MEGAMOX MEDIATES ECO-FRIENDLY SYNTHESIS OF SELENIUM NANOPARTICLES AS IMMUNOMODULATORY, ANTIOXIDANT AND ANTIMICROBIAL AGENTS

Ahmed A. Askar[a], Asmaa M. Abouzaid[b], Akhilesh Dubey[c]* and Sally A. El-Zahaby[d]

(Received 29 December 2020) (Accepted 27 May 2022)

ABSTRACT

The research herein includes methodology for eco-friendly preparation of selenium nanoparticles (SeNPs) using the broad-spectrum antibiotic, Megamox (Mega.). Characterization of SeNPs was done using UV-Visible spectroscopy, FTIR, XRD, DLS and TEM imaging. Additionally, the immunomodulatory, antioxidant and antimicrobial activities of both SeNPs and Mega. were checked against different strains of bacteria and fungi. TEM and DLS images showed that SeNPs are polydisperse spheres with mean diameter of 22.4 nm. FTIR analysis indicated that the hydroxyl and nitrogen moieties in Mega. were effective for reduction plus binding manner. According to the results of the nitro blue tetrazolium reduction test, both SeNPs and Mega. presented high intracellular killing activity, which confirmed their immunostimulatory effect. Antioxidant activity of SeNPs and Mega. were 90 and 82 %, respectively. SeNPs presented great activity facing multi-drug resistant bacteria and TB. SeNPs are considered promising cost-effective and eco-friendly anti-bacterial and anti-fungal agents that can represent a new potential nano-platform in both medical and infectious diseases control.

Keywords: Antimicrobial, *C. albicans*, Eco-friendly, MRSA, Megamox, Nanoparticles,TEM

ABBREVIATIONS

SeNPs: Selenium nanoparticles, Mega.: Megamox, FTIR: Fourier-transform infrared spectroscopy, XRD: X-Ray Diffraction, TEM: Transmission Electron Microscopy, MRSA: Methicillin-resistant *Staphylococcus aureus,* NPs: Nanoparticles, mM: Millimolar, TB: Tuberculosis, RCMB: The Regional Center for Mycology and Biotechnology, ATCC: American Type Culture Collection, MIC: Minimal Inhibitory Concentration, DPPH: 2, 2-diphenylpicrylhydrazyl, HBSS: Hank's balanced salt solution, NBT: Nitroblue Tetrazolium, UV-Vis: UV-Visible Spectroscopy, Cu: Copper , Au: Gold , Ag: Silver , Se: Selenium, SPR: Surface Plasmon Resonance (SPR), O.D: Optical Density, DLS: Dynamic Light Scattering, PS: Particle Size, ppm: Parts Per Million, TBHQ: Tertiary-Butyl Hydroquinone, FCC: Face Centered Cubic

INTRODUCTION

Diverse forms of applications can be obtained through using nanotechnology. This originates from the ability of nanotechnology to work on different matters at very miniature dimensions; 1-100 nanometer[1]. Novel nanotherapeutics and diagnostic tools as well as drug delivery systems can be developed as applications of nanotechnology[2,3]. Achieving targeted therapy is another application that has gained a lot of interest in researchers[4]. Nanomaterials possess their own antimicrobial activity[5,6] or augment or elevate the usefulness and the safety margin of antibiotics administration[7].One of the very promising applications of nanoparticles (NPs) in medicine is their usage in modulating the immune system's functions either by immunosuppression or immunostimulation effects. Compositions, sizes and surface chemistry of the NPs affect these immunomodulations. These modulating effects may bring benefits or dangers[8]. Therefore, monitoring of the effect of the developed NPs

[a] Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo-11884, Egypt
[b] Botany and Microbiology Department, Faculty of Science (Girls), Al-Azhar University, Cairo-11884, Egypt
[c] Department of Pharmaceutics, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Mangaluru-575 018, Karnataka, India
[d] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, King Salman International University, South Sinai, Egypt
*For Correspondence: E-mail:akhilesh@nitte.edu.in

https://doi.org/10.53879/id.59.10.12817

must be performed to ensure their successful clinical outcomes for immunological diseases. Additionally, there are currently NPs-based platforms for the efficacious delivery of vaccines in cancer immunotherapy and those include liposomes, lipid-based NPs, polymeric NPs and nanospheres[9]. These NPs help to deliver vaccine to immune cells. The improvement in the use of immunomodulatory agents as an alternative therapy in cancer treatment to strengthen the immune response had gained a lot of interest lately[10].Dendritic cells platform was the most successful cell-based therapeutic modulator employed as vaccination for cancer immunotherapy that enabled improvement of the immune response for cytotoxic T-cells through delivering tumor specific antigens to lymphatic organs[11]. More recently, SeNPs had been used as immunostimulatory agents on the 4T1 breast cancer mode[10]. In the field of anti-microbial and anti-fungal therapies there are many reasons behind the strong need to have newly developed agents capable to solve the raised problems in last decades[12]. Knowing that, infants are subjected usually to antibiotics administration in most cases of infantile diarrhea (gastroenteritis), even sometimes, prior to hospital attendance, explaining the high prevalence of antibiotic resistance which is continually increasing and widely reported as a major problem, most especially among children in developing countries[13].Furthermore, many body regions can possess fungal infections. Aspergillus infection is not simple to be handled and consequently can produce difficulties like lung disorders similar to invasive and permanent pulmonary aspergillosis. Another prevalent fungal infection is *Candida*, which can influence the epidermis and mucous layers either externally or in an invasive state that develops life-threatening situations[14]. In this regards, biogenic SeNPs toward *Candida albicans* and *Aspergillus fumigatus* were developed as anti-fungal agents. They had size rage of 120–140 nm and had been synthesized by *Bacillus* sp. MSh-1[15].

