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Antioxidant and antimicrobial efficiency of curcumin nanoparticles against pathogenic microorganisms

Amira E. Sehim[1]; Rasha Y. Abdel-Ghafar[1], Zeinab K. Hamza[2]; Aziza A. El-Nekeety[2]; Mosaad A. Abdel-Wahhab[2]

[1]Botany and Microbiology Dept, Faculty of Science; Benha University, Benha, Egypt

[2]Food Toxicology & Contaminants Dept., National Research Centre, Cairo, Egypt.

Abstract

Curcumin (Cur) is a pivotal compound widely used in pharmaceutical and medical applications such as antioxidant, antimicrobial, anticancer, and anti-inflammatory. However, some limitations faced its application in different purposes owing to its insolubility, low bioavailability, and fast degradation. These limitations can be overcome by the formulation of Cur nanoparticles (Cur-NPs) to boost its bioactivity and enhance its solubility. This study aimed to synthesize and characterize Cur-NPs and evaluate the antioxidant activity and antimicrobial efficiency against different pathogenic microorganisms. Cur-NPs were prepared, characterized and the antimicrobial efficiency was evaluated against E. coli, S. typhimurium, and Y. enterocolitica (Gram-negative), S. aureus, B. cereus (Gram-positive), and the pathogenic fungi (Aspergillus niger, A flavus, Penicillium expansum, and Candida albicans). The synthesized Cur-NPs showed a rounded shape with 44 ± 8 nm average size and 43 ± 4 mV ξ-potential. Cur-NPs showed potent antioxidant (IC50 of 1550 µg/ml) and antimicrobial properties against the tested bacteria and fungi in a dose-dependent manner. It could be concluded that Cur-NPs are a promising candidate to be used as an antimicrobial in food preservation, or medical and pharmaceutical applications to replace antibiotics.

Keywords: Curcumin nanoparticles; antioxidant; antibacterial; antifungal; antimicrobial.

1. Introduction

The arising global changes from climatic change and globalization have an extreme impact on human health, mainly infectious diseases [1]. The raise in people's mobility, the emissions of greenhouse gas, urbanization, global warming, pollution, deforestation, sea-level rise, loss of sea ice, the extreme events of weather with floods and droughts have increased the prevalence, and transmission of the existing infections, like vector-borne diseases, and the evolution of new pathogens [2]. These infections sometimes may lead to epidemics such as COVID-19 that the world is facing nowadays [3]. Despite the existence of several anti-infective medications, there are other concerns are the drug-resistance due to the misuse of antimicrobial and antibiotic agents and the up growth of new multidrug-resistant organisms [4].

These issues present a challenge to humanity, particularly when considering that developing new drugs requires money and time. Hence, the establishment of novel drugs from natural resources is an alternative approach [5,6].

Curcumin (Cur) is an active compound of turmeric extracted from Curcuma longa rhizome. It is widely used in food as a spice, coloring and flavoring agent, and food preservative. Previous studies showed that Cur has a powerful anti-inflammatory [7,8], antioxidant [9,10], antitumor [11], and antimicrobial activities [12,13]. Cur also inhibits lipid peroxidation and showed a scavenging property against reactive oxygen species (ROS) such as nitric oxide, hydroxyl radicals, and singlet oxygen [14-16]. Additionally, Cur can reduce the pro-inflammatory cytokines such as IL-6, and IL-1β in HaCaT cells exposed to TNF-α [17]. Cur showed antibacterial effect against the

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Gram-positive bacteria (Staphylococcus aureus and Enterococcus faecalis) and Gram-negative bacteria (Pseudomonas aeruginosa and E. coli) as reported by Tyagi et al. [18]. Moreover, Mandroli and Bhat [19] reported that Cur has antibacterial properties against the endodontic bacteria (Actinomyces viscosus, Streptococcus mutans, Lactobacillus casei, Prevotella intermedia, and Porphyromonas gingivalis). Additionally, several reports indicated that Cur has antifungal activity since it inhibited the growth of different fungi including Microsporum gypseum, Trichophyton mentagrophytes, T. rubrum, Epidermophyton floccosum [20], Aspergillus parasiticus, A. flavus, Penicillium digitatum and Fusarium moniliforme [21].

