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In Vitro Applications of the Biosynthesized Nickel Oxide Nanoparticles from Safflower

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Abstract

The present study aimed to prepare nickel oxide nanoparticles using readily available, inexpensive, and environmentally friendly materials, so this work was done using the extract of the petals of safflower (*Carthamus tinctorius L.*) to obtain NiO-NPs. The work involved the utilization of two different genotypes of safflower [Dincer 5-18-1 (red flower) and Remzibey-05 (orange flower)] to experiment with which one worked best. The studying of structure, shape and particles size was determined by (FTIR, UV-Vis., XRD and TEM) analysis and all of the acquired data revealed that the nanoparticles were effectively formed. Nickel oxide particles were evaluated biologically in comparison to its precursor of hydroxide $Ni(OH)_2$ under three dilution series (25%, 50%, and 75%) against some microbes [Candida albicans, Staphylococcus aureus, and Escherichia coli]. The results show that the prepared oxides are less effective than their hydroxides, and in general, all compounds show the inhibition effect of microorganism growth at high concentrations.

Introduction:

Nowadays, we can note that how the discoveries and inventions in nanoscience in different branches of science have made life easier. Both of nanoscience and nanotechnology are emerging domains concentrating on used technologies, structures, and systems with unparalleled characteristics and functionalities a scale of 1–100nm, so the ability to apply nanoscience theory by monitoring, measuring, and manipulation of each of the shape and size, aggregation, regulating, and creating materials on a nanoscale is known as nanotechnology [1,2].

Biosynthesis (green synthesis) of metal nanoparticles is presented as a cost-effective and environmentally acceptable alternative to chemical toxicity. The use of natural resources such as microorganisms, plant extracts, bio-polymers, and so on, provides many benefits in terms of eco-friendly and biocompatible materials and methods can be used for several different medical and pharmaceutical implementations, in contrast to the use of hazardous substances in the manufacturing process. Plant extract synthetic techniques have gained favor over

traditional approaches such as physio-chemical ways for creating nanomaterials. Sustainable nanomaterials production has gained popularity due to several advantages such as low cost, safe to use, and applicable procedures with a diverse variety of applications in nanotech, biomedicine, and nano-electronics, and others [3, 4].

One of the plant based-extracts that derived from various parts Safflower plant as Safflower waste (leaf and stem) [5], fresh flower [6], seeds [7] due to the fact that it contains many active phenolic compounds [8]. Safflower scientifically known as *Carthamus tinctorius L.*, a member of the Asteraceae family, has traditionally been used in many nations for colouring agent and as an ingredient for food, healthful drinks, and cosmetics. The dried petals of *C. tinctorius*, a classic medicinal herb, have yielded a wide range of glycosides. *C. tinctorius* contains around 200 chemicals, including alkaloids, alkane diols, flavonoids, riboflavin, lignans, steroids, and quinochalcones C-glycosides. In addition, the main pigments found in safflower petals divided in two types, the first of these is soluble in water and found in large quantities known as hydroxysafflor yellow A and safflor yellow B while the other type is insoluble in water and called red (Carthamin) [9].

Nanoparticles of nickel oxide (NiO NPs) have piqued the interest of researchers because of its many bendable features, and many studies have dealt with their preparation by various methods like biosynthesis from bacteria such as *Euphorbia heterophylla (L.)* [10], microwave assisted method [11], green synthesis using plant extracts [12-15], anodic arc plasma method [16], etc. This work involves the use of the biosynthesis of (NiO NPs) from dry safflower petals, which was used in some previous studies to prepare nanoparticles.

Nagaraj B. et al. [6] have been reported synthesis of gold nanoparticles by using aqueous extracts fresh flower of safflower, where the nanostructure confirmed by UV-Vis., and TEM. It was found that the size of these particles ranging about 40-200 nm. The synergistic effect of AuNPs various antibiotics (Imipenem, Norfloxacin, and Vancomycin) were studied verses some pathogenic microbes (*Aspergillus niger*, *Aspergillus flavus*, *E.coli and Streptobacillus sp.*) and tests showed that AuNPs have more inhibition activity towards bacteria than fungi.

The study conducted by Sreekanth Tvm and Kap Duk Lee [17] showed the synthesis of silver nanoparticles using safflower flowers extract with evaluated of their cytotoxic and antimicrobial activities, finding them to be toxic vs. *E. coli, S. aureus, B. substilis, C. albicans and B. cerevisiae* both in liquid and solid group media. AgNPs also had a cytotoxic effect on SGT oral cancer cell line in the range of concentration (0.2 to 1.0) mM.

In another study, Francisco Rodríguez-F elix and co-worker [5] have been used an extract of safflower waste involved a mixture of stems and leafs to prepare silver nanoparticles from silver nitrate. The particles investigated by some techniques such as FTIR, SEM, and HR-TEM that confirmed the spherical shape of particles with average size (8.67 ± 4.7) nm. AgNPs tested as a growth inhibitor for some bacteria that showed high activity in low concentration.

Monika Choudhary and her research team [7] were used seeds of safflower to extract steroidal saponin which then utilized in the preparation of silver nanoparticles. After characterized the particles used to exam their activity as anti-acne and showed good results.