Table I: Experimental factorial design for the optimization of SeNPs production

Run number	Conc. of selenious acid (mM)	Conc. of Mega. (mg mL^{-1})	Temperature (°C)	Responses: absorbance (O.D.) at λ_{max} 415.0 nm
I	0.5	4	25	0.042
II	1	1	25	0.065
III	0.5	2	25	0.094
IV	1	1	30	0.104
V	1.5	1	30	0.308
VI	1	1	37	0.108
VII	1.5	2	30	1.312
VIII	1	4	37	0.999
IX	0.5	1	25	1.048
X	1	4	25	0.195
XI	0.5	4	30	0.849
XII	1.5	1	37	1.126
XIII	0.5	4	37	1.112
XIV	1	2	30	1.082
XV	1.5	4	37	1.067
XVI	1.5	2	25	1.051
XVII	1.5	4	25	0.017
XVIII	1	2	37	0.899
XIX	1	4	30	1.232
XX	1.5	4	30	0.917
XXI	0.5	2	37	1.032
XXII	1.5	1	25	0.129
XXIII	0.5	1	30	1.089
XXIV	1	2	25	0.066
XXV	1.5	2	37	1.615
XXVI	0.5	2	30	0.993
XXVII	0.5	1	37	0.96

From the manufacturing aspect for NPs, they can be prepared through chemical, physical and biological procedures[16]. Chemical procedures have been extensively used in many researches; though it showed successful results, unfortunately it had many drawbacks. Chemical synthesis can affect drug integrity[17], the heat used in the procedure might negatively affect the components used in the NPs manufacture[18]. Additionally, synthetic methods ordinarily require multi-steps intricate procedures; particularly when combining the antibiotics among metal NPs. To accomplish this conjugation, NPs obtained initially are modified on the srface and subsequently capping of activated groups take place[19]. Developing eco-friendly manufacturing techniques to decrease or totally abolish the use of chemical agents are strongly needed[20]. Mega. which consists of amoxicillin + clavulanic acid 228 mg and/or 156 mg showed the lowest overall bacterial resistance (0.0-8.3% and 0.0-17.1%) according to a study performed by Adenike Ogunshe[13].

In this regard, in the current research, Mega. was applied for the initial time as an archetype form to demonstrate an uncomplicated post-assembly of SeNPs, as well as producing their capping action. Additionally,

Table II: FT-IR wave numbers of characteristic bonds and corresponding assignments for Mega., without and with SeNPs

Peak number	Mega. ƛ (cm^{-1})	Mega. + SeNPs (cm^{-1})	Corresponding to
I	3430.12	3430.12	OH and NH- group band vibration
II	2913.64	2913.64	The broad peaks are characteristic to the presence of–NH amino group and –OH stretching group sin alcoholic and phenolic compounds. The presence of this peak may be due to binding of Se^{2+} ions to OH group
III	1410.28	1420.52	Corresponding to aliphatic C-H stretching. And for stretching vibration of aliphatic C-H group.
IV	1125.71 1075.23	1125.71 1075.23	May be ascribed for the presence of C=O stretching group and attributed to vibrations of aromatic ring C=C of amide C=O and/ or of COO- groups.

Table III: Antibacterial activity of SeNPs synthesized by Mega.

Bacterial test strain		Mean diameter of inhibition zone (mm)/ minimum inhibitory concentration (MIC) (µg mL^{-1})					
		Mega.		SeNPs		Tetracycline (standard)	
		Inhibition zone	MIC	Inhibition zone	MIC	Inhibition zone	MIC
B. subtilis (ATCC 6633)	Standard strains (ATCC)	31±0.84	15.62 ±0.05	34±0.15	0.27 ±0.85	25±0.005	31.25±0.005
S. aureus (ATCC 29213)		25±0.33	31.25 ±0.45	27±0.23	0.27±0.37	25±0.005	62.5±0.005
E. coli (ATCC 25922)		29±0.11	3.9 ±0.77	33±0.85	0.03±0.46	23±0.005	15.62±0.005
P. aeruginosa (ATCC 27853)		28±0.61	15.62 ±0.02	25±0.37	0.015 ±0.87	20±0.01	62.5±0.005
E. faecalis (ATCC 29212)		24 ±0.02	62.5±0.33	26±0.64	0.13±0.56	0.0±0.01	0.0±0.01
S. typhi (ATCC 6539)		35±0.01	3.90±0.08	32±0.45	0.03±0.14	30±0.01	7.81±0.005
V. cholera (Clinical strain)		38±0.015	1.95±0.99	30±0.33	1.09±0.56	24±0.01	31.25±0.005
M. tuberculosis (RCMB 010126)		0.0±0.01	0.0±0.01	36±0.92	0.067 ±0.48	0.0±0.01	0.0±0.01
S. aureus (MRSA)	Clinical resistance strains	20±0.01	62.5±0.01	28±0.31	0.27±0.35	0.0±0.01	0.0±0.01
S. epidermis		28±0.67	7.81 ±0.005	31±0.34	0.03±0.47	0.0±0.01	0.0±0.01
E. coli		27±0.48	3.9 ±0.078	25 ±0.79	0.13±0.89	0.0±0.01	0.0±0.01
P. aeruginosa		21±0.01	15.62 ±0.22	22 ±0.52	0.54±0.42	0.0±0.01	0.0±0.01
A. baumannii		25±0.15	3.9 ±0.005	27 ±0.48	0.27±0.32	0.0±0.01	0.0±0.01
K. pneumonia		30±0.41	1.95 ±0.005	32±0.51	0.13±0.55	0.0±0.01	0.0±0.01

Table IV: Antifungal activity of SeNPs synthesized by Mega.

Fungal test strain	Mean diameter of inhibition zone (mm)/ minimum inhibitory concentration (MIC) (µg mL^{-1})					
	Mega.		SeNPs		Amphotericin B (standard)	
	Inhibition zone	MIC	Inhibition zone	MIC	Inhibition zone	MIC
C. albicans (ATCC 10231)	0.0	0.0	40 ±0.05	0.03 ±0.63	0.0±0.01	0.0 ±0.01
A. niger (RCMB 002007)	0.0	0.0	37 ±0.05	0.06 ±0.48	16 ±0.15	62.5±0.05
A. flavus (ATCC 16883)	0.0	0.0	28±0.11	2.18 ±0.94	17 ±0.12	31.25±0.01
F. oxysporum (RCMB 008002)	0.0	0.0	45±0.05	0.03 ±0.85	18±0.17	15.62±0.005
P. citrinum (RCMB 001011)	0.0	0.0	26±0.41	4.37±0.39	20 ±0.20	62.5±0.05

Table V: Antioxidant activity for SeNPs and Mega.

