carson *et al.,* 2002) as follow: 100 μL of each bacterium including nearly 0.5×10^6 colony-forming units (CFU/mL) was speared out onto the Muller Hinton Agar medium (Merck, Germany) by a sterile swab and the stock solution was dilated to 25, 50 and 100 mg in DMSO. Then, the sterile discs (6 mm in diameter) were impregnated with trial serial solutions

(including 25, 50, and 100 mg/mL of compounds per disk) and placed on the surface of the inoculated medium. At the end, plates were incubated at 37 °C for 24 h and zone of inhibitions were measured in millimeters (mm) with a caliper and compared with the control (Lalli *et al.,* 2008).

Minimum inhibitory concentration (MIC) determination

MIC and MBC were determined using broth dilution assay method (Carson *et al.,* 2002). For the dissection of the minimum inhibitory concentration of the extracts and nanoparticles against these bacteria, serial dilution technique was applied. The samples (0.01 mg) were dissolved in 2 ml distilled DMSO in order to obtain a stock solution (50mg/ml). Afterward, 1 ml of this solution was transferred to a test tube containing 1ml Mueller Hinton broth medium to reach a concentration of 25 mg/mL. After 24 h of incubation at 37 °C, bacterial growth was examined. The MIC of the samples was taken as the lowest concentration inhibited bacterial growth. DMSO and two antibiotics (Vancomycin, Gentamicin) were reference as negative and bactericidal drugs, respectively.

Minimum bactericidal concentration

Minimum bactericidal concentration (MBC) of samples also were investigated as same as MIC. MBC shows the lowest concentration of antibacterial agent that kills all bacterium. In this method, the bacterial growth was observed on the surface of agar medium. For this reason, a loop full of each bacterial cultured in Muller Hinton broth was cultured on MHA medium and incubated at 37 °C for 24 h (Carson, Mee, Riley, 2002).

Antifungal activity

The selected isolates were grown on Sabouraud Dextrose Agar (SDA). Next, 7 days-old culture of fungi was scraped with a sterile sculpture, macerated with sterile distilled water. Antifungal test was conducted using the disc diffusion protocol by applying 100 µL of a suspension containing (10^5 CFU/mL) of *A. oryzae* on the SDA medium (Oxoid, Hampshire, England). The disks (6 mm in diameter) were impregnated with samples solution (equivalent to 25, 50, and 100 mg /disc) and DMSO (as a negative control) was placed on the inoculated agar. The inoculated plates were incubated for 72 h at 25 °C and inhibition zones (antifungal activity) was measured and compared with the positive control (Clotrimazol and Amphotericin B); each assay was repeated twice (Hammer, Carson, Riley, 2004).

DNA cleavage experiment

Preparation of culture media

DNA cleavage experiments were performed according to the literature (Lalli *et al.,* 2008). Luria-Bertani broth (10 g/L of peptone, 5 g/L of yeast extract, and 10 g/L of NaCl) was used for the culturing of *E. coli*. Subsequently, 50 mL of medium was prepared and autoclaved for 20 min at 121 °C under 15 lb of pressure. The autoclaved medium was inoculated with the seed culture and incubated at 37 °C for 24 h.

Isolation of DNA

To obtain the pellet, the fresh bacterial culture of *E. coli* contained chromosomal DNA (1.5 mL) was centrifuged. DNA was extracted base of the Kit protocol (gram-negative extraction CINNAPURE, Iran) and chromosomal DNA was stored at -40 °C for the following test.

Agarose gel electrophoresis

Cleavage products were analyzed by the agarose gel electrophoresis method (Lalli *et al.,* 2008). Test samples (5 mg/mL) were prepared in DMSO, added to the isolated chromosomal DNA of *E. coli* (5 µL/mL), and incubated for 1.5 h at 37 °C. Next, 10 µL of compound/DNA samples (mixed with bromophenol blue dye at a 5: 1 ratio) was loaded carefully into the electrophoresis chamber wells along with a standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0; 0.5 M EDTA/1 L) and finally loaded onto the agarose gel (1% gel was stained with 10 µg/mL of ethidium bromide). A gel containing samples was connected to power supply (100 V) for 45 min, and the DNA bands under the UV transilluminator were observed to determine the extent of DNA cleavage. Also, H_2O_2 combined/incubated with DNA were treated and used as negative control.