Experimental

Material and Instrumentation:

In this study, the salt of nickel nitrate hexahydrate ($Ni(NO_3)_2.6H_2O$) from CDH Corporation was used without additional purification. Both types of Safflower petals were purchased from the Istrabadi market in Al-Kadhimiya, Baghdad. The bioactivity tests were carried out in the BPC analysis center, in addition to the different measurements that were performed using the following instruments: ChemTech CT Muffle furnace, UV-1900 Shimadzu spectrometer in the range (190–800) nm; Shimadzu FTIR spectrophotometer with the model IRAffinity-1 was used in the range (4000–400) cm^{-1}; BIOBASE Ultrasonic Cell Disrupter UCD-150; Philips XRD-PW1730; FEI/Philips CM120 TEM.

Procedure:

1- Preparation method for NiO-SR[10]:

Firstly, the extract of *C. tinctorius L.* (red safflower petals) was prepared in aqueous medium (2 g of petals in 100 ml of distilled water), heated and stirred for two hours in 60 ℃, after which the extract was filtered twice until a clear solution was obtained. Meanwhile, the nickel(II) nitrate hexahydrate salt solution was prepared in the concentration (1mM). To preparation of NiO-NPs, added (10 ml) of plant extract to (90 ml) of used nickel salt and mixed vigorously on stirring hotplate for 2 hrs. at 60 ℃ with observation the change in degree of green color of salt solution, the resulting mixture was kept for overnight at room temperature. In the next day, the mixture was heated again with mixing at 85 ℃ until dried, the green precipitate was collected and washed with distilled water and dried at 100 ℃ followed by calcination at 300 ℃ for 3 hrs. in open air furnace. Finally, a greyish black-colored result was collected and stored in an airtight container for future investigation.

2- Preparation method for NiO-SY2[10]:

The work was carried out in a similar way to the preparation process above in NiO-SR sample from orange safflower extract with the help of ultrasound by using ultrasound probe for 15 min. at 80 ℃ and power rate 100%. Ultrasound was used to mix the resulting mixture of nickel salt with the orange safflower extract to get rid of the turbidity of the obtained total solution from mixing and heating of (10 ml) of plant extract with (90 ml) of ($Ni(NO_3)_2.6H_2O$) for 2 hrs. at 60 ℃.

3- Assay of antibacterial and antifungal activity [18]:

Agar diffusion method was used to assay the biological activity of prepared compounds. The activity was tested against (*Escherichia coli* as a gram-negative bacteria and *Staphylococcus aureus* as a gram-positive bacteria) and a naturally occurring fungus *Candida albicans*, by using a method that included uniformly inoculating the surface of Moller-Hinton agar via spreading 100µl of (1×10^9 cells/ml) of microorganisms suspension in a petri dish (90 mm diameter) for one day and using Macfarland solution as a standard to adjust the turbidity of the suspension. After this, used three dilution series (25% , 50% , 75%) of (standard antibiotic, prepared nanoparticles, and their precursor of $Ni(OH)_2$ dissolved in DMSO) to immerse 0.1 ml of each concentration into the wells (5mm diameter) and leave the Petri-dishs for a ½ hrs. and followed by incubation at 37 ℃ of the Petri-dishs for one day, after this their

biological effectiveness may be assessed by measuring the inhibitory zone in millimetre. Figure (1) shows a graphical abstract of the biosynthesize of nickel oxide nanoparticles.

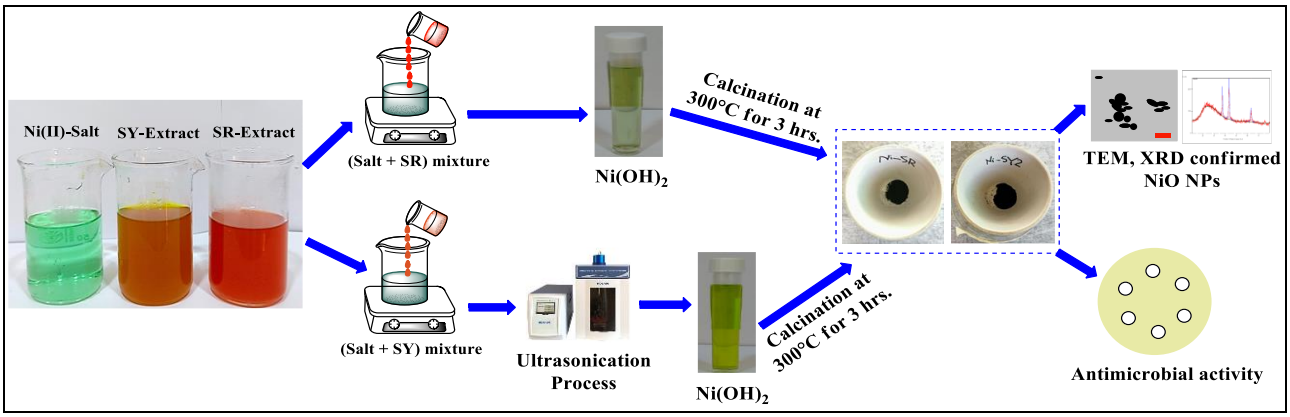

Fig. 1 Schematic route to biosynthesize of NiO-NPs.

Results and discussion:

1- Characterization of biosynthesized NiO-NPs:

The prepared NiO-NPs were examined by the techniques: Uv-Vis. spectrum; FTIR spectrum, XRD, and TEM. Investigations of these nanoparticles showed approach results to previous studies.

