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The Antibacterial Activities of NiO Nanoparticles Against Some Gram-Positive and Gram-Negative Bacterial Strains

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Abstract

Introduction: Given the increasing rate of antibiotic resistance among bacterial strains, many researchers have been working to produce new and efficient and inexpensive antibacterial agents. It has been reported that some nanoparticles may be used as novel antimicrobial agents. Here, we evaluated antibacterial properties of nickel oxide (NiO) nanoparticles.

Methods: NiO nanoparticles were synthesized using microwave method. In order to control the quality and morphology of nanoparticles, XRD (X-ray diffraction) and SEM (scanning electron microscope) were utilized. The antibacterial properties of the nanoparticles were assessed against eight common bacterial strains using agar well diffusion assay. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were measured. Antibiotic resistance pattern of the bacteria to nine antibiotics was obtained by Kirby-Bauer disk diffusion method.

Results: The crystalline size and diameter (Dc) of NiO nanoparticles were obtained 40-60 nm. The nanoparticles were found to inhibit the growth of both gram-positive and gram-negative bacteria with higher activity against gram-positive organisms. Among bacterial strains, maximum sensitivity was observed in *Staphylococcus epidermidis* with MIC and MBC of 0.39 and 0.78 mg/mL, respectively. The bacteria had high resistance to cefazolin, erythromycin, rifampicin, ampicillin, penicillin and streptomycin.

Conclusion: NiO nanoparticles exhibited remarkable antibacterial properties against gram-positive and gram-negative bacteria and can be a new treatment for human pathogenic and antibiotic-resistant bacteria.

Keywords: Nickel Oxide Nanoparticles, Bacteria, Antibacterial Activity.

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Introduction

Widespread and inappropriate utilization of antibiotics for either preventive or therapeutic purposes has resulted in the emergence of remarkable antibiotic resistance rates worldwide.[1] The increasing emergence of antibiotic resistance among common bacterial strains has urged the scientific community to seek out new antimicrobial agents.[2] In general, antibiotic resistant bacteria develop suddenly and may even challenge newly introduced antibiotics.[3] Two problematic nosocomial pathogens are *Pseudomonas aeruginosa* and *Staphylococcus aureus*, both of which commonly exhibit multidrug resistance. The important role of *P. aeruginosa* has been discovered since 1975.[4] *S. aureus* is also one of the main causes of bacterial infections in hospital settings and community.[5] *P. aeruginosa* and *S. aureus* are 2 important pathogens, which have antibiotic resistance plasmids to suppress the antibacterial properties of the antibacterial agents. Additionally, antibiotic resistance of *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Staphylococcus epidermidis*, *Micrococcus luteus* and *Bacillus subtilis* have also been reported.[2,4] Therefore, new antibacterial strategies need to be expanded to fight antibiotic resistant bacteria.

Among the most promising of new antimicrobial agents, nanoparticles have shown potent antibacterial activity.[6,7] Nanoparticles are very small-scale molecules and have several physical and chemical properties.[8] Nanoparticles can be metals ions such as Cu, Pt, Zn, Pd, Ag, Fe, Au or metal oxides such as nickel oxide (NiO), CeO2, CuO, FeO, Ag2O and ZnO.[9] The most important effects of nanoparticles are antibacterial, antiviral, antioxidant, antiparasitic, anti-neoplastic and anti-inflammatory effects.[10,11] The use of nanoparticles as potential antibacterial agents is due to the fact that bacteria are unable to exhibit resistance to the nanoparticles in several cases.[3] Currently, silver and gold nanoparticles are widely used as antimicrobial agents.[12,13] However, the information regarding then antibacterial effects of NiO nanoparticles remains to be completely clarified. Thus, this study was aimed to study the antibacterial effects of NiO nanoparticles on the *P. aeruginosa*, *S. aureus*, *E. coli*, *K. pneumoniae*, *S. marcescens*, *S. epidermidis*, *M. luteus* and *B. subtilis*. Additionally, the techniques used to synthesize NiO nanoparticles (e.g. hydrothermal, sol-gel, solid-state reaction, electrochemistry, micro emulsions, spray pyrolysis and precipitation methods) are usually complex and expensive.[14] Therefore, we also introduced a new simple and cheap method to produce NiO nanoparticles.