RESULTS AND DISCUTION

Identification of components

Identification of components was assigned by matching their mass spectra with Wiley Registry of Mass Spectral Data (7th edition, Agilent Technologies, Inc.) and National Institute of Standards and Technology 08 MS (NIST) library data. The identification was also confirmed by comparison of the retention indices with data in the literature (Lalli *et al.,* 2008). The percentages of compounds were calculated by the area normalization method, without considering response factors. The GC analysis of the ethanolic extract of *Euphorbia*

prostrata showed 14 different components. The identified components with their relative percentages and Kovats indices are given in Table (I, II). The main constituents of *Euphorbia prostrata* volatile oil were phytol (53.8%), stearic acid (10.1%) and palmitic acid (5.0%). Also, 24 compounds were identified in *Pelargonium graveolens* extract, with total abundance of 98.9% and the major constituents of the extract were β-citronellol (47.1%), trans-geraniol (6.8%) and δ-cadinene (5%).

TABLE I - Chemical composition of the ethanolic extracts of *Euphoria prostrate*

NO	Components	KI	%
1	Undecane	1102	1.2
2	Dodecane	1204	3.6
3	Tetradecane	1302	2.4
4	Naphthalene, decahydro-1,5-dimethyl-	1356	1.9
5	β-bourbonene	1390	1.4
6	Nepetalactone	1401	2.0
7	Dodecanoic acid	1570	1.8
8	Hexadecane	1602	3.67
9	Octadecane	1800	2.3
10	Neophytadiene	1845	2.8
11	2-Pentadecanone, 6,10,14-trimethyl-	1848	2.4
12	Palmitic acid	2005	5.0
13	Stearic acid	2160	10.1
14	Phytol	2164	53.8
Total percentage			94.4

TABLE II - Chemical composition of the ethanolic extracts of *Pelargonium graveolens*

1	Linalool	1103	1.2
2	Rose oxide	1114	1.3
3	*trans*-p-Menthan-3-one	1157	1.8
4	cis-p-Menthan-3-one	1168	1.7
5	β-Citronellol	1238	47.1
6	*trans*-Geraniol	1260	6.8
7	Citronellyl formate	1279	1.0
8	Citronellol hydrate	1366	2.8
9	(-)-β-Bourbonene	1390	1.0
10	*trans*-Caryophyllene	1425	4.3
11	Citronellyl propanoate	1447	1.5
12	α-Humulene	1458	1.2
13	Geranyl propionate	1478	1.3
14	δ-Cadinene	1527	4.8
15	Citronellyl iso-valerate	1531	2.8
16	Geraniol butyrate	1563	1.8
17	Phenyl ethyl tiglate	1588	2.0
18	Citronellyl tiglate	1669	3.2
19	Geranyl tiglate	1704	4.0
20	Neophytadiene	1838	1.0
21	Cyclotetradecane	1879	1.5
22	Hexadecanoic acid	2005	1.8
23	Phytol	2108	1.6
24	Linolenic acid	2165	1.4
Total percentages			98.9

Morphology of the nanoparticles

The morphology of the obtained Mn-Ni@Fe_3O_4 particles investigated by FE- SEM (Figure 1). Results show that Mn-Ni@Fe_3O_4 particles have an amorphous and porous structure. The XRD pattern of the prepared Mn-Ni@Fe_3O_4 particles indicate very week peak at $2\theta = 35.5°$ attributed to 311 lattice plane of magnetite (Fe_3O_4) cubic structure. The XRD pattern for confirm an amorphous structure or very poor crystalline of Mn-Ni@Fe_3O_4 particles (Figure 2). The FE- SEM images of the Mn: Fe $(OH)_3$-NPs with different magnifications (Figure 1). Show that prepared Mn: Fe $(OH)_3$ nanoparticles are spherical with approximately size of 65 nm, It while are uniform in the shape and size.