A) Uv-Vis spectra of biosynthesized NiO-NPs:

The biosynthesized NiO-NPs were tested by Uv-Vis spectrophotometer at λ_{range} = (190-800) nm for the NiO-SY2 sample and in the range λ_{range} = (200-800) nm for the NiO-SR sample. In general, the Uv-Vis absorption study is a useful technique for estimating the energy structures and optical characteristics of nanoparticles [19]. The recorded absorption maxima of NiO nanoparticles varied in previous studies according to the shape of particles, their size, synthetic procedure and calcination temperature [20]. Uv-Vis. spectra of NiO-SR and NiO-SY2 shown in figure (2a,b) respectively, where the absorption edge of the NiO-SR sample appears at 302 nm while the NiO-SY2 sample shows two absorption peaks at (273 and 324) nm, the maximum absorption at the wavelength equal to 273nm [21,22]. By comparison between the spectra of the two types of prepared NiO-NPs and according to the cut-off wavelength of the NiO-SY2 spectrum at 273nm that showed a blue shift towards the ultraviolet region, which can be attributed to the fact that the particle size of the NiO-SY2 sample is smaller than the particle size of the NiO-SR sample [23].

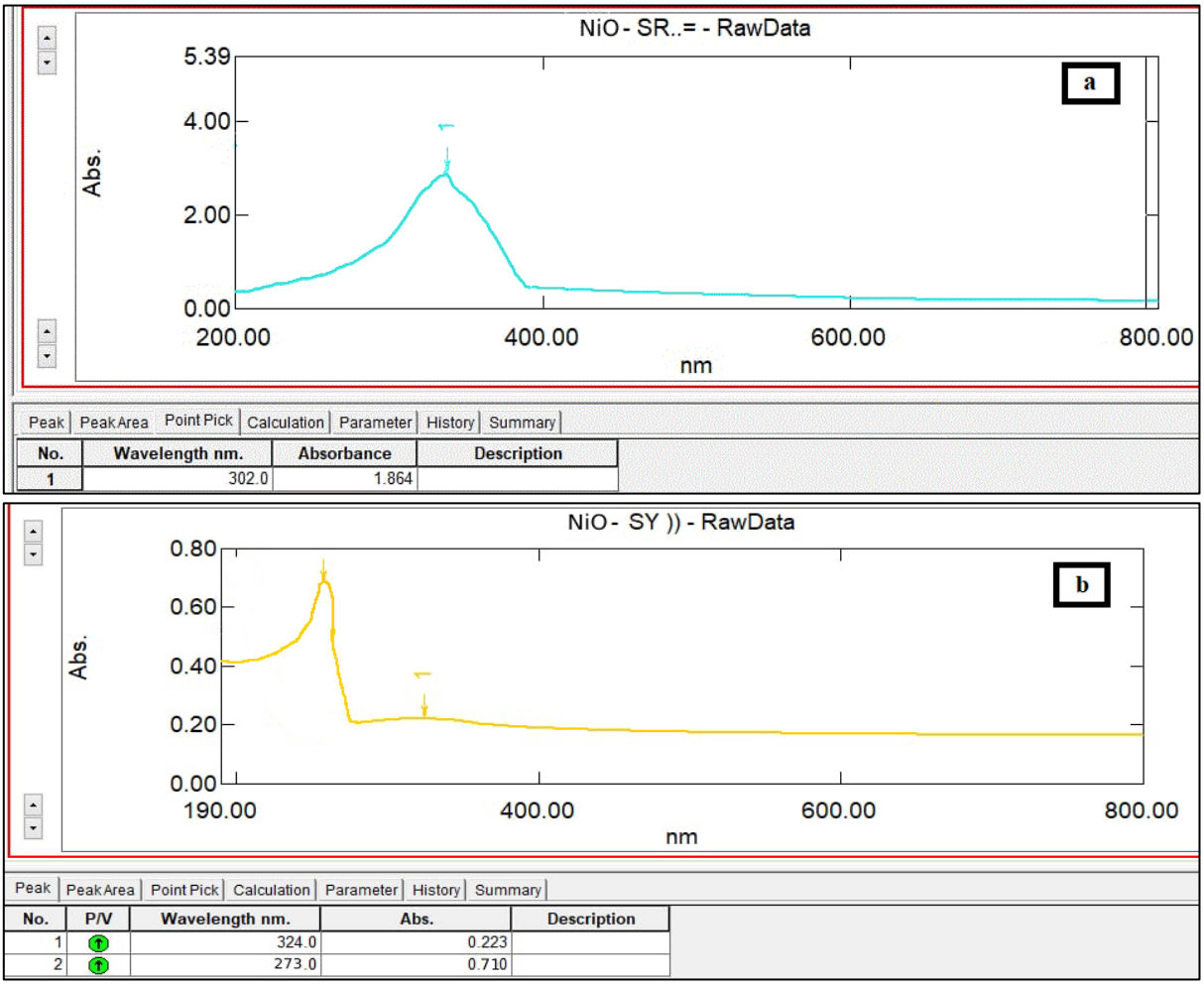

Fig. 2 Uv-Vis spectra of bio-synthesized NiO-NPs a) NiO-SR spectrum b) NiO-SY2 spectrum.

B) FTIR spectra of biosynthesized NiO-NPs:

The infrared spectra (FTIR) of prepared compounds were recorded in the range of wavenumber (4000-400) cm^{-1}, as shown as in the figures (3and 4). Because safflower extract contains many bioactive compounds which have multifunctional groups and the oxidation reaction depends on the phenolic content of extract to convert the nickel salt into hydroxide, then the last convert to oxide by heat, so we can note many bands attributed to diverse functional groups and the occurrence of a displacement in the hydroxyl group band compared with -OH position (at 3293 cm^{-1}) in extract that shown in literature [5], in addition to the appearance of a distinctive band indicating the presence of the (Ni-O) bond.