Materials and Methods

Synthesizing and Characterizing the NiO Nanoparticles

NiO nanoparticles were synthesized using the microwave method as a faster and cheaper strategy than other conventional methods. Briefly, the mixture of Ni $(OAc)_2.4H_2O$ (1 mM), NaOH (2 mM) and thiourea (4 mM) was prepared by dissolving in propylene glycol (30 mL) and agitated for 20 minutes. The solution was exposed to microwave irradiation in a domestic microwave oven (2450 MHz at the output powers of 600-900 watt for 10 minutes). The obtained powders were washed with distilled water and absolute ethanol. Finally, the precipitate was dried in vacuum (50°C, 48 hours).[15] The NiO nanoparticles were scanned using XRD (X-ray diffraction) and SEM (scanning electron microscope). XRD patterns of NiO nanostructure sublimated at 50°C. SEM imaging of NiO nanoparticles was performed using SEM (LEO 1455VP, London). Images were prepared using a through lens detector (TLD) in the immersion mode (80 000x magnification). The working distance was adjusted at 3 mm using an accelerating voltage of 10 kV. The resolution was adjusted so that the particles could be clearly distinguished from the background. The NiO nanoparticles size distribution plots were drawn by measuring the diameter of at least 100 nanoparticles in SEM images.

Bacterial Strains

Lyophilized cultures of *E. coli* (PTCC 1330), *K. pneumoniae* (PTCC 1053), *S. marcescens* (PTCC 1621), *P. aeruginosa* (PTCC 1074), *S. aureus* (PTCC 1112), *S. epidermidis* (PTCC 1114), *M. luteus* (PTCC 1110) and *B. subtilis* (PTCC 1023) were procured from Persian Type Culture Collection (PTCC), Iran.

Antimicrobial Assay

The antibacterial properties of NiO nanoparticle was were by agar well diffusion assay. Bacterial cultures were prepared to match the turbidity of a McFarland 0.5 standard (1.5×10^8 CFU/mL) in sterile distilled water prior to the assay and inoculated on Mueller-Hinton agar (Merck Company, Germany). Ten serial dilutions yielding concentrations of 100, 50, 25, 12.5, 6.25 3.12, 1.56, 0.78, 0.39 and 0.195 mg/ml for NiO nanoparticle were prepared using two-fold dilution.[16] Six mm diameter wells were cut in the culture medium at a distance of 20 mm from each other and then 20 µL of each concentration of nanoparticles was added to each of the 5 wells. The cultures were incubated [(36 ± 1°C), 24 hours] under aerobic condition. Afterwards, the inhibition zone around the wells (mm) was measured.[17,18] Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (i.e. the lowest concentration of nanoparticles inhibiting the bacterial colonies) were determined on Mueller-Hinton agar.[19] All antibacterial activity tests were performed in triplicate. Antibiotic resistance pattern of bacterial strains was performed by Kirby-Bauer antibiotic sensitivity test according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI).[20] Mueller-Hinton agar (Merck, Germany) was inoculated with the bacterial suspension in normal saline (0.5 McFarland turbidity). Antibiotic discs (Padtan Teb Co, Iran) were placed on medium cultures. The following antibiotic discs (per unit disc) were applied for performing antimicrobial susceptibility test: cefazolin (30 µg), erythromycin (15 µg), gentamicin (10 µg), rifampicin (5 µg), imipenem (10 µg), tobramycin (10 µg), ampicillin (10 µg), penicillin (10 µg), and streptomycin (10 µg). The resistance data was interpreted according to the guidelines of the CLSI.[20]

Statistical Analysis

The data was analyzed using one-way ANOVA in the SPSS version 18.0. The *P* values less than 0.05 were considered as statistically significant.

Results

Synthesis and Characterization of NiO Nanoparticles

Following the synthesis of NiO nanoparticles, the appearance of the nanoparticles was assessed by XRD (Figure 1), energy-dispersive analysis of X-ray and SEM.