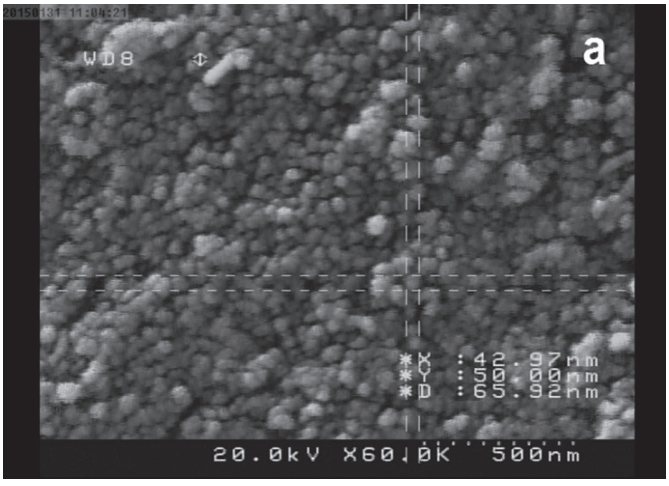

FIGURE 1 - FE-SEM images of the prepared Mn-Ni@Fe_3O_4.

Antibacterial activity of Plant extracts

Antibacterial activities of the nanoparticles and plant extracts were evaluated based on the disc diffusion methods (Figure 3). DMSO did not show inhibition against the tested bacteria. The most of medical extracts and nanoparticles showed moderate to high activities at 25 to 100 mg/mL concentration. *Euphorbia prostrata*

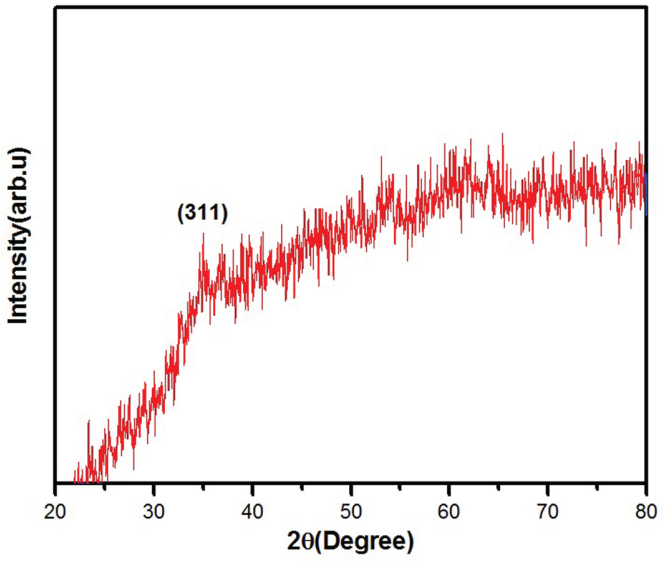

FIGURE 2 - XRD pattern of the prepared Mn-Ni@Fe_3O_4 particles.

showed higher antibacterial activity against *E. coli, P. aeruginosa* and *S. aureus* than *Pelargonium graveolens*, while it was lower against *B. subtilis* at 25, 50 and 100 mg/mL concentration. About nanostructures; Mn: $Fe(OH)_3$ showed higher antibacterial activity against *E. coli* and *S. aureus*, while it was lower for *B. subtilis* and *P. aeruginosa* at 25, 50 and 100 mg/mL concentration. Also, combination of nanoparticles with medical extract showed the highest biological activity. As seen in Table III, IV, combination of *Euphorbia prostrata &* Mn: Fe(OH)3 showed the highest activity with 21.00 (mm) diameter zone at 100 mg/mL concentration against *E. coli.* Finally, inhibitory effects of all compounds were lower than standard antibiotics.