A medium broad band appears in the spectrum of each NiO-SR and NiO-SY2 at the position (3448 cm^{-1} and 3348 cm^{-1}) identical to stretching mode of (OH/NH) groups. Bands in the ν = 2966 cm^{-1} (for NiO-SR) and (2920+2850) cm^{-1} (for NiO-SY2) attributed to C–H bond. A band at 1732 cm^{-1} in the NiO-SY2 spectrum belongs to carbonyl group (C=O). The aliphatic and aromatic (C=C) groups appear as a weak band at 1635 cm^{-1} and medium band at 1543 cm^{-1} respectively (for NiO-SR), and at 1631 cm^{-1} and 1539 cm^{-1} (for NiO-SY2). Strong bands at 1384 cm^{-1} in both compounds associated with ν(-NO) group. The C-O groups in both compounds show their bands at 1018 cm^{-1} and 1037 cm^{-1}. The main characteristic bands of Ni-O appeared at 420 cm^{-1} as a strong and sharp band for NiO-SR and a weak band at 416 cm^{-1} for NiO-SY2 [5,24].

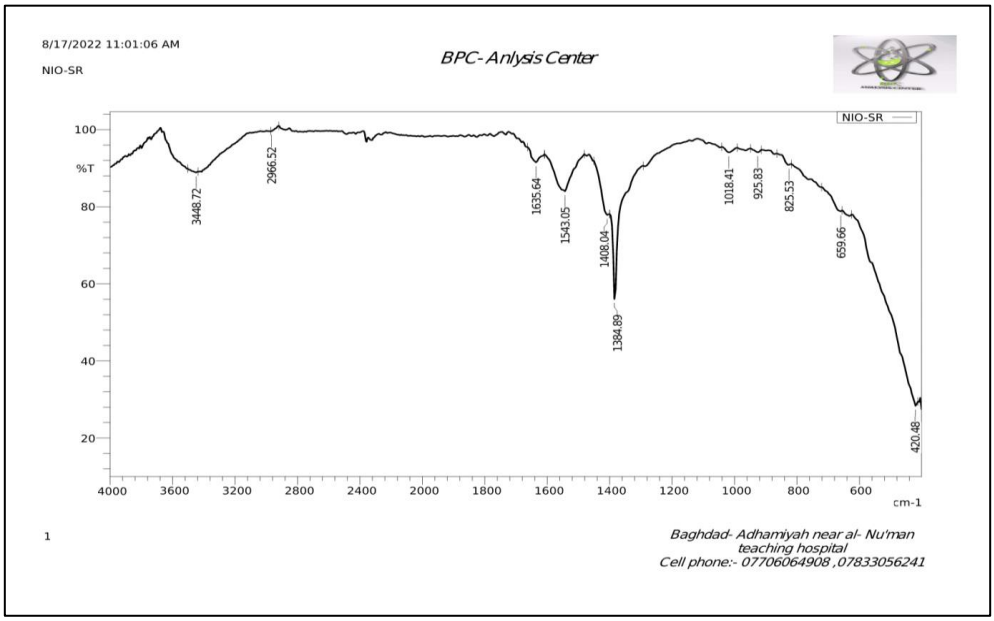

Fig. 3 The infrared spectrum of NiO-SR.

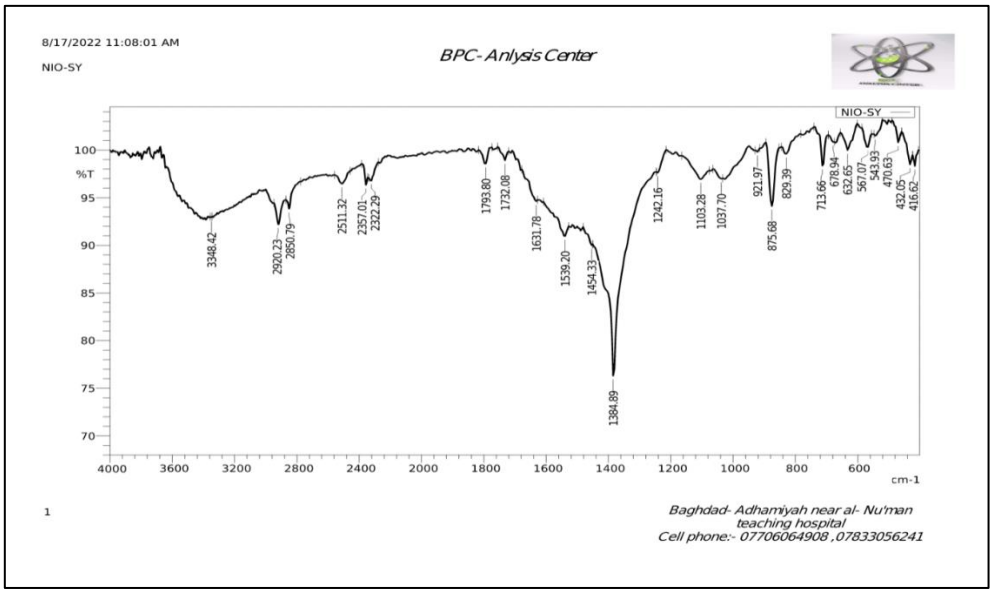

Fig. 4 The infrared spectrum of NiO-SY2**.**

C) PXRD pattern of biosynthesized NiO-NPs:

Both NiO-SR and NiO-SY2 samples were studied by powder X-ray diffraction technique to describe their purity and phase structure. The XRD pattern of prepared NiO-NPs has been shown in figures (5 and 6), the pattern of both compounds display only three diffraction peaks located in the position (2θ) between 37° and 64° compatible with miller indices (hkl) = (111), (200), and (220) for cubic structure (space group symbol Fm−3m; COD 96-101-0096)[25]. All crystalline parameters are summarized in table (1) and the crystallite size D was calculated by Debye-Scherrer's equation [26,27]:

$$D = (0.94\lambda)/(\beta \cos\theta)$$

Where *D* act the crystallite size calculated in (nm) unit, λ= 1.5406A° = 0.15406 nm which act the wavelength of source of CuKα, β = full width at half maximum (FWHM) in radian, θ = Bragg's angle in radian.