The crystalline size and diameter (Dc) of nanofibers were obtained 40-60 nm by analyzing the diffraction patterns of full width of the half maximum (FWHM) using the Debay-Scherer equation (Equation 1):

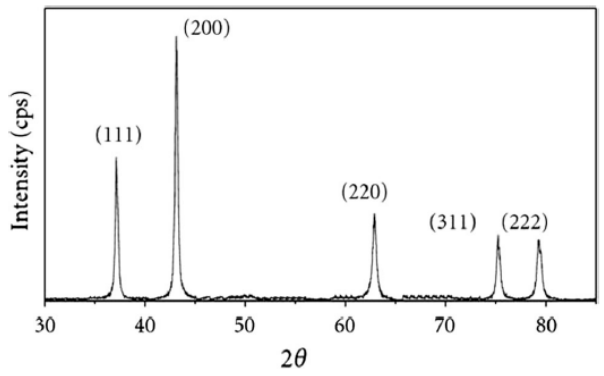

Figure 1. XRD Pattern of NiO Nanoparticles.

$$D_{xrd}=0.9\lambda\ (57.3)\ /W_{size}\cos\Theta \qquad (1)$$

Figure 2 illustrates the SEM images of the NiO nanoparticles sublimated within the range of 50°C. Image was obtained using the TLD (80 000× magnification). The NiO nanoparticles size distribution plots were prepared by measuring the diameter of at least 100 nanoparticles in SEM images. The morphology of all samples was particle-like with an average size of 50 nm. The nanoparticles surface had a relatively good dispersion.

Antibacterial properties of NiO nanoparticles

The bacterial growth was determined using agar well diffusion assay in culture media loaded with different concentrations of NiO nanoparticles by measuring the diameter of inhibition zone. Gram-positive bacteria showed higher sensitivity to NiO nanoparticles at lower concentrations compared to gram-negative bacteria ($P<0.05$, Figure 3). The results showed that NiO nanoparticles inhibited bacterial growth in a dose-dependent manner (Figure 4). The antibacterial activity (MIC and MBC) of NiO nanoparticles are demonstrated in Table 1. Among bacterial strains, maximum sensitivity was observed in *S. epidermidis* with MIC of 0.39 mg/mL. Based on antibiogram results, all gram-negative bacteria were resistant to cefazolin, erythromycin and rifampicin and all gram-positive bacteria were resistant to cefazolin.

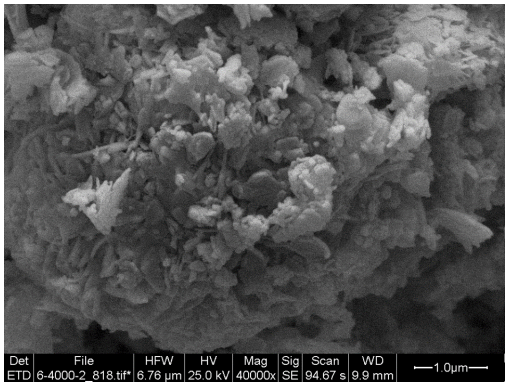

Figure 2. SEM Image of the Synthesized NiO Nanoparticles.

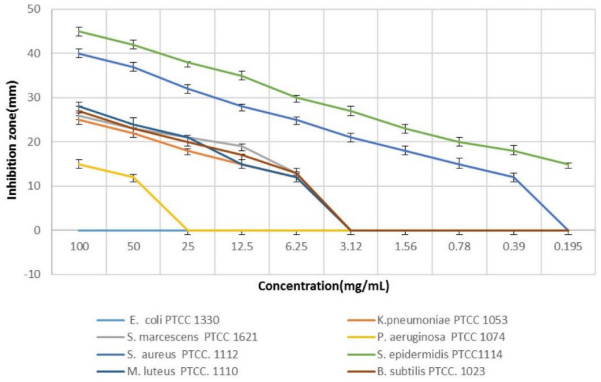

Figure 3. The Antibacterial Properties of Nickel Oxide Nanoparticles Against Various Bacterial Strains.

The antibiotic resistance pattern of the bacterial strains to common antibiotics of different classes is shown in Table 2.