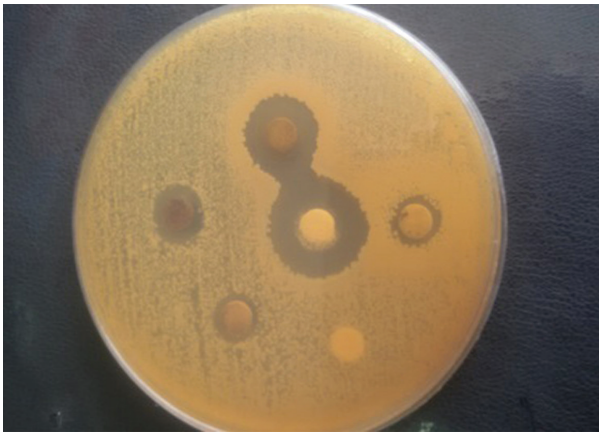

FIGURE 3 - Disc diffusion methods and their hollows around discs against *S. aureus.*

Antifungal activity

To evaluate the antifungal activity of nanoparticles and extracts, disk diffusion method was performed. The most of the medical plants and nanoparticles showed moderate to high antibacterial activities against *C. albicans*. *Pelargonium graveolens* showed higher antifungal activity against *C. albicans* at 50 and 100 mg/mL concentration. Also, Mn: Fe(OH)3 showed higher antifungal activity against *C. albicans* at 25, 50 and

TABLE III - Anti bacterial activity as diameter of zone of inhibition* (mm) around the constructed and standard discs.** All data are the mean of three measurements

Samples	*E. coli* (-)			*Pseudomonas aeruginosa* (-)			*Bacillus subtilis* (+)			*Staphylococcus aureus* (+)		
	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)
Pelargonium graveolens	11.46	10.28	8.00	17.50	14.12	11.00	17.00	15.12	12.00	13.50	9.00	8.60
Euphorbia prostrata	13.64	12.00	9.20	20.40	11.50	10.00	13.20	9.36	7.30	18.30	10.00	8.00
Mn: Fe(OH)$_3$	8.40	8.00	7.00	12.00	9.10	7.00	10.00	9.00	8.00	10.00	8.00	7.30
Mn-Ni@Fe_3O_4	10.60	8.00	7.00	9.22	8.54	7.00	8.46	8.00	6.28	10.34	9.66	8.20
Pelargonium graveolens & Mn: Fe(OH)$_3$	16.00	11.00	9.00	16.38	14.40	12.28	14.00	9.00	7.00	14.30	10.40	7.46
Pelargonium graveolens & Mn-Ni@Fe_3O_4	17.00	13.00	12.06	19.38	12.48	10.58	15.38	9.00	6.60	10.00	8.00	6.60
Euphorbia prostrate & Mn: Fe(OH)$_3$	21.00	16.60	14.22	15.20	10.10	8.00	13.28	12.16	8.42	14.10	13.40	12.00
Euphorbia prostrate & Mn-Ni@Fe_3O_4	20.00	15.28	10.38	19.50	17.00	11.26	19.00	16.48	12.00	19.16	16.00	8.00
Gentamicin (10 µg/disc)	20.00			20.60			-			-		
Vancomycin (30 µg/disc)	-			-			25.00			20.40		

TABLE IV - Antibacterial effects of extract and nanoparticles performed by Broth dilution (MIC (mg/mL) and MBC (mg/mL) methods

Samples	*E. coli* (-)		*P. aeruginosa* (-)		*B. subtilis* (+)		*S. aureus* (+)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Pelargonium graveolens	25.00	50.00	0.78	3.125	12.50	25.00	0.78	3.125
Euphorbia prostrate	6.25	25.00	0.39	0.39	0.195	0.78	25.00	25.00
Mn: $Fe(OH)_3$	25.00	50.00	25.00	25.00	12.50	50.00	25.00	50.00
Mn-Ni@Fe_3O_4	25.00	50.00	25.00	25.00	12.50	50.00	25.00	50.00
Pelargonium graveolens & Mn: $Fe(OH)_3$	1.56	3.125	0.78	3.125	3.125	12.50	6.25	12.50
Pelargonium graveolens & Mn-Ni@Fe_3O_4	1.56	3.125	0.39	0.78	50.00	50.00	1.56	3.125
Euphorbia prostrate & Mn: $Fe(OH)_3$	25.00	50.00	25.00	25.00	12.50	50.00	25.00	50.00
Euphorbia prostrate & Mn-Ni@Fe_3O_4	0.39	0.39	1.56	1.56	0.195	0.39	0.195	0.39

100 mg/mL concentration. Compounds showed no antifungal effects on *A. oryzae.* Combination of nanoparticles with medical extract showed the highest biological activity. As seen in Table V, combination of *Pelargonium graveolens* & Mn: Fe(OH)3 showed the highest antifungal activity with 17.6 (mm) diameter zone at 100 mg/mL concentration against *C. albicans*. Finally, inhibitory effects of all compounds were weaker than standard antibiotics.