The average of crystallite size for NiO-SR = 10.56 nm and for NiO-SY2 = 10.05 nm as expected from the pattern where the NiO-SY2 sample show low intensity due to its smaller particle size compared to NiO-SR sample [28]. NiONPs that generated by biosynthesis method used in previous works [10,15,25,29] showed variation in the size of the prepared particles, some of them were approach in size while others were smaller or larger.

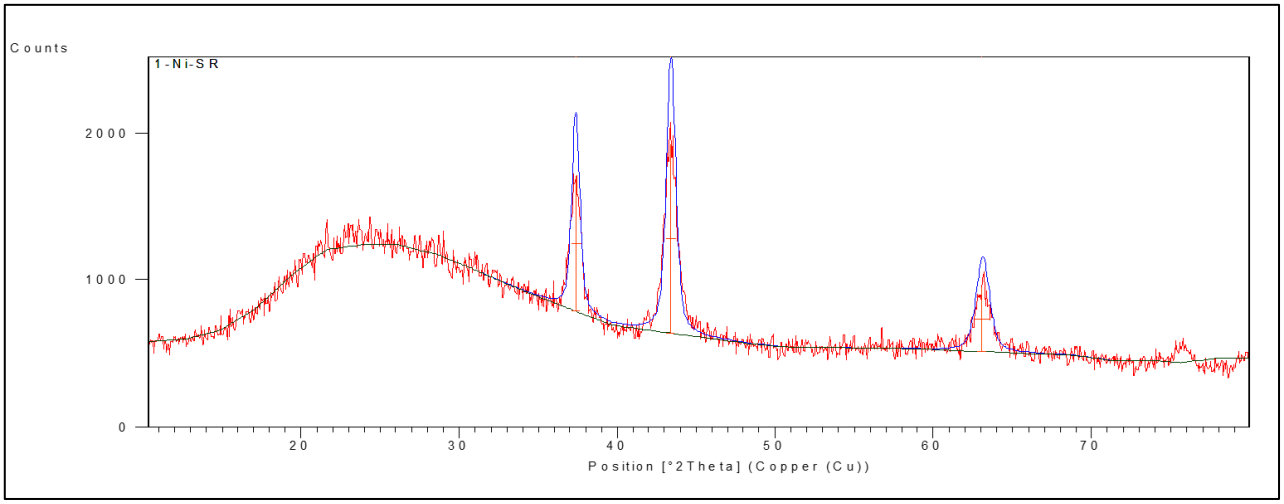

Fig. 5 PXRD patterns of NiO-SR.

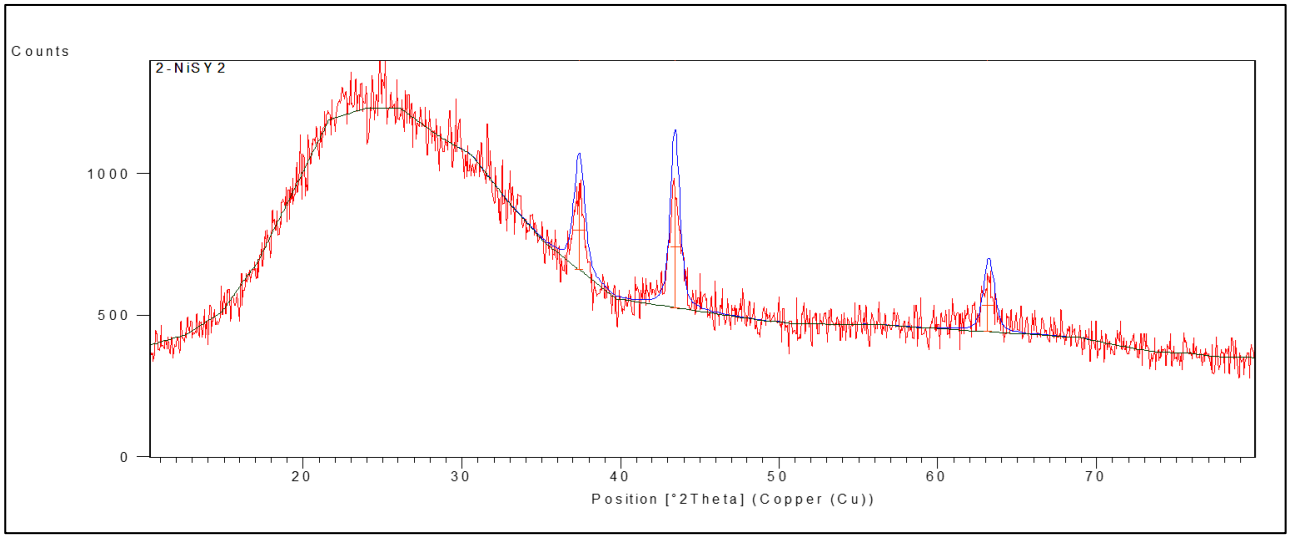

Fig. 6 PXRD patterns of NiO-SY2.