Despite the medical benefits and inherent advantages of Cur, its application in the food or pharmaceutical industry faces several challenges due to the water insolubility at normal pH, poor bioavailability, limited absorption rapid metabolism, and excretion [22]. To overcome such challenges, especially low bioavailability, and poor solubility, nanotechnology and nano-drug delivery may be the best options [23,24]. Cur was successfully encapsulated in chitosan [25], bovine serum albumin [26], and liposomes [27] or complexed with cyclodextrin [28], and phospholipids [29].

2. Materials and methods

2.1. Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl), and Ascorbic acid were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Czapek-Dox's agar was obtained from DSMZ (GmbH, Germany). Cur was purchased from Mepaco Arabian Pharmaceutical Co. (Cairo, Egypt). All reagents and chemicals used in the current study were supplied by Sigma Aldrich (Taufkirchen, Germany) and were of analytical grade.

2.2. Microorganisms

Fungal strains of Aspergillus niger, A flavus, Penicillium expansum, and Candida albicans were isolated from cereals samples collected from local markets in Benha city, Egypt. Staphylococcus aureus, Salmonella typhimurium, and Escherichia coli 0157:H7 have been isolated from serological survey identified by Dairy Microbiological Lab., National Research Centre, Cairo, Egypt. Yersinia enterocolitica was provided by the Hungarian National Collection of Medical Bacteria (Budapest, Hungary) and Bacillus cereus B-3711 was obtained from the Northern Regional Research Laboratory (NRRL, Illinois, USA).

2.3. Preparation of curcumin nanoparticles (Cur-NPs)

Cur-NPs were prepared as described in our previous work [8]. One hundred mg (0.27 mmol) of Cur was dissolved in dichloromethane (20 mL) then 1 ml of this solution was sprayed into boiling water (50 mL) dropwise with a flow rate of 0.2 ml/min for 5 min under ultrasonic conditions using an ultrasonic power of 100 W and a frequency of 30 kHz (Shenzhen Langee Ultrasonic Electric Appliance Co., Guangdong, China). After sonication, the contents were then stirred at 800 rpm for 20 min at room temperature until a clear yellow-colored solution appeared. The solution was concentrated under low pressure at 50 OC and then freeze-dried to obtain a yellow powder. The morphology of Cur-NPs was determined by TEM (transmission electron microscopy, 2100-HR, JEOL, CA, USA). An Orius 1000 CCD camera was used for image acquisition (GATAN, Warrendale, PA, USA). Zeta potential measurement was based on nanoparticle electrophoretic mobility and calculated from Smoluchowski's equation [30]. Particle sizes were determined using a dynamic light scattering (DLS; Zetasizer™ 3000E, Malvern Instruments Worcestershire, UK). All measurements were preformed in triplicate at 25°C.

2.4. Antibacterial activity

The diameter of inhibition zones was estimated by the disc diffusion procedure [31] with minor modifications. The solutions of the examined sample were prepared at different concentrations (1000, 500, 250, 125 µg/ml) for antibacterial assay. Sterile discs were impregnated with 10 µL of each (10µg/disk), and a loading control was also prepared to contain 10 µL of Dimethyl sulfoxide (DMSO) for each inoculated spread plate. The discs of samples were placed on the agar plate surface using sterile forceps, gently pressing down each disc to ensure complete contact with the agar surface. The assessment of antimicrobial efficiency was conducted against a wide range of human pathogenic microorganisms, Gram-positive bacteria (B. cereus and S. aureus), and Gram-negative bacteria (Y. enterocolitica, E. coli, and, S. typhimurium). Cefoperazone (100 µg/ml) was used as a reference antibacterial, while the negative control was DMSO solution (10% v/v). The plates were incubated for 18 h at 37°C after the discs were applied. The diameter of complete inhibition zone was measured to the nearest ml, including the disc diameter, using sliding calipers, which were held on the back of the inverted Petri plate. Plates were examined for growth inhibition and the diameter of inhibition zone was measured. The strength of the activity was classified as high activity for the inhibition zone having

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diameters of (10-15 mm), low activity for the diameter ranging from (7-10 mm), and no activity for those with less than 7 mm diameter.