DNA cleavage experiment

DNA interaction with compounds has been illustrated at Figure 4. Lanes A–H refer to (A): *Pelagonium graveolen* & Mn-Ni@Fe_3O_4; (B): Mn-Ni@Fe_3O_4; (C): *Euphorbia prostrata*; (D): *Pelagonium graveolen*; (E): Mn: Fe(OH)3 ; (F): *Euphorbia prostrata* & Mn: $Fe(OH)_3$; (G): *Euphorbia prostrata* & Mn-Ni@Fe_3O_4; (H): *Pelagonium graveolen* & Mn: Fe(OH)3 ; (I): DNA; (J): Lader; (K): H_2O_2 respectively. Lanes of I, J and K point out pure DNA (positive control), ladder (or marker) and DNA treated with H_2O_2 (denatured control), respectively. It is to be noted that if the lane of a sample test is similar to lane I, it means that no cleavage has been occurred. So, *Pelagonium graveolen* & Mn-Ni@Fe_3O_4 and *Euphorbia prostrata* & Mn: Fe(OH)3 Nanoparticles notably degraded DNA structure. The DNA cleavage of other compounds was weaker and can be ordered as D > E > B >G.

Disc diffusion method and MIC and MBC data suggest that the antimicrobial activities of the plant extract were more effective than nanoparticles and decreasing the concentration of the extract and nanoparticles had a directly proportional to reduce the zone of inhibitions.

TABLE V - Antifungal activity as diameter of zone of inhibition* (mm) around the constructed and standard discs.* All data are the mean of three measurements

Samples	Fungi (cm)					
	Candida albicans			*Aspergillus oryzea*		
	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)	100 (mg/mL)	50 (mg/mL)	25 (mg/mL)
Pelargonium graveolens	15.48	12.20	8.00	6.00	6.00	6.00
Euphorbia prostrata	15.00	9.60	9.00	6.00	6.00	6.00
Mn: $Fe(OH)_3$	11.00	8.14	7.14	6.00	6.00	6.00
Mn-Ni@Fe_3O_4	8.32	7.30	7.00	6.00	6.00	6.00
Pelargonium graveolens & Mn: $Fe(OH)_3$	17.16	10.60	8.00	6.00	6.00	6.00
Pelargonium graveolens & Mn-Ni@Fe_3O_4	15.00	13.20	8.62	6.00	6.00	6.00
Euphorbia prostrate & Mn: $Fe(OH)_3$	16.46	12.00	8.42	6.00	6.00	6.00
Euphorbia prostrate & Mn-Ni@Fe_3O_4	13.40	11.50	10.28	6.00	6.00	6.00
Clotrimazole (100µg/disc)	20.10			-		
Amphotericin B (100 µg/disc)	-			11.00		

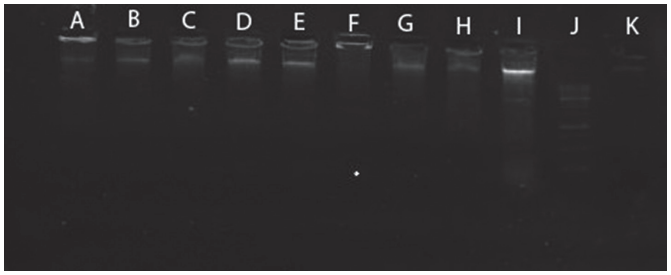

FIGURE 4 - DNA Cleavage of materials; (A): *Pelagonium graveolen* & Mn-Ni@Fe_3O_4; (B): Mn-Ni@Fe_3O_4; (C): *Euphorbia prostrata*; (D): *Pelagonium graveolen*; (E): Mn: Fe(OH)3 Nanoparticles; (F): *Euphorbia prostrata* & Mn: Fe(OH)3 Nanoparticles; (G): *Euphorbia prostrata* & Mn-Ni@ Fe_3O_4; (H): *Pelagonium graveolen* & Mn: Fe(OH)3; (I): DNA; (J): Lader; (K): H_2O_2.