Discussion

To overcome the increased resistance of bacteria to common antibiotics and their side effects, there is a need to create new antimicrobial drugs.[21] How nanoparticles promote their numerous antibacterial effects remains to be sufficiently explained.[10] There are reports on the antibacterial activities of nanoparticles demonstrated by binding of nanoparticles to bacterial surface, disrupting cell membrane, modulating respiration chain and other permeability-dependent reactions, generating reactive oxygen species (ROS), promoting electrostatic attraction between the positively charged nanoparticles and negatively charged bacterial cells and finally degrading bacterial essential proteins.[19,22] In this study, we assessed the antibacterial properties of NiO nanoparticles. The highest inhibitory effect was recorded against gram-positive bacteria, while the growth of gram-negative bacteria was influenced less pronouncedly. This observation can be partly explained by different cellular wall structures of gram-positive and gram-negative bacteria, as well as changes in cellular physiology and metabolism.[23] The opposite charges of bacteria and nickel ions released from NiO nanoparticles are thought to cause adhesion and bioactivity due to electrostatic forces. Since bacterial cell walls are negatively charged molecules, they are the potential targets of Ni^{2+} ions, which are released from NiO nanoparticles. Gram-negative bacteria are generally less susceptible and more resistant to antimicrobial agents and antibacterial pharmaceuticals than gram-positive bacteria mainly due to their impermeable cell wall. The envelope surrounding gram-negative bacteria has two lipid membranes with high concentrations of lipopolysaccharides, lipoprotein and porin channels in the outer leaflet of the outer membrane.[24] Almost all the components will be affected by multidrug efflux pumps.[19] Finally, it can be concluded that gram-positive

Figure 4. The Antibacterial Properties of Nickel Oxide Nanoparticles Against Staphylococcus epidermidis by Agar Well Diffusion Method.

Table 1. The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Nickel Oxide Nanoparticles Against Bacterial Strains

Bacteria	**MIC** (mg/mL)	**MBC** (mg/mL)
Escherichia coli (PTCC 1330)	MIC>100	MBC>100
Klebsiella pneumoniae (PTCC 1053)	12.5<MIC≤25	50
Serratia marcescens (PTCC 1621)	12.5<MIC≤25	50
Pseudomonas aeruginosa (PTCC 1074)	50<MIC≤100	MBC>100
Staphylococcus aureus (PTCC 1112)	0.78<MIC≤1.56	3.125
Staphylococcus epidermidis (PTCC 1114)	0.195<MIC≤0.39	0.78
Micrococcus luteus (PTCC 110)	0.78<MIC≤1.56	3.125
Bacillus subtilis (PTCC 1023)	3.125<MIC≤6.25	12.5

Table 2. The Antibiotic Resistance Pattern of Bacterial Strains to Antibiotics

Bacteria	**Antibiotics**								
	CZ	**S**	**P**	**AM**	**TB**	**IM**	**RA**	**G**	**E**
Escherichia coli (PTCC 1330)	R	R	R	R	SM	S	R	SM	R
Klebsiella pneumoniae (PTCC 1053)	R	R	R	R	R	S	R	R	R
Serratia marcescens (PTCC 1621)	R	R	R	R	R	S	R	S	R
Pseudomonas aeruginosa (PTCC 1074)	R	R	R	R	SM	S	R	R	R
Staphylococcus aureus (PTCC 1112)	R	R	S	R	SM	S	R	S	S
Staphylococcus epidermidis PTCC 1114	R	R	S	R	R	S	S	R	SM
Micrococcus luteus (PTCC 110)	R	R	S	R	R	S	R	S	S
Bacillus subtilis (PTCC 1023)	R	R	R	R	SM	S	S	S	S

Abbreviations: S: Sensitive, SM: Semi-sensitive, R: Resistant, CZ: Cefazolin, E: Erythromycin, GM: Gentamicin, RA: Rifampicin, IM: Imipenem, TB: Tobramycin, AM: Ampicillin, P: Penicillin, S: Streptomycin.