2.5. Antifungal activity

Pathogenic fungal strains were grown on potato dextrose agar (PDA) medium at 30 ºC for 7 days until mycelia growth covered the entire dishes, from which, a spore suspension was obtained in 10 ml (0.85 % NaCl 0.1% Tween 80). Then it was diluted to 106 spore /ml while Candida albicans was grown on sabouraud dextrose agar (SDA) at 35 oC for 24 h. The inoculum was adjusted to optical density 0.5 McFarland standard (1.5 ×108 CFU/ml).

2.5.1. Determination of antifungal activity by agar well diffusion method

Antifungal efficiency of the synthesized Cur-NPs was performed by the agar well diffusion assay as described by Ben Hsouna et al. [32]. The freshly prepared fungal suspensions were inoculated onto the surface of PDA and SDA media using a sterile swab. Wells of 7 mm were drilled in the center by a sterile well puncher at equal distances. Each well was then filled with a various concentration (1, 2, and 3 mg/ml) of Cur-NPs. The plates were placed in the refrigerator for 30 min to let the diffusion of Cur-NPs into the agar and then incubated at 35oC for 24h for C. albicans and 30oC for five days for the filamentous fungi. The antimicrobial efficiency was determined by the measurement of inhibition zone (including the wells diameter) that appeared after incubation period. DMSO (10%) was used as a negative control. The antifungal activity was measured in triplicate and was determined as very strong, moderate, and weak when the zones of inhibition were 15, 10, and 5 nm, respectively [33].

2.6. Antioxidant activity

In vitro antioxidant activity of the synthesized Cur-NPs was evaluated by 2.2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay described previously [34] with slight modification. A 24 mg of DPPH was dissolved in 80% methanol (100 ml) to prepare the stock solution and it was kept at 20 °C. The working solution was adjusted to an absorbance of about 0.750 ± 0.02 at 517 nm using a spectrophotometer. An aliquot of 1 ml DPPH solution was mixed with 100 µl of Cur-NPs at varying concentrations (2000, 1000, 500, 250, 125, 62.5 µg/ml). The reaction mixture was mixed strenuously and kept for 30 min in the dark at room temperature. The shift between the initial violet (oxidized product) and the yellow end from (reduced product) of DPPH was followed by recording the decrease of absorption at 517 nm against a blank (without DPPH) with UV-Vis Shimadzu spectrophotometer (UV-1601, PC) using 10 mm polystyrene. Methanol was used for the zero spectrophotometer and the measurement was performed in triplicate. The activity of radical scavenging was expressed as percentage of DPPH inhibition using the following formula:

% Inhibition = [(A control – A treatment/A control)] X 100

Where:

A control: is the absorbance of the control;

A treatment is the absorbance of the treatments.

Ascorbic acid was utilized as a reference compound, the inhibition percentage was plotted against concentration, and the IC50 value was calculated by graphical method.

2.7. Statistical analysis

The data were statistically analyzed by one-way ANOVA using the ''SPSS-24'' a computer software program, and then the Duncan posthoc test ($p < 0.01$). The results were expressed as Mean ± SD.