Active compounds / Nanoparticles	Absorbance (λ_{max} 517nm)	Scavenging activity %
Mega.	0.17±0.4	80
SeNPs from Mega.	0.06±0. 5	92
Standard tert-Butyl hydroquinone (TBHQ)	0.14±0.93	86

Table VI: The intracellular killing activities of SeNPs and Mega.

Intracellular killing activity %	Mega.		SeNPs	
	4 µg mL^{-1}	8 µg mL^{-1}	4 ppm	8 ppm
	46.2 ± 0. 46	52.6 ± 0. 85	112.9 ± 0. 99	168.3 ± 0. 67

evaluation of the impact of SeNPs as an antimicrobial steward was achieved facing both MDR and standard bacterial strains. Moreover, the same was reviewed against TB, filamentous and unicellular fungi. The immunomodulatory furthermore antioxidant action of incorporated SeNPs was additionally explained herein.

MATERIALS AND METHODS

Materials

Media ingredients were obtained of Oxoid (Hampshire, United Kingdom) and Difco (Plymouth, England). Megamox 1 g (Amoxycillin (as trihydrate) 875 mg + Clavulanic acid (as potassium) 125 mg) was obtained from JPI-KSA. Chemicals (Selenious acid) including reagents appropriated in the studies and biological investigations were collected at the scientific conventional category of Sigma-Aldrich (Missouri, United States).

Synthesis of SeNPs

SeNPs were manufactured using Mega. (as reducing and capping agent). Experimental factorial study (Table I) practiced examining the influence of the concentration plus temperature approaching this SeNPs construction. The factorial study was consisting of three variables in two levels, the concentration of selenium (0.5, 1 and 1.5 mM) and concentration of Mega. (1, 2 and 4 mg mL^{-1}) and temperature (25, 30 and 37°C). The selenious acid suspension (by various conc. as manifested in Table I) was mixed with Mega. suspension (by conc. presented in Table I) by quotient (1:1) V/V. The mixtures were stirred at controlled room temperature of around 25°C. The absorption color of the individual specimen was reported practicing a JASCO V-560 UV-visible spectrometer performing on a resolution at 415 nm.

Characterization of SeNPs

Characterization of SeNPs was done by using following methods:

UV-visible spectrophotometer (UV-Vis.)

JASCO V-560, UV-Vis. Spectrophotometer, Tokyo, Japan, at wavelength range from 200-900 nm and at a resolution of 1 nm using Mega. without SeO_2 addition as a baseline blank (negative) for auto zero support. Toward particle size investigation, the specimens were diluted ten moments by deionized water before estimations.

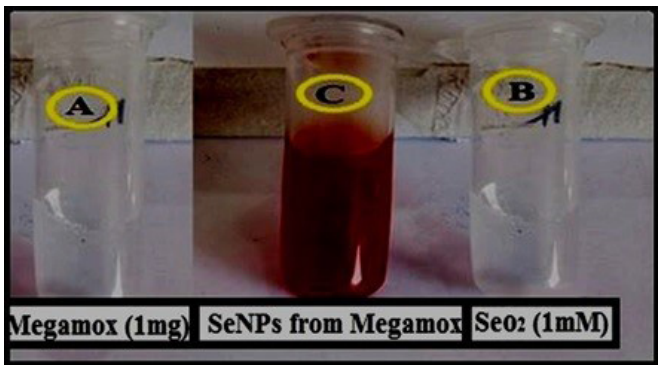

Fig. 1: Color difference from transparent (Mega. and Se2+) to reddish color (SeNPs)

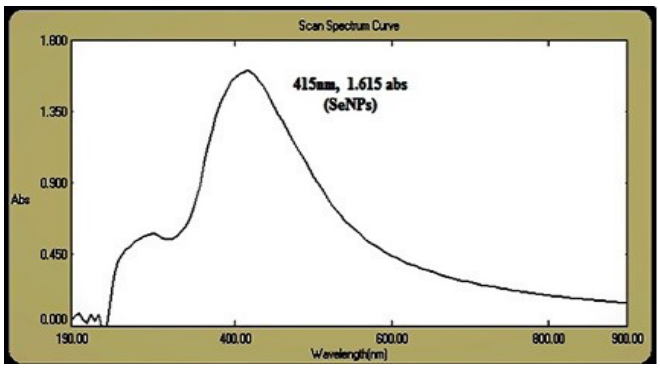

Fig. 2: UV-Visible spectrum of SeNPs synthesized by using Mega. at 37 °C

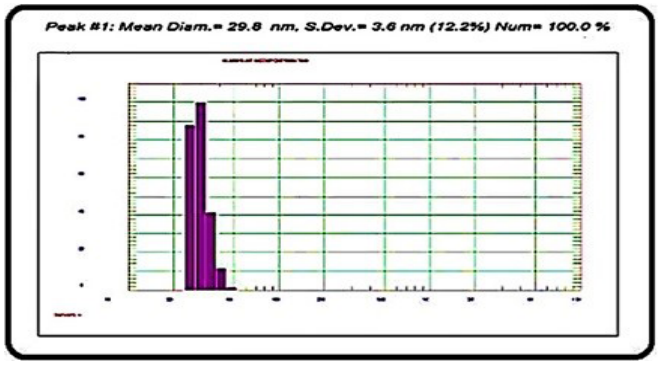

Fig. 3: DLS pattern of PS distribution of the SeNPs synthesized by Mega. at 37 °C