bacterial strains are more sensitive to NiO nanoparticles than the gram-negative bacterial strains because of their membrane structure. Agar well diffusion assay confirmed that NiO nanoparticles inhibited the growth of bacterial strains dose-dependently. In this regard, the highest antibacterial activity was observed at the 100 mg/mL concentration. The antibacterial activity of nanoparticles can also be attributed to the nickel ions released from NiO nanoparticles. The released ions in turn may increase the membrane permeability and promote oxidative stress, which in turn activates cell death pathways. These arguments are supported by previous reports on the antibacterial properties of other nanoparticles such as silver and zinc oxide.[25,26] In other studies, the antimicrobial properties of nickel nanoparticles synthesized with *Ocimum sanctum* leaf extract (NiGs) were evaluated against gram-negative and gram-positive pathogenic bacteria and fungi. The NiGs nanoparticles showed higher antimicrobial efficiency against tested microorganisms compared to leaf extract alone and antibiotics.[18,19] Another investigation showed that bimetallic Cu–Ni nanoparticles had a significant effect on *Streptococcus mutans*, making

them suitable to be used in dentistry.[27] In a recent study, hydroxide and NiO nanoparticles were successfully synthesized by the chemical precipitation, in which, in accordance with our study, NiO nanoparticles showed inhibitory activity against both gram-positive and gram-negative bacteria with excellent selectivity against gram-positive bacteria.[14] Suresh et al. also suggested that the NiO nanoparticles exhibited excellent antibacterial and antifungal activities.[28]

Conclusion

In conclusion, our findings revealed the potential role of NiO nanoparticles as antibacterial agents, so that they could be used as antiseptic agents in various environmental and biomedical industries. However, the toxicity of these nanoparticles against eukaryotic cells should be explored before approving them as new antibacterial agents.