Table 1: Parameters of XRD of biosynthesized NiO-NPs.

Sample	[°2Th.]	β [°2Th.]	$d_{spacing}$ [Å]	(hkl)[25]	Rel. Int. [%]	Crystallite size [nm]	Size average
NiO-SR	37.32638	0.5904	2.40915	111	71.79	13.31	10.56
	43.4001	0.6888	2.08504	200	100	11.19	
	63.07015	0.984	1.47401	220	34.35	7.18	
NiO-SY2	37.35025	0.7872	2.40766	111	64.75	9.98	10.05

43.42221	0.6888	2.08403	200	100	11.19
63.15213	0.7872	1.47229	220	42.11	8.98

D) TEM of biosynthesized NiO-NPs:

The microscopic study of the prepared compounds was performed using the TEM image as shown in figure (7), which displays the spherical-shaped particles that aggregate to form a cluster shape. Most particles exhibited a small size and the diameter of the particles ranges about (3-20) nm and few particles show diameter >20nm in the image of NiO-SR, while D = (3-17) nm for NiO-SY2 sample [30].

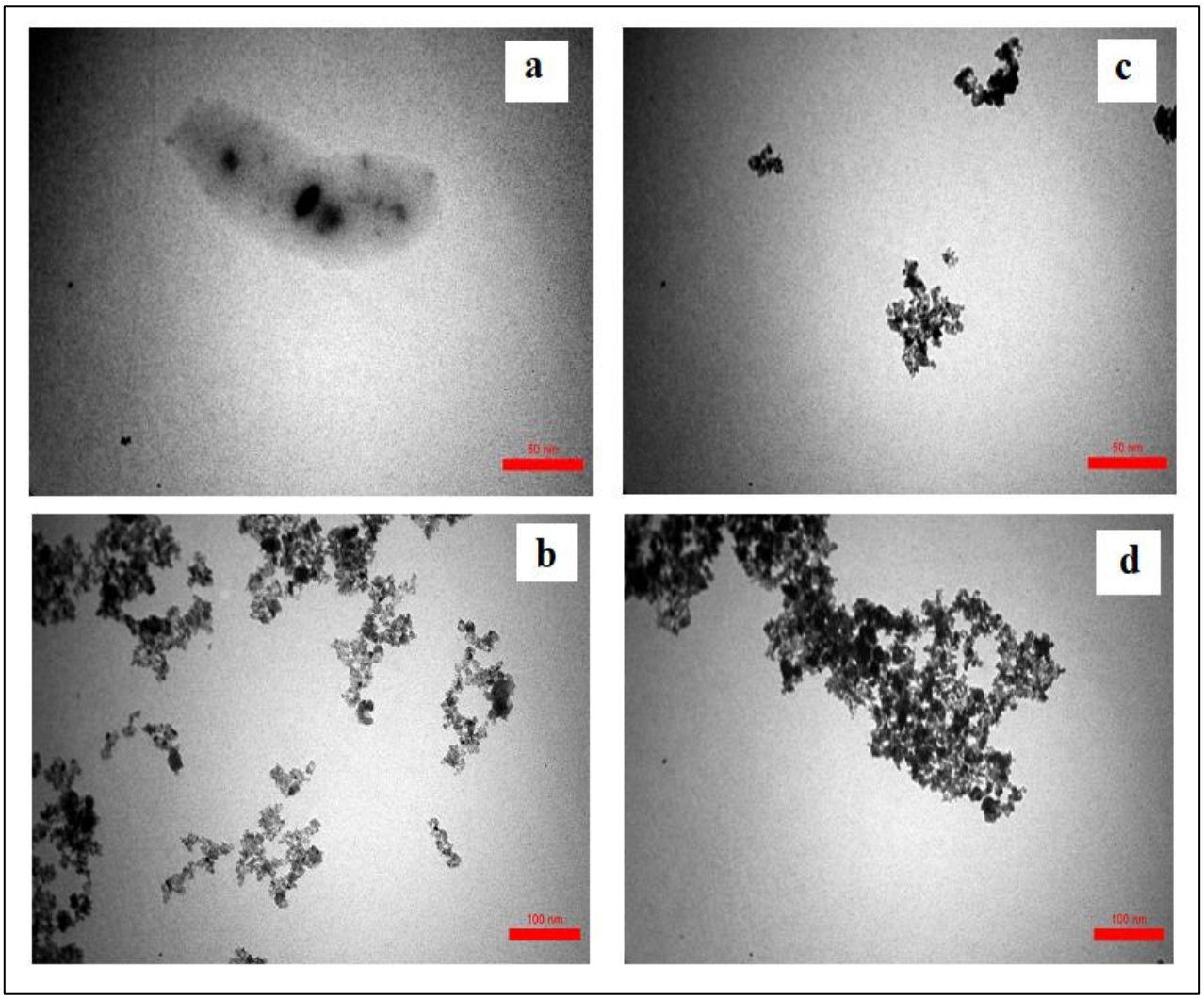

Fig. 7 Assorted magnification of TEM-image of NiO-SR (a,b) and NiO-SY2 (c,d) particles.