3. Results and Discussion

3.1. Characterization of the prepared Cur-NPs

The current results indicated that Cur-NPs showed a higher yellow visual color intensity which was probably due to the significant light scattering (Fig. 1A). The TEM image showed that Cur-NPs are round-shape (Fig. 1B); however, the DLS analysis indicated that the average particle size of the synthesized Cur-NPs was 44 ± 8 nm (Fig. 1C) and the ZetaSizer analysis showed that the ξ-potential of Cur-NPs was 43 ± 4 mV (Fig. 1D). The shape and size of Cur-NPs reported in this study are similar to those reported in the literature using the same or other techniques for the preparation [8,35,36]. In this concern, it was reported that the material solubility is increased due to the hyperbolic relation between the particles size and the surface-specific dissolution rate as a result of the large surface area which promotes homogeneous dispersion [37,38]. Moreover, the particle charge is a very important factor for the determination of the physical stability of emulsions and suspensions since the electrostatic repulsion among the particles is increased when the particles bear the same charge, and hence, it lends higher physical stability as a result of the reduction of the aggregation likelihood [37]. The ξ-potential of Cur-NPs recorded herein (43 ± 4 mV) suggests the higher stability of nanoparticles [39-41].
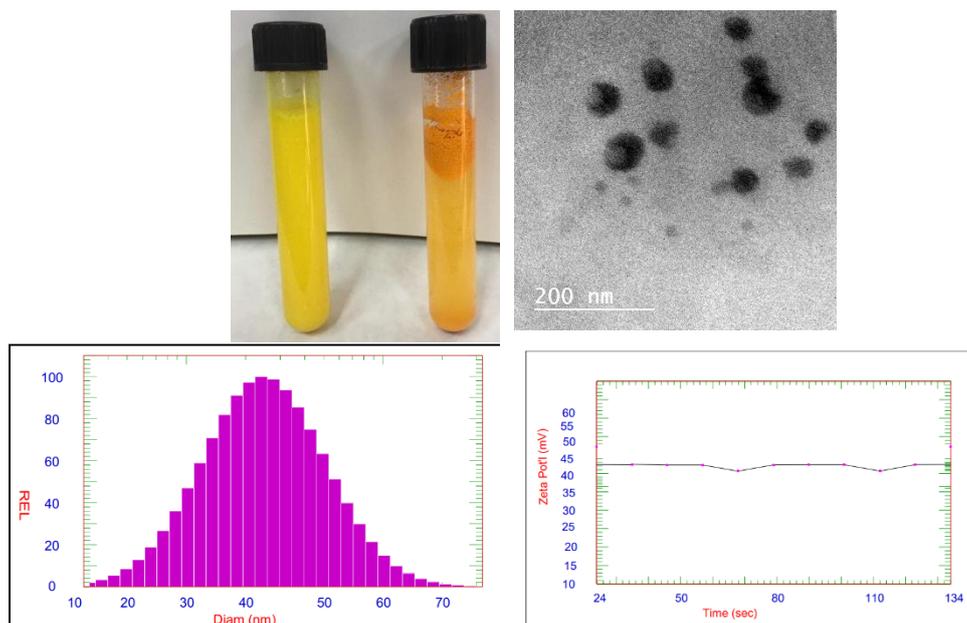
Fig. 1. A. Photographs of aqueous Cur dispersions after preparation showing Cur-NPs on the right and crude Cur on the left. (Note the flakes of non-dissolved Cur on top of the liquid on the right), (B) TEM image showing the round shape of Cur-NPs, (C) The DLS analysis of Cur-NPs showing the size distribution (44 ± 8 nm), and (D) ZetaSizer chromatogram showing the zeta potential of Cur-NPs (43 ± 4 mV).

3.2. Assessment of the antibacterial activity

The antibacterial influence of Cur-NPs was examined on bacteria strains including gram-positive (*S. aureus*, *B. cereus*) and gram-negative bacteria (*E. coli*, *S. typhimurium, and Y. enterocolitica*) using different concentrations of Cur-NPs (1000 µg/ml to 125 µg/ml). The obtained results (Table 1) showed that there are variations in the bioactivity of Cur-NPs depending on the microbial species and strains. Our findings showed a significantly higher efficiency of Cur-NPs against Gram-positive than the selected Gram-negative bacteria which may be due to differences in their cell membrane and structure. A similar regularity was found for all tested concentrations (1000 µg/ml to 125 µg/ml) against the microbial species. As clearly shown in Fig (2) and Table (1), there is a difference in the antibacterial effectiveness of Cur-NPs between the microbial species. In turn, Cur-NPs at a concentration of 1000 µg/ml recorded the highest inhibition against *S. aureus* (13.5 mm) compared to cefoperazone, which was used as a standard reference drug (13 mm). The difference between the antimicrobial property of Cur-NPs in Gram-negative and Gram-positive bacteria is attributed to the difference in cell membrane structure which contains peptidoglycan in the outer layer in the case of Gram-positive bacteria. Meanwhile, Gram-negative contain an outer phospholipidic membrane.