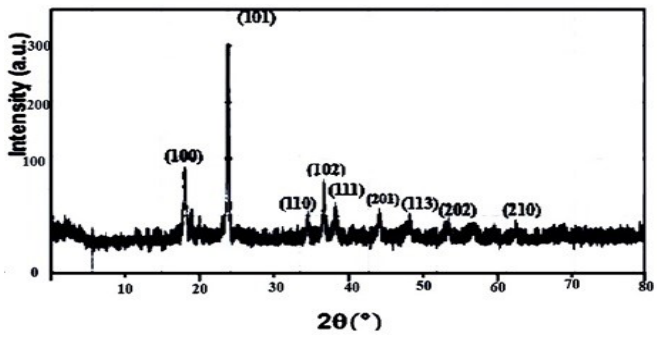

Fig. 4: XRD pattern for the SeNPs synthesized by Mega. at 37°C

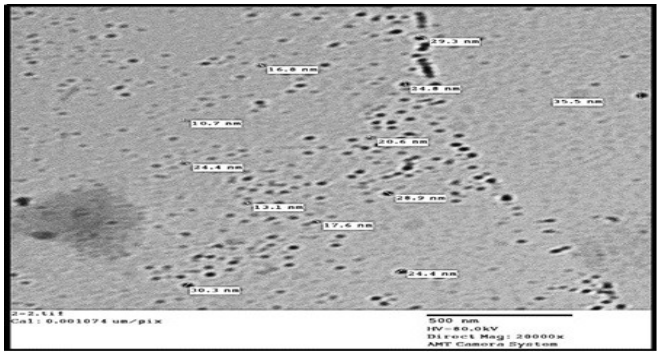

Fig. 5: TEM image for SeNPs synthesized by Mega. at 37°C

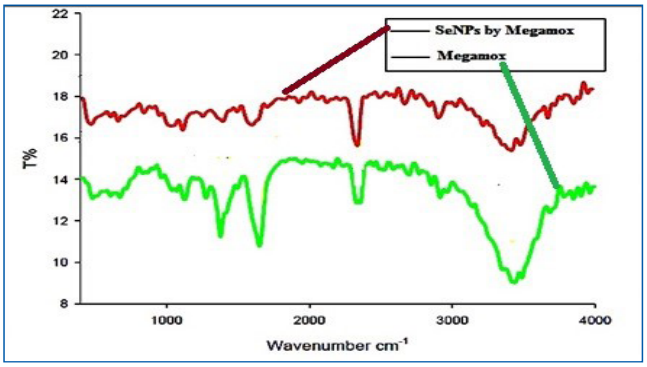

Fig. 6: FT-IR spectrum for Mega. andSeNPs synthesized by Mega. at 37°C

Transmission electron microscopy (TEM)

The morphology and size of the manufactured NPs were examined by TEM microscopy, JEOL JEM-100 CX, Peabody, USA. TEM images were scouted by drop covering the SeNPs upon carbon-coated TEM layers.

X-ray diffraction (XRD)

Specimens were centrifuged before XRD examination. X-Ray diffraction models were achieved by the Shimadzu equipment, XRD-6000 line, Tokyo, Japan. The estimations included stress investigation, remaining austenite quantitation, crystallite capacity, crystallinity consideration, and materials examination through overlain X-ray diffraction models.

Fourier transform infrared (FTIR) examination

Finally, FT-IR investigation provided information about the utilitarian groups inhabiting in the Mega. drug. The trials were conducted out applying JASCO FT-IR 3600 Infra-red spectrometer, Easton, USA, through using

KBr pellets. A resolution of 4.0 cm^{-1} in the wave number range of 400-4000 cm^{-1} was used in the measurements. SeNPs concentration assessment was performed using UNICAM939 Atomic Absorption Spectroscopy, Cambridge, UK, was implemented with deuterium experience improvement. All suspensions were prepared by applying ultra-pure water.

Assessment of the antimicrobial activity of SeNPs and Mega.

SeNPs 70 ppm (manufactured by Mega.; 0.1 mg mL^{-1}), the negative control Mega. 0.1 mg mL^{-1} and the positive control selenium ions (Se^{+}; 1mM) were examined for their antimicrobial action, utilizing the agar paper diffusion method. Additionally, SeNPs and Mega. were reviewed upon different isolates of MDR bacteria (expand) provided helpful from Bacteriology Lab in Botany and Microbiology Department, Faculty of Science, Al-Azhar University. Cairo, Egypt. The investigated pathogens involved Gram-positive bacteria (*Enterococcus faecalis, MRSA, Staphylococcus aureus, and Staphylococcus epidermidis*) and Gram-negative bacteria (*Enterobacter cloacae, E. coli, and Acinetobacter baumannii*) following the same method adopted by El-Sherbiny et al.[21]. The MDR bacteria were observed and, identified through Vitek® two methods (biomarkers, Marcy-L Etoile, France) following the procedure of Funke et al[22]. On the other hand, SeNPs and Mega. were checked for the antibacterial response upon official strains of Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 4352) and Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213). Furthermore, *Penicillium citrinum* (RCMB 001011), *Aspergillus flavus* (ATCC 16883), *Fusariu moxysporum* (RCMB 008002), *Aspergillus niger* (RCMB 002007), and *C.albicans* (ATCC 10231) were used to study the antifungal action[23].

Anti-mycobacterial activity of SeNPs and Mega.

Additionally, SeNPs and Mega. were examined for their potential as antibacterial factors against tuberculosis (TB) producing bacteria (*Mycobacterium tuberculosis* RCMB 010126) obtained kindly from The Regional Center for Mycology and Biotechnology (RCMB), Faculty of science, Al-Azhar University, Cairo, Egypt. The separate *M. tuberculosis* (RCMB 010126) clone was grown following anxiety on LB medium at 37°C for 72 h. The anti-tuberculosis activity was predicted by estimating the diameter of the inhibitory zone, using paper disk diffusion method and measurement of MIC using serial dilution technique. The zones of inhibition were analyzed after 72 h of incubation at 37°C. Each test was repeated three times.

Minimum inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) of the of SeNPs and Mega. was determined by the conventional paper disk diffusion methodby applying paper disk (266812 W. Germany 12.7 mm in diameters). Bacteria were grown on nutrient agar medium, while fungi and yeast were grown on Sabouraud agar medium. The purified Mega. was dissolved in water and loaded on paper disks with different concentrations as the following (250, 125, 62.50, 31.25, 15.63, 7.81, 3.90, 1.95, 0.98, 0.49, 0.24 and 0.12 µg mL^{-1}). Drying disks were loaded on surface of agar plates inoculated with test organism. Growth inhibition was examined after 24 h. from incubation at 37°C for bacteria and after 72 h. incubation at 27°C for fungi and yeast. Each test was repeated three times. MIC was expressed as the lowest concentration inhibiting test organism's growth.

Antioxidant activity for SeNPs and Mega.