2- Bio-application of biosynthesized NiO-NPs:

Table 2 and Figures (8 and 9) illustrated the ability of prepared nanoparticles of NiO to inhibit some selected bacteria (*Escherichia coli* as a gram-negative and *Staphylococcus aureus* as a gram-positive) and fungi (*Candida albicans*), and compared with their precursor of $Ni(OH)_2$, antibiotic, and solvent (DMSO). Evaluation was done by applied the agar diffusion method by utilization of three series of dilution as follows (25% , 50% , 75%). By observing the results, it was found that nickel hydroxide showed better resistance toward Candida albicans than NiO nanoparticles. A high concentration of NiO-SR in the sample showed an almost similar inhibition effect to what the antibiotic showed. Also, sample NO-SY2 gave

better results than NiO-SR, which may be due to its smaller particle size and its large surface area. The results obtained showed agreement with many previous studies [21,31,32] . The compounds can be arranged as follows according to their inhibiting ability: [$Ni(OH)_2$-$SY2_{(25\%, 75\%)}$ > $Ni(OH)_2$-$SR_{(50\%, 75\%)}$, NiO-$SR_{(75\%)}$ > NiO-$SY2_{(75\%)}$, Used antibiotic] vs. *S. aureus* ; [NiO-$SY2_{(75\%, 50\%)}$ > $Ni(OH)_{2(75\%,50\%)}$ > $Ni(OH)_{2(25\%)}$, NiO-$SR_{(75\%)}$, Used antibiotic] vs. *E.* coli ; [$Ni(OH)_{2(75\%,50\%,25\%)}$ > NiO-$SY2_{(75\%)}$ > NiO-$SR_{(75\%)}$ > Used antibiotic] vs. *C. albicans*.

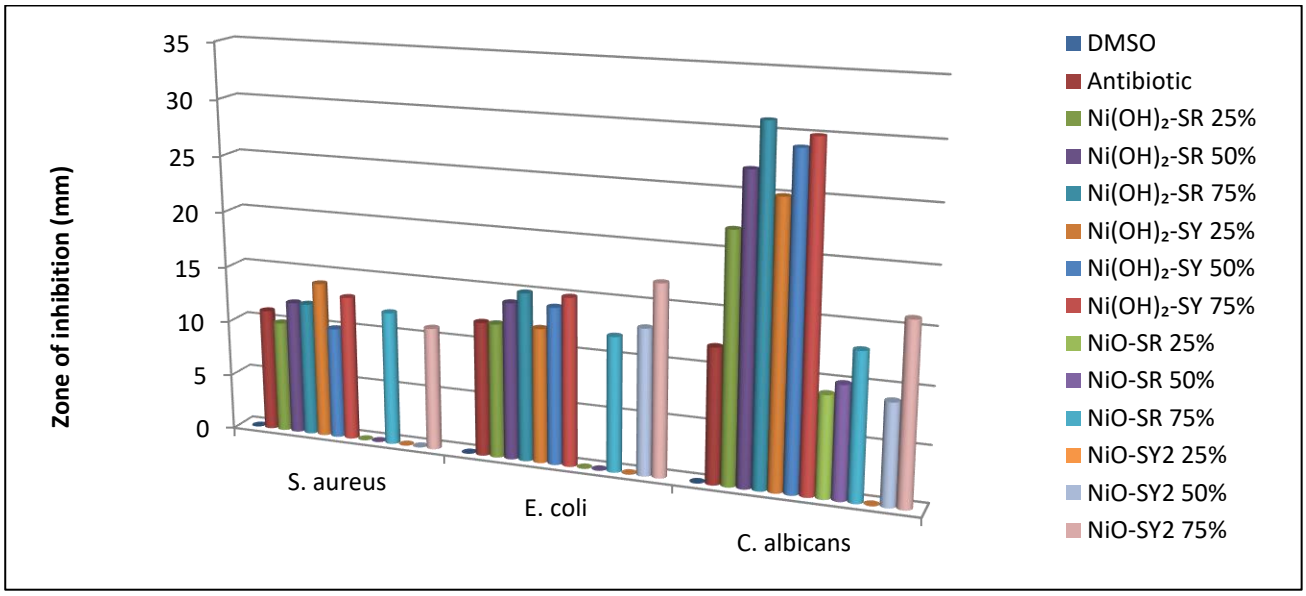

Fig. 8 Chart showing the inhibition activity vs. three chosen microorganisms.