These two layers have different types of interaction when encountered by Cur-NPs [42]. The formation of the zone of inhibition around the bacterial discs suggested the antibacterial property of Cur-NPs. It was reported that Cur-NPs showed antibacterial activity against several pathogenic bacteria such as *S. aureus* [43], *K. pneumoniae*, *P. aeruginosa, and E. coli* [44]. The mechanism by which Cur-NPs induced their antibacterial activity was proposed by the attachment of Cur-NPs to the cell wall of bacteria or membrane and inhibits the respiratory process [45]. The current results are similar to that reported previously and indicated that Cur-NPs are responsible for the membrane destruction of *E. coli* and cause apoptosis as indicated by various markers including the depolarization of cell membrane, ROS accumulation, and an increase of calcium ions influx [46]. Moreover, Cur-NPs are involved in cellular processes via targeting proteins and DNA and inhibiting bacterial quorum sensing which is responsible for the communication process mediated by biochemical signals that regulate microbial behavior and cell density [47]. These effects are responsible for the inhibition zone formation and suggested the antimicrobial properties of Cur-NPs [48]. Additionally, Cur-NPs were reported to be responsible for the reversible process of the resistance of methicillin in the *S. aureus* strain [49].

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Table 1 Antibacterial activity of Cur-NPs against Gram positive and Gram negative bacteria in terms of inhibition zone

Cur-NPs (µg/ml)	Inhibition zone (mm)				
	Gram +ve bacteria		Gram -ve bacteria		
	S. aureus	*B. cereus*	*E. coli*	*S. typhmirum*	*Y. enterocolitica*
100 0	13.50 ± 0.71	12.50 ± 0.12	10.50 ± 0.71	12.00 ± 0.22	11.00 ± 0.41
500	12.00 ± 1.42	11.25 ± 0.35	10.50 ± 0.71	12.50 ± 0.71	11.00 ± 0.71
250	12.50 ± 0.71	9.00 ± 0.24	9.25 ± 0.35	11.00 ± 0.14	10.50 ± 0.61
125	10.50 ± 0.71	8.00 ± 0.31	0	9.00 ± 0.26	10.00 ± 0.71
Reference	13.00 ± 2.82	12.75 ± 0.35	12.25 ± 0.35	11.50 ± 0.71	10.50 ± 0.71

diameter in (mm)

The zone of inhibition values is expressed as mean ± SD

Fig. 2. Antibacterial activity of Cur-NPs against some pathogenic bacteria **(A)** *B. ceruse*, **(B)** *S. aureus*, and **(C)** *Y. enterocolitica*

3.3. Assessment of antifungal activity

The results illustrated in Table (2) indicated that Cur-NPs were effective against all tested pathogenic fungi. The inhibitory activity of Cur-NPs varied among tested fungi depending on fungal species. Moreover, the suppression of fungal growth increases by increasing Cur-NPs concentrations. It was observed that the maximum growth inhibition (51.0 ± 0.1 mm) occurred in the case of *C. albican*s at 3 mg/ml of Cur-NPs followed by *P. expansum* (43.3 ± 0.05 mm) while, the minimum growth inhibition (13.3 ± 0.01mm) was observed in case of *A. niger* (Fig. 3). Previous studies revealed the broad-spectrum property of Cur as an antimicrobial agent against fungi, virus and bacteria [50-53]. The antifungal potency of Cur has been demonstrated mostly against Candida spp. Cur-NPs are more easily dispersed in water as compared with Cur without any formulation and show more effective antimicrobial potency against *E. coli, B. subtilis*, *S. aureus*, *P. aeruginosa,* and two known pathogenic fungi; *P. notatum* and *A. niger* [54]. It was investigated that Cur-AgNPs showed an antifungal effect against fluconazole-resistant *Candida* spp. isolated from patients with HIV [55]. The largest inhibition zone was observed for *C. glabrata* and *C. albicans* (20.6 ± 0.8 mm and 20.1 ± 0.8 mm, respectively), while the