Antioxidant activity for SeNPs and Mega. was carried out by measuring scavenging activity of 2, 2-diphenylpicrylhydrazyl (DPPH) free radicals according to procedure adopted by Cappuccino and Sherman[24].Briefly, 2 mL of distilled water, 1 mL of 0.1 mM DPPH solution in ethanol and 0.5 mL of the each nanoformulation were shaken vigorously and allowed to reach a steady state for 30 min at controlled room temperature of about 25°C. Decolorization of DPPH was determined by measuring the decrease in absorbance at 517 nm and the DPPH radical scavenging effect was calculated according to the following equation:

$$I\ (\%) = (A\ blank\ /A\ sample - 1) \times 100$$

where I (%) is the inhibition percent, (A blank) is the absorbance of the control reaction (containing all reagents except the test compound) and (A sample) is the absorbance of the test compound.

Immunomodulatory assay

Isolation of neutrophils

Preservative-free heparin and 2 mL of dextran B 4.5% in saline were combined to each 15 mL peripheral blood specimens received from healthful volunteers. The mixture was mildly agitated then permitted to stand for 60 min at 37°C to provide the sedimentation of erythrocytes. Neutrophils were separated by centrifugation according to by Ferrante and Thong technique[25]. Subsequent discharge

of the remaining erythrocytes was by hypotonic lysis, the neutrophils were washed by Hank's balanced salt suspension (HBSS) (Sigma, St Louis, USA) plus next suspended at a concluding concentration of 25×10^6 cells mL^{-1} in HBSS for intracellular killing action. The viability of neutrophils was examined by trypan blue suspension.

Intracellular killing activity

The intracellular killing action or the respiratory rupture of neutrophils was estimated by the nitroblue tetrazolium (NBT) reduction inspection by the modified method of Baehner and Nathan[26]. Secluded neutrophils were incubated in HBSS among latex particles, NBT (Sigma, St Louis, USA), and the separated flavonoids in alike concentrations as used in chemotaxis for 30 min at 37°C, then the reduced stain, blue formazan was obtained with pyridine and estimated spectrophotometrically on 515 nm. The issues were correlated with the negative control consisting of all the reagents except the neutrophil suspension. The variation in the absorbance among the cultures of cells that actively phagocyte latex bits and the negative control was estimated as a sign of intracellular killing action of neutrophils.

Statistical analysis

The averages of three replications and standard deviation (SD±) were determined for each sample and obtained results and the data were studied for an investigation of variance. Midpoints are viewed significantly different at ($P < 0.05$).Design-Expert® software was used to perform the statistical analysis of the full factorial design (3^3). The predicted R^2 was evaluated as an action of the accuracy of a predicted pattern response value[2]. Statistical results revealed that the predicted R^2 rates were in order among the adjusted R^2 in all rejoinders (data are not shown).

RESULTS

The results of the experimental factorial design used for optimization of SeNPs production are shown in Table I. The powerful absorption band observed at 415.0 nm ensured the formation of SeNPs. Run no. 25 in Table I showed the optimized condition (1.5 mMSeO_2, 2.0 mg mL^{-1} Fluc. and 37°C) with record raised the O.D (1.615) higher than other runs, including the raised SeNPs yield. Fig. 1 showed progressive color difference from transparent (Mega. and Se^{2+}) to the reddish color showing the assembly of SeNPs. As shown in Fig. 2, UV-Vis spectrum of SeNPs synthesized by Mega. had maximum absorption of 1.615 at λ_{max} 415 nm. FT-IR wave numbers of the characteristic bonds and corresponding assignments for Mega., without and with SeNPs are represented in Table II. The average PS was found to be 29.8±1.6 nm in SeNPs combined by Mega as illustrated in Fig. 3.The XRD model concerning the SeNPs is shown in Fig. 4. Examining the solution containing SeNPs prepared using Mega. by TEM, demonstrated the presence of spherical NPs ranged in size from 10.7 to 35.5 nm with the average main diameter was equal to 22.4 nm as presented in Fig. 5. Several peaks are observed, these being at SeNPs showing the diffraction characteristics, and it appeared at 9 theta (point) as 23.2°, 30.5°, 41.7°, 44.3°, 46.4°, 52.3°, 56.7°, 62.5° and 72.6°. Those peaks corresponded to the (100), (101), (110), (102), (111), (201), (113), (202) and (210) planes of the standard cubic phase of Se, respectively.

SeNPs synthesized by Mega. presented a excellent impact as antibacterial agents for fighting multidrug-resistant bacteria, including the large inhibition zone against *K. pneumonia* (32.0 mm), followed by *S. epidermidis* (31.0 mm) as given in Table III. On the other hand, it revealed a maximal inhibition zone covering the standard strains of bacteria including *B. subtilis* ATCC 6633 (34.0 mm), followed by *E. coli* ATCC 25922 (33.0 mm).The synthesized SeNPs were outstandingly very promising anti-TB acting against *M. tuberculosis* RCMB 010126 (36 mm). Additionally, all tested bacterial pathogenic strains were sensitive to the functionalized Mega. and the treated MDR bacteria were also resistance to the standard antibiotic tetracycline and sensitive to the SeNPs synthesized by Mega.From the results illustrated in Table IV, it could be concluded that SeNPs synthesized by Mega. were further active upon unicellular fungi *C. albicans* ATCC 10231 (40 mm), while amphotericin B alone, didn't show any activity. SeNPs incorporated by Mega. presented a strong impact as antifungal agent toward filamentous fungi including inhibition zone against *F.oxysporum*(45.0 mm), followed by *A. niger*(37.0 mm) as depicted in Table IV. The MIC were determined and the effects pointed that MIC toward multicellular and unicellular fungi extending from 0.03 ppm upon *F. oxysporum* and *C. albicans* to 4.37 ppm against *P. citrinum,* while the activity of standard amphotericin B was ranging from 16.0 mm against *A. niger* to 20.0 mm *P. citrinum,* with MIC ranging from 15.62 µg mL^{-1} toward *F. oxysporum* to 62.5 µg mL^{-1} *P. citrinum* and *A. niger.*

Antioxidant activity of Mega. and SeNPs synthesized by Mega. were evaluated using DPPH scavenging assay, a meaningful variation was recognized amongst the corresponding values achieved. The results in Table V showed that Mega. and SeNPs synthesized by Mega. had potential antioxidant activities when compared with the standard tertiary-butyl hydroquinone (TBHQ), at

percentage 80 %, 92 % and 86 %, respectively.SeNPs and Mega.were tested with the NBT reduction test and showing to increase the intracellular killing activity of neutrophils, data show in Table VI.

