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Effect Of Iron Oxide Nanoparticles (Fe_2O_3) On Candida Albicans And Candida Glabrata

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Abstract.