In this regard, Lalli *et al.* (2008) examined chemical composition, antifungal, and antioxidant activity of *Pelargonium graveolens* essential oil and showed that chemical composition of geranium oil contains citronellol and geraniol as dominant compounds, which is in agreement with the results of current research. The ethanolic extract of *Euphorbia prostrata,* which contains some active components, has been reported to exhibit antibacterial activities. Phytol, the major component of this ethanol extract, has shown antimicrobial activity against pathogen microorganisms. Phytol, that is one of the major compounds in both extract, is one of the most important di-terpenes and is one of the products in chlorophyll metabolism in plants. It possesses both antimicrobial and anticancer activities. Some researchers reported that fatty acids such as palmitic acid and stearic acid (present in this ethanol extract) had antibacterial activity (McGaw, Jäger, Van Staden, 2002). High proportions of β-citronellol and phytol in this extract make it interesting considering its high antibacterial activity. The mechanism of essential oils action may contain: 1) Damage or destruction of the cell wall; 2) Disturbances in the cytoplasmic membrane; 3) Leakage cell contents; 4) Membrane protein destruction and defeat in transports; and 5) Coagulation of cell contents (Luqman *et al.,* 2008). The nanoparticles can either directly interact with the microbial cells (e.g. interrupting transmembrane electron transfer, disrupting/penetrating the cell envelope, and oxidizing cell components) or produce secondary products (e.g. reactive oxygen species (ROS) and dissolved heavy metal ions) that cause damage to bacteria (Emamifar *et al.,* 2010). Anti-bacterial properties of metal oxide nanoparticles (according to the surface to volume ratio) are very different. This antibacterial activity is due to their high surface-to-volume ratio rather than to the sole effect of metal-ion release (Thukkaram *et al.,* 2014**).**

Arif Khan *et al.* (2015a) worked on pharmacological characterization of methanol extract of *Calligonum polygonoides* from District Bannu Dried plant and measured growth inhibition of *Aspergillus niger*. They showed *C. polygonoides* possess significant antioxidant, antifungal and cytotoxic bioactive compounds. Also in another study conducted by Imran Khan *et al.* (2016), pure compound of *Lonicera quinquelocularis* plant were studied for antioxidant, antimicrobial and phytotoxic activities. They reported that crude extract inhibits the growth of *Aspergillus fumigatus* and *Fusarium solani* 65 and 70%, respectively. Also, antimicrobial activities of *Calligonum polygonoides*, *Albezia lebeck* and *Piper nigrum* were screened through the agar tube dilution method (Khan *et al.,* 2015b). They reported that growth of the Gram-positive bacteria (*S. aureus*) as well as Gram-negative bacteria (*E. coli*) was markedly inhibited by *C. polygonoides.* The *A. lebeck* and *P. nigrum* extracts showed activity against *Aspergillus niger* followed by *A. flavis*; while the highest activity were shown by *A. lebeck* against *A. niger* and by *P. nigrum* against *A. flavis*. Nanoparticles can cause peroxidation of the phosphor-lipid compounds of bacterial membrane; therefore, the integrity of the cell membrane reduces, normal cellular activities in a healthy cell structure such as the respiratory activities disappear, and cell death becomes unavoidable; in agreement with the results of current research. Due to the difference between the negatively charged bacteria and the positively charged nanoparticles, the nanoparticles act as an electromagnetic absorbing the microbes causing the nanoparticles to bind cell surface. Also, the electrostatic forces or hydrophobic iron oxide nanoparticles are capable of the binding bacterial cell wall and connecting to surface bonding agents (Chifiriuc *et al.,* 2011; Ebrahiminezhad *et al.,* 2012).

CONCLUSION

The combination of *Euphorbia prostrata* and *Pelargonium graveolens* extracts with nanostructures showed synergic effects to eliminate the bacteria via DNA destruction and others mechanisms. Furthermore, it seems that the synergistic effect of nanoparticles with plant extracts leads to new choices for the treatment of infectious diseases.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests

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