DISCUSSION

Synthesis of SeNPs using Mega.

Metabolites from microbial and plant extracts design a more desirable alternative to synthetic methods; the application of microorganisms achieves the growing requirement for non-hazardous NPs assembly route. Potential reducing factors of selenium to nano selenium can obtain phenolic compounds, tannins, saponins, carbohydrates, flavonoids minerals, iron, calcium, potassium, and vitamins[27]. Based on our recent discovery Mega, (broad-spectrum antibiotic) was able to reduce Se^{2+} ions to SeNPs. Moreover, the bio reductive formation of SeNPs, which may be employed for the safe large-scale production of these NPs, was first investigated herein. Results in Table I represented the experimental factorial design applied for the optimization of SeNPs generation. The determinants decided herein in the prevailing investigation and their levels were determined after conducting preliminary trials (details not presented) to designate the expected ranges of the objective variables. The optical density (O.D.) of the prepared SeNPs extended the peak intensities with contemporary blue shifts. This implied production of SeNPs with extraordinary yields and smaller sizes[28]. It is realized that SeNPs exhibit a reddish color in aqueous solution due to the Surface Plasmon Resonance (SPR) of element NPs[29]. The color change can be explained based upon the reducing power of Mega. antibiotic that leads to the redox reaction; selenium ions (Se^{2+}) were reduced into nanosize (SeNPs). Additionally, Mega. acted as capping agent being responsible for the stability of the formed SeNPs[30].The factors selected herein in the current research and their levels were chosen following doing preliminary trials (data not displayed) to designate the expected spectra of the sovereign variables.

Characterization of SeNPs

Spectroscopic techniques

By using UV-Visible spectroscopy examination (UV-Vis.)

It was previously mentioned that the dispersion of SeNPs created intense color due to SPR. The surface of metal appears similar plasma, having both available electrons in the conduction band and positively charged nuclei that make the mineral NPs becoming characteristic optical reflection spectrum in the UV-Vis range. The most observable property of NPs is their change in color at different sizes when observed in the visible region of the spectrum. It is well known that metal NPs such as Cu, Au, Ag, Se, etc. can absorb a certain wavelength of light in the visible region of the spectrum, therefore, UV-Vis spectrum is the most basic and important technique for identification and characterization of the prepared NPs[30]. The technique is simple, selective and sensitive to NPs. It is also time saving, easy to operate and involved easy sample preparation. On the other hand, there are certain limitations to this technique such as clear solution is a must for perfect analysis, the colored solutions are usually applied for the exposure and the procedure does not define the morphology of the particles. SPR is a common excitation of the electrons in the conduction band; near the outside of the NPs. Electrons are limited to specific oscillations styles by the particle's capacity and appearance. Consequently, metallic NPs had a characteristic optical absorption spectrum in the UV-Visible range[31]. The colloidal suspension of NPs, especially dark appearance, was presented and considered bias of their low dimensionality and their fitting to cover the SPR. Commonly, the SPR bands are controlled by the size, appearance, morphology, configuration and dielectric background of the developed NPs[32].

By using dynamic light scattering (DLS) technique

Aqueous specimens are applied for defining the hydrodynamic radius of the particle i.e. the medium size of the particle (PS) using laser rays. Unity and polydispersity sign of the particle size i.e. the area of the size of the particles present in the medium, can additionally be discovered.To examine the distribution of SeNPs PS, DLS was conducted and its results were associated with the TEM images.

By checking the X-Ray diffraction (XRD) pattern

The XRD pattern indicated that SeNPs were in the face-centered cubic (fcc) structure and crystalline in nature. Data concerning diamond lattice spacing among atoms, the orientation of a particular crystal, structure of the particle, size, and internal force estimation were obtained through XRD. While leaving the crystal, the incident beam of X-rays was caused by the atomic planes, which then interfered with one another exploring the diffraction pattern of the crystal. Size is calculated using Scherrer's equation $a=0.9\lambda/B \cos \theta$[30].

By imaging through transmission electron microscopy (TEM)

The particle size obtained from DLS estimation (29.8 nm) was obviously a bit larger than TEM image results (22.4), because DLS measures the hydrodynamic radius which take into consideration the organic free radical on the surface of SeNPs as well[16,23].

Fourier transform infrared (FTIR) spectroscopy

From the results of FTIR spectrum of Mega. and SeNPs synthesized by Mega.at room temperature, it was noticed that there were slight changes in % T between both Mega. and SeNPs synthesized by Mega (Fig. 6). On the other hand, there were no differences between the wave number of both samples, suggesting that, the Mega., structure didn't breakdown and it was keeping its stability even after being mixed with selenious acid solution. Additionally, upon comparing the IR spectrum of both Mega. alone and the SeNPs synthesized by Mega. there were no changes in all peaks position. Some changes in % T had been detected that might be attributed to the incorporation of the SeNPs along with its stabilizer Mega. in the active sites through van der Waals forces. The changes in peaks were associated to amide groups (positively charged) and hydroxyl groups (negatively charged), which was interpreted by the reducing power of the derived Mega. to selenium acid. Additionally, Mega. was coating SeNPs and, consequently, preserved and prevented SeNPs from precipitation and aggregation.

Antimicrobial potential of the synthesized SeNPs

In vitro antibacterial activity

The MIC determinations of SeNPs manifested strong activity towards MDR bacteria involve *E. coli, A. baumannii, P. aeruginosa, K. pneumonia, S. epidermis, S. aureus (MRSA),* and *M. tuberculosis* the results were listed as: 0.27 ppm, 0.03 ppm, 0.13 ppm, 0.54 ppm, 0.27 ppm, 0.13 ppm and 0.067 ppm, respectively. The MIC results of SeNPs against the standard ATTC bacteria remained in the range from 0.015 ppm against *P. aeruginosa* to1.09 ppm *Vibrio cholera*. The unique features of inorganic NPs are their large outside to volume ratios and their nano-scale dimension, which enhance the response beside pathogenic microorganisms[33].