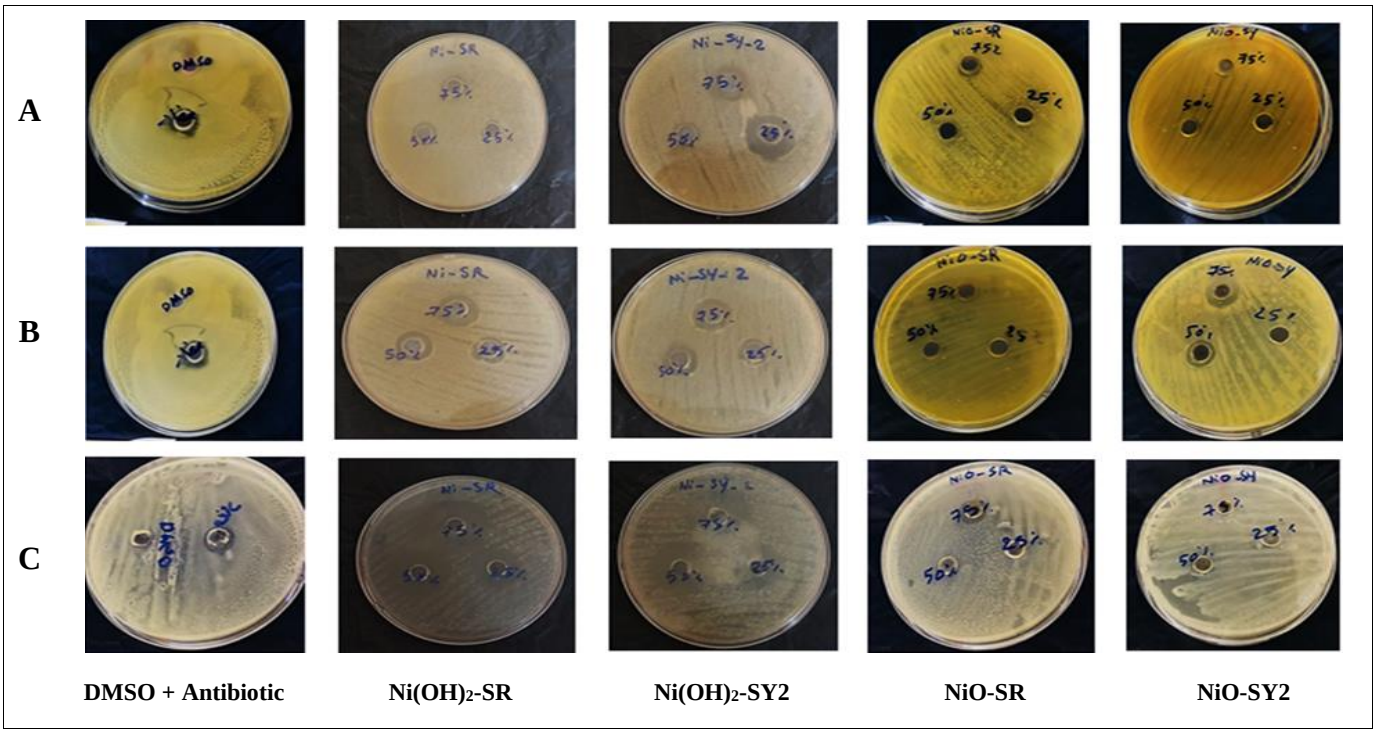

Fig. 9 Antimicrobial efficacy versus A) S. aureus , B) E. coli, C) C. albicans

Table 2: Measurements of inhibition zone (mm) for the three chosen microorganisms.

Sample	Dilution	Inhibition Zone (mm)		
		S. aureus	*E. coli*	*C. albicans*
DMSO (solvent)	-	–ve	–ve	–ve

Antibiotic (stand.)	-	11	12	12
$Ni(OH)_2$-SR	25%	10	12	22
	50%	12	14	27
	75%	12	15	31
$Ni(OH)_2$-SY2	25%	14	12	25
	50%	10	14	29
	75%	13	15	30
NiO-SR	25%	–ve	–ve	9
	50%	–ve	–ve	10
	75%	12	12	13
NiO-SY2	25%	–ve	–ve	–ve
	50%	–ve	13	9
	75%	11	17	16

Conclusion:

Nickel oxide nanoparticles were successfully prepared by biosynthesized method involving the reaction of nickel(II) nitrate hexahydrate with aqueous extracts of safflower petals of two species Dincer 5-18-1 (red flower) and Remzibey-05 (orange flower). The prepared nanoparticles were analyzed by several techniques (FTIR, UV-Vis., XRD and TEM), the results of the analysis showed agreement with previous studies. The average diameter of nanoparticles obtained from PXRD is about (10± 0.5) nm, and TEM-images displayed the spherical-shaped particles that aggregate to form a cluster shape with a size distribution of approximately (3- 20) nm.

Bioactivity assay of the prepared NiO-NPs was performed against some selected microbes [*Escherichia coli* (GNP), *Staphylococcus aureus* (GPB) and *Candida albicans* (fungus)] in different concentrations and compared it with their precursor of $Ni(OH)_2$; an antibiotic; and solvent of DMSO. The result show that nickel hydroxid was more affective to inhibit fungi (*C. albicans)* than prepared nickel oxide, while the particles of 25% NiO-SY2 was appeared a highest zone of inhibition = 17 mm versus *Escherichia coli*.

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اجراء تطبيق خارج جسم الكائن الحي لدقائق اوكسيد النيكل النانوية المحضرة حيوياً من نبات القرطم

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الخلاصة:
هدفت الدراسة الحالية إلى تحضير جزيئات أوكسيد النيكل النانوية باستخدام مواد متوفرة بسهولة وغير مكلفة وصديقة للبيئة، لذلك تم هذا العمل باستخدام مستخلص بتلات العصفر (*Carthamus tinctorius L.*) للحصول على الدقائق النانوية لاوكسيد النيكل. تضمن العمل استخدام نوعين وراثيين مختلفين من العصفر [Dincer 5-18-1 (زهرة حمراء) وRemzibey-05 (زهرة برتقالية)] لتجربة أيهما يعمل بشكل أفضل. تم دراسة التركيب والشكل وحجم الجزيئات باستخدام التحاليل الطيفية والمجهرية كلا من: (FTIR وUV-Vis وXRD وTEM) وتبين من خلال النتائج ان الدقائق النانوية قد تكونت بنجاح. تم تقييم جزيئات أكسيد النيكل بيولوجيًا بالمقارنة مع سلائفها من هيدروكسيد النيكل تحت ثلاث سلاسل تخفيف (25٪، 50٪، 75٪) ضد بعض الميكروبات [المبيضات البيضاء، المكورات العنقودية الذهبية، والإشريكية القولونية]. أظهرت النتائج أن الأكاسيد المحضرة أقل فعالية من الهيدروكسيدات الخاصة بها وبصفة عامة جميع المركبات تظهر تأثير تثبيط نمو الكائنات الحية الدقيقة عند استخدام تراكيز عالية منها.