smallest one was verified for *C. tropicalis* which recorded 16.4 ± 0.7 mm. The antifungal potency of Cur-NPs was also reported against *Trichosporon, Candida, Cryptococcus, Paracoccidioides,* and *Aspergillus* [45]. The antifungal potency of Cur was extensively studied against *Candida* spp [56]. The growth of *C. parapsilosis*, *C. glabrata*, *C. albicans*, and *C. dubliniensus* was inhibited by Cur [57]. Similar to the current results, a previous study showed that different Cur-NPs formulations were able to exhibit strong or moderate antifungal effect against *F. oxysporum*, *C. albicans*, *A. flavus*, *A. niger,* and *C. neoformans* [58]. These authors suggested that possible mechanisms as Cur-NPs have a binding affinity to sterol 14α-demethylase (CYP51), leading to the impairment of fungal growth.

Several reports suggested that Cur has powerful antifungal potency against different fungal species including *T. rubrum*, *M. gypseum*, *T. mentagrophytes*, and *E. floccosum* [20]. Moreover, turmeric oil showed powerful antifungal activity against *A. parasiticu*s, *A. flavus*, *P. digitatum*, and *F. moniliforme* [21], *C. gloeosporioides, C. musae*, and *F. semitectum* [59]. Interestingly, Cur is also effective against the infection by *C. albicans* which is known to be resistant to the available antifungal drugs [60]. It is well documented that antifungal activity is due to

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its role in the reduction of ergosterol production; which preserves the membrane fluidity, integrity, and asymmetry and is contributed to the proper functioning of membrane-bound enzymes [17]. Thus, the antifungal potency of Cur-NPs reported in this study may be explained by the inhibition of

oergosterol biosynthesis. Additionally, Cur-NPs affect the mechanism of ATPase-dependent efflux to bring down the secretion of Sap. Consequently, these nanoparticles affect the biosynthesis of ergosterol, PM-ATPase, and the secretion of proteinase [17].

Table 2 Effect of different concentrations of Cur-NPs on growth diameter of pathogenic fungi

Pathogenic fungal isolates	Cur-NPs Concentration (mg/ml) DIZ(mm)			
	DMSO (control)	1 mg/ml	2 mg/ml	3 mg/ml
Aspergillus niger	0.0	9.6 ± 0.05	11.6 ± 0. 5	13.3 ± 0.01
Aspergillus flavus	0.0	20.3 ± 0.11	23.0 ± 0.17	24.6 ± 0.05
Penicillum expansum	0.0	37.0 ± 0.20	39.3 ± 0.30	43.3 ± 0.05
Candida albican	0.0	46.6 ± 0.28	50.6 ± 0.03	51.0 ± 0.1

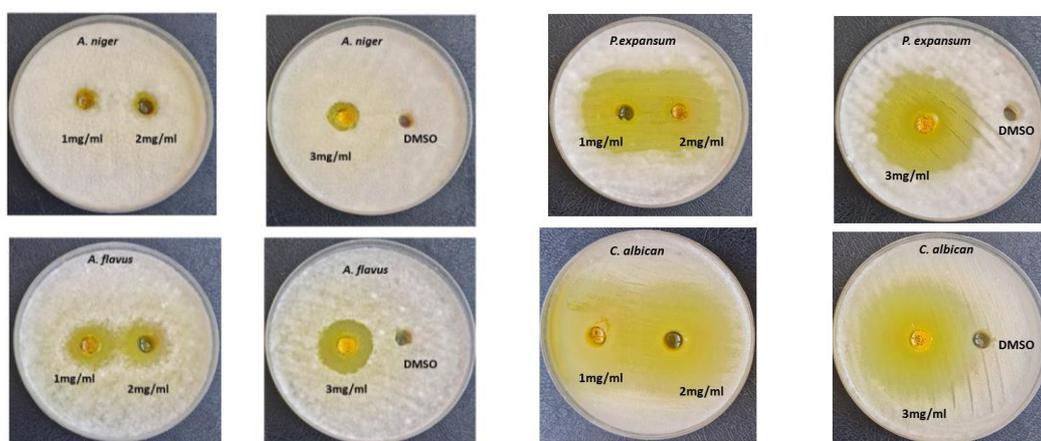The zone of inhibition values are expressed as mean ± SD