In vitro antifungal activity

From the last stated antifungal results (Table IV), SeNPs synthesized by Mega. could possess synergistic effect upon *C. albicans, A. niger, A.flavus*, *F.oxysporum* and *P. citrinum*. These results agreed with those found by Shakibaie et al. who reported the antifungal activity of biogenic SeNPs against both *A. fumigatus* and *C. albicans*[34].The biogenic SeNPs had PS range from 120–140 nm and was synthesized by *Bacillus* sp. MSh-1. The MIC measurements of the antifungal activity of the SeNPs were found to be 70 µg mL^{-1} against *C. albicans* and 100 µg mL^{-1} against *A. fumigates* highlighting their potential use as antifungal agents.

Antioxidant activity for SeNPs and Mega.

From these assays, the potential mechanism of antioxidant action of SeNPs and Mega. included reductive ability, electron donating ability and radicals' scavengers'capabilities, Data is shown in Table V.

Immunomodulatory activity for SeNPs and Mega.

In this investigation, the immunomodulatory action of the Mega. and SeNPs manufactured by Mega. were examined by *in vitro* experiments. The neutrophils act as the primary role of an effector or killer cell for various types of contaminations[35]. Defects in neutrophil support, especially chemotaxis, phagocytosis and intracellular killing action, were found to be correlated with a kind of infectious complication[36]. Optimal host defense toward infection is recognized to be a large number of neutrophils to reply by chemotaxis. Chemotaxis is induced by the movement of the phagocytic cells in response to chemotactic incentives, creating them to move in the direction of progressing concentration of the attractant[37]. Neutrophils are susceptible to the chemotactic elements at nanomolar concentrations and progress towards them. Zymosan is allowed as a chemo-attractant factor in several *in vitro* and *in vivo* bioassays[38]. The primary purpose of the neutrophil in host resistance is the intracellular killing of microbes. This final step of phagocytosis depends upon the successful achievement of the preceding steps: motility (chemo toxic progress), identification, ingestion, degranulation and intracellular killing.

CONCLUSION

In the current research, an innovative method for SeNPs synthesis through the use of antibacterial Mega. was introduced. The prepared SeNPs had size 22.4 nm and were spherical in shape when examined by TEM. The present study placed emphasis on the innovative synthesis of biogenic SeNPs using Mega. Antimicrobial studies involved inhibition zone and MIC investigations and highlighted the synergistic impact between Mega. and SeNPs as antimicrobial agents. SeNPs presented powerful action toward *Klebsiella pneumonia* (32.0 mm),

followed by *S. epidermis* (31.0 mm), and showed excellent antifungal action toward unicellular fungi *C.albicans* ATCC 10231 (40 mm). SeNPs, additionally presented potential antioxidant activity when compared with the standard tertiary-butyl hydroquinone (TBHQ) and high intracellular killing capability which confirmed the immune stimulatory role. The important role of the synthesized SeNPs as anti-TB induced by *M. tuberculosis* was also an outstanding finding.

REFERENCES

1. Nikalje A.P.: Nanotechnology and its applications in medicine. **Med. Chem.**, 2015, 5, 081-089.
2. Abd-Elsalam W.H., El-Zahaby S.A. and AL-Mahallawi A. M.: Formulation and *in vivo* assessment of terconazole-loaded polymeric mixed micelles enriched with Cremophor EL as dual functioning mediator for augmenting physical stability and skin delivery. **Drug. Deliv.**, 2018, 25, 484-492.
3. Dubey A., Shetty A., Ravi G.S., Kiritkumar M.C., Prabhu P., Hebbar S. and El-Zahaby S.A.: Development and investigation of novel solid self-nanoemulsifying system loaded with hydrochlorothiazide for the treatment of hypertension. **Int. J. Pharm. Investig.**, 2018, 8, 83-91.
4. Hafner A., Lovrić J., Lakoš G.P. and Pepić I.:Nanotherapeutics in the EU: an overview on current state and future directions. **Int. J. Nanomed.,** 2014, 19,1005-1023.
5. Wei D., Sun W., Qian W., Ye Y. and Ma X.: The synthesis of chitosan-based silver nanoparticles and their antibacterial activity. **Carbohydr. Res.**, 2009,344, 2375-2382.
6. Zhang L., Pornpattananangkul D., Hu C.M. and Huang C.M.: Development of nanoparticles for antimicrobial drug delivery. **Curr. Med. Chem.**, 2010, 17, 585-594.
7. Abeylath S.C. and Turos E.: Drug delivery approaches to overcome bacterial resistance to beta lactam antibiotics. **Expert Opin. Drug Deliv.**, 2008, 5, 931-949.
8. Jiao Q., Li L., Mu Q. and Zhang Q.: Immunomodulation of Nanoparticles in Nanomedicine Applications. **Bio Med. Res. Int.**, 2014,1-19.
9. Park Y.M., Lee S.J., Kim Y.S., Lee M.H., Cha G.S. and Jung I.D.:Nanoparticle-based vaccine delivery for cancer immunotherapy. **Immune Netw.**, 2013,13,177-183.
10. Yazdi M., Mahdavi M., Kheradmand E. and Shahverdi A.: The preventive oral supplementation of a selenium nanoparticle-enriched probiotic increases the immune response and lifespan of 4T1 breast cancer bearing mice. **Arzneimittelforschung**, 2012, 62, 525-531.
11. Lee J.S., Kim D.H., Lee C.M., Ha T.K., Noh K. T. and Park J.W.: Deoxypodophyllo toxin Induces a Th1 Response and Enhances the Antitumor Efficacy of a Dendritic Cell-based Vaccine.**Immune Netw.**, 2011,11, 79-94.
12. El-Zahaby S.A., Kassem A.H. and El-Kamel.: Non-antibiotic Therapies for Treatment of *Helicobacter pylori* Infection. **Pharm. Biotech. Microbiol.,** 2016, 2,1-5.
13. OgunsheAdenike A. O.: Comparative bacteriostatic potentials of oral paediatric antibiotics sold in two countries Running head: antibiotics from different countries. **Arch. Clin. Microbiol.**, 2014, 5,:3.
14. Brown G.D., Denning D.W., Gow N.A., Levitz S.M., Netea M.G. and White T.C.: Hidden killers: human fungal infections. **Sci. Transl. Med.**, 2012, 194,13.
15. Shakibaie M., Forootanfar H., Golkari Y., Mohammadi-Khorsand T. and Shakibaie M. R.: Anti-biofilm activity of biogenic selenium nanoparticles and selenium dioxide against clinical isolates of *Staphylococcus aureus,Pseudomonas aeruginosa* and *Proteus mirabilis*.**J. Trace Elem. Med. Biol.**, 2015, 29,235-241.
16. El-Batal A.I., El-Sayed M.H., Refaat B.M. and Aska A.A.Z.:Synthesis of selenium nanoparticles by *Bacillus laterosporus* using gamma radiation. **British J. Pharm. Res.**, 2014b, 4,1364.
17. Ranghar S., Sirohi P., Verma P. and Agarwal V.: Nanoparticle-based drug delivery systems: promising approaches against infections. **Braz. Arch. Biol. Technol.**, 2014,57, 209-222.
18. Sharma V.K., Yngard R.A. and Lin Y.: Silver nanoparticles: green synthesis and their antimicrobial activities. **Adv. Colloid Interface Sci.**, 2009, 145, 83-96.
19. Jagannathan R., Poddar P. and Prabhune A.: Cephalexin-mediated synthesis of quasi-spherical and anisotropic gold nanoparticles and their *in situ* capping by the antibiotic. **J. Phys. Chem. C.**, 2007, 111, 6933-6938.
20. Iravani S., Korbekandi H., Mirmohammadi S.V. and Zolfaghari B.: Synthesis of silver nanoparticles: chemical, physical and biological methods. **Res. Pharm. Sci.**, 2014, 9,385-406.
21. EL-Sherbiny G., EL-Batal A., EL-Sherbiny I.M. and Askar A.A.: Antibacterial potential with molecular docking study against multi-drug resistant bacteria and *Mycobacterium tuberculosis* of streptomycin produced by *Streptomyces atroverins*, strain Askar-SH50. **J. Chem. Pharm. Res.**, 2017, 9, 189-208.
22. Funke G., Monnet D., Von Graevenitz A. and Freney J.: Evaluation of the VITEK 2 system for rapid identification of medically relevant gram-negative rods**. J. Clin. Microbiol.**,1998, 36,1948-1952.
23. El-Batal A.I., El-Sayed M.H., Refaat B.M. and Aska A.A.Z.:Marine *Streptomyces cyaneus* strain alex-sk121 mediated eco-friendly synthesis of silver nanoparticles using gamma radiation. **British J. Pharm. Res.**, 2014, 4,2525.
24. Cappuccinoj G.A.S.N. 2004. Microbiology, Laboratory Manual, New Delhi, India: Pearson Education.
25. Baehner R. L. and Nathan D.G.: Quantitative nitroblue tetrazolium test in chronic granulomatous disease.**N. Engl. J. Med.**, 1968, 278, 971-976.
26. Ferrante A. andThong Y.H.:Optimal conditions for simultaneous purification of mononuclear and polymorphonuclear leucocytes from human blood by the Hypaque-Ficoll method. **J. Immunol. Methods,** 1980, 36, 109-117.
27. Sharma P.R. and Varma A.J.: Thermal stability of cellulose and their nanoparticles: effect of incremental increases in

carboxyl and aldehyde groups. **Carbohydrate Polymers.,** 2014, 114, 339-343.

28. Liu F.K., Hsu Y.C., Tsai M.H. and Chu T.C.:Using γ-irradiation to synthesize Ag nanoparticles. **Mater. Lett.**, 2007, 61, 2402-2405.
29. Link S. and EL-Sayed M.A.: Optical properties and ultrafast dynamics of metallic nanocrystals.**Annu. Rev. Phys. Chem.,** 2003, 54, 331-366.
30. Khurana J.M. and Vij K.:Nickel Nanoparticles as Semi heterogeneous Catalyst for One-Pot, Three-Component Synthesis of 2-Amino-4 H-pyrans and Pyran Annulated Heterocyclic Moieties. **Synth.Commun.,** 2013,43, 2294-2304.
31. Sheikh F.A., Barakat N.A., Kanjwal M.A., Chaudhari A.A., Jung I.H., Lee J.H. and Kim Y.H.:Electrospun antimicrobial polyurethane nanofibers containing silver nanoparticles for biotechnological applications. **Macromol. Res.,** 2009, 17, 688-696.
32. Sadasivudu P., Shastri N. and Sadanandam M.: Development and validation of RP-HPLC and UV methods of analysis for fluconazole in pharmaceutical solid dosage forms**. Int. J. Chem. Tech. Res.**, 2009, 1, 1131-1136.
33. Xiu Z.M., Ma J.and Alvarez P.J.:Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions. **Environ. Sci. Technol.**, 2001, 45, 9003-9008.
34. Shakibaie M., Mohazab N.S. and Mousavi S.A.A.: Antifungal activity of selenium nanoparticles synthesized by bacillus species Msh-1 against *Aspergillus fumigatus* and *Candida albicans.* **Jundishapur J. Microbiol.**, 2015b, 8,e26381.
35. Akbay P., Basaran A.A., Undeger U. and Basaran N.: *In vitro* immunomodulatory activity of flavonoid glycosides from Urticadioica L. **Phytother. Res.**, 2003,17,34-37.
36. Lehrer R.I., Ganz T., Selsted M.E., Babior B.M. and Curnutte J.T.:Neutrophils and host defense. **Ann. Intern. Med.**, 1988,109,127-142.
37. Wagner H.and Jurcic K.: Immunologic studies of plant combination preparations. *In vitro* and *in vivo* studies on the stimulation of phagocytosis. **Arzneimittelforschung.,**1991, 41, 1072-1076.
38. Ahmet başaran A., Ceritoğlu I., Ündeğer Ü.and Başaran N.: Immunomodulatory activities of some Turkish medicinal plants. **Phytother. Res.**, 1997, 11, 609-611.