Ethical Approval

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Khameneh B, Diab R, Ghazvini K, Fazly Bazzaz BS. Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them. Microb Pathog. 2016;95:32-42. doi:10.1016/j.micpath.2016.02.009
2. Slavin YN, Asnis J, Häfeli UO, Bach H. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J Nanobiotechnology. 2017;15(1):65. doi:10.1186/s12951-017-0308-z
3. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine. 2017;12:1227-1249. doi:10.2147/ijn.s121956
4. Cross A, Allen JR, Burke J, et al. Nosocomial infections due to *Pseudomonas aeruginosa*: review of recent trends. Rev Infect Dis. 1983;5 Suppl 5:S837-845.
5. Valaperta R, Tejada MR, Frigerio M, et al. *Staphylococcus aureus* nosocomial infections: the role of a rapid and low-cost characterization for the establishment of a surveillance system. New Microbiol. 2010;33(3):223-232.
6. Singh R, Lillard JW Jr. Nanoparticle-based targeted drug delivery. Exp Mol Pathol. 2009;86(3):215-223. doi:10.1016/j.yexmp.2008.12.004
7. Huh AJ, Kwon YJ. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. J Control Release. 2011;156(2):128-145. doi:10.1016/j.jconrel.2011.07.002
8. Patil SP, Kumbhar ST. Antioxidant, antibacterial and cytotoxic potential of silver nanoparticles synthesized using terpenes rich extract of *Lantana camara* L. leaves. Biochem Biophys Rep. 2017;10:76-81. doi:10.1016/j.bbrep.2017.03.002
9. Zare Y, Shabani I. Polymer/metal nanocomposites for biomedical applications. Mater Sci Eng C Mater Biol Appl. 2016;60:195-203. doi:10.1016/j.msec.2015.11.023
10. Teow SY, Wong MM, Yap HY, Peh SC, Shameli K. Bactericidal properties of plants-derived metal and metal oxide nanoparticles (NPs). Molecules. 2018;23(6). doi:10.3390/molecules23061366
11. Stankic S, Suman S, Haque F, Vidic J. Pure and multi metal oxide nanoparticles: synthesis, antibacterial and cytotoxic properties. J Nanobiotechnology. 2016;14(1):73. doi:10.1186/s12951-016-0225-6
12. Beyth N, Houri-Haddad Y, Domb A, Khan W, Hazan R. Alternative antimicrobial approach: nano-antimicrobial materials. Evid Based Complement Alternat Med. 2015;2015:246012. doi:10.1155/2015/246012
13. Panyala NR, Peña-Méndez EM, Havel J. Gold and nano-gold in medicine: overview, toxicology and perspectives. J Appl Biomed. 2009;7(2):75-91. doi:10.32725/jab.2009.008.
14. Shanaj BR, John XR. Effect of calcination time on structural, optical and antimicrobial properties of nickel oxide nanoparticles. J Theor Comput Sci. 2016;3(2):149. doi:10.4172/2376-130X.1000149
15. El-Kemary M, Nagy N, El-Mehasseb I. Nickel oxide nanoparticles: Synthesis and spectral studies of interactions with glucose. Mater Sci Semicond Process. 2013;16(6):1747-1752. doi:10.1016/j.mssp.2013.05.018
16. Shahidi Bonjar GH, Karimi Nik A, Heydari MR, et al. Anti-pseudomona and anti-bacilli activity of some medicinal plants of Iran. DARU J Pharm Sci. 2003;11(4):157-163.
17. Valgas C, de Souza SM, Smânia EF, Smânia A Jr. [Screening methods to determine antibacterial activity of natural products]. Braz J Microbiol. 2007;38(2):369-380. doi:10.1590/S1517-83822007000200034
18. Helan V, Prince JJ, Al-Dhabi NA, et al. Neem leaves mediated preparation of NiO nanoparticles and its magnetization, coercivity and antibacterial analysis. Results Phys. 2016;6:712-718. doi:10.1016/j.rinp.2016.10.005
19. Jeyaraj Pandian C, Palanivel R, Dhanasekaran S. Screening antimicrobial activity of nickel nanoparticles synthesized using *Ocimum sanctum* leaf extract. Journal of Nanoparticles. 2016;2016:4694367. doi:10.1155/2016/4694367
20. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; 16th informational supplement (M100-S16). Wayne, PA, USA: CLSI; 2006.
21. Richter MF, Hergenrother PJ. The challenge of converting Gram-positive-only compounds into broad-spectrum antibiotics. Ann N Y Acad Sci. 2019;1435(1):18-38. doi:10.1111/nyas.13598
22. Reidy B, Haase A, Luch A, Dawson KA, Lynch I. Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications. Materials (Basel). 2013;6(6):2295-2350. doi:10.3390/

ma6062295
23. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr. 2016;4(2). doi:10.1128/microbiolspec.VMBF-0016-2015
24. Maria-Neto S, de Almeida KC, Macedo ML, Franco OL. Understanding bacterial resistance to antimicrobial peptides: From the surface to deep inside. Biochim Biophys Acta. 2015;1848(11 Pt B):3078-3088. doi:10.1016/j.bbamem.2015.02.017
25. Kim JS, Kuk E, Yu KN, et al. Antimicrobial effects of silver nanoparticles. Nanomedicine. 2007;3(1):95-101. doi:10.1016/j.nano.2006.12.001
26. Jin T, Sun D, Su JY, Zhang H, Sue HJ. Antimicrobial efficacy of zinc oxide quantum dots against *Listeria monocytogenes, Salmonella Enteritidis,* and *Escherichia coli* O157:H7. J Food Sci. 2009;74(1):M46-52. doi:10.1111/j.1750-3841.2008.01013.x
27. Argueta-Figueroa L, Morales-Luckie RA, Scougall-Vilchis RJ, Olea-Mejía OF. Synthesis, characterization and antibacterial activity of copper, nickel and bimetallic Cu–Ni nanoparticles for potential use in dental materials. Progress in Natural Science: Materials International. 2014;24(4):321-328. doi:10.1016/j.pnsc.2014.07.002
28. Suresh S, Karthikeyan S, Saravanan P, Jayamoorthy K. Comparison of antibacterial and antifungal activities of 5-amino-2-mercaptobenzimidazole and functionalized NiO nanoparticles. Karbala International Journal of Modern Science. 2016;2(3):188-195. doi:10.1016/j.kijoms.2016.05.001