Fig. 3. Inhibition zone (mm) of different species of pathogenic fungi after treatment with different concentration of cur-NPs

3.4. Antioxidant activity

The DPPH radical scavenging property of Cur-NPs at different concentrations compared with vitamin C as a standard antioxidant are presented in (Table 3). These results indicated that the scavenging property of Cur-NPs was increased by increasing the concentration and the recorded values were 41.84, 31.44, 29.24, and 28.21 for the concentrations of 1000, 500, 250, and 125 µg/ml, respectively with IC_{50} of 1550 µg/ml (Fig. 4). Similar data were recorded previously using different methods for the

preparation of Cur-NPs and suggested that the nano-formulations enhance the antioxidant property of Cur [61-63]. Additionally, several studies reported that Cur-NPs showed a higher antioxidant and free radical scavenging property than that of crude Cur [64-66]. Generally, the small size of Cur-NPs and the amorphous state formation with the hydrogen-binding are the key factors for the increase of antioxidant property of Cur [67].

Table 3 DPPH radical scavenging activity of Cur-NPs and standard ascorbic acid as a reference

Concentration (µg/ml)	% DPPH radical scavenging activity	
	Ascorbic acid	Cur-NPs
1000	98.55 ± 0.084	41.84 ± 7.07
500	98.24 ± 0.13	31.44 ± 1.045
250	80.28 ± 0.36	29.24 ± 0.62
125	62.69 ± 0.60	28.21 ± 2.52

Values are expressed as mean (n=3) of the percent inhibition of the absorbance of DPPH radicals
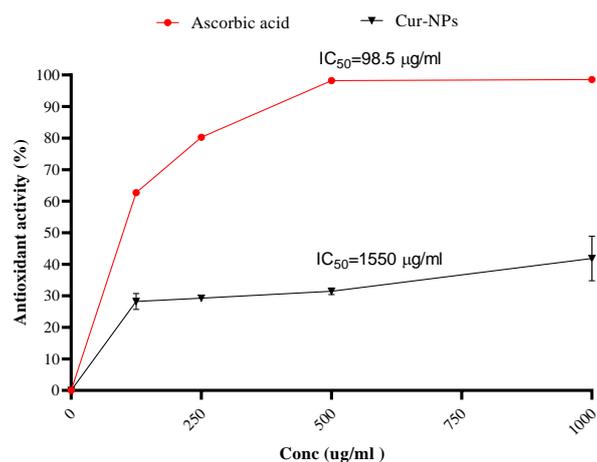
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Fig. 4. DPPH radical Scavenging activity of Cur-NPs and standard ascorbic acid as a reference
Values are expressed as mean (n=3) of the percent inhibition of the absorbance of DPPH radicals.

4. Conclusion

Cur-NPs with a particle size of 44 ± 8 nm and zeta potential of 43 ± 4 mV can be synthesized. These nanoparticles showed dose-dependent antibacterial potency against *S. aureus*, and *B. cereus* (gram-positive) and *E. coli*, *S. typhimurium, and Y. enterocolitica* (gram-negative). However, the antibacterial potency in the case of gram-positive was higher than that of Gram-negative bacteria. Cur-NPs also showed strong antifungal effect against the tested pathogenic fungi in the order of *Candida albican> Penicillum expansum> Aspergillus flavus > Aspergillus niger*. Cur-NPs also showed antioxidant property and DPPH radical scavenging properties with IC$_{50}$ of 1550 μg/ml. Therefore, Cur-NPs have antimicrobial and antioxidant properties against Gram-positive, Gram-negative and pathogenic fungi and can be used as an antimicrobial agent in food preservation, medical and pharmaceutical applications to replace antibiotics.

5. Acknowledgment

6. Declarations

Conflict of interest :The authors declare no competing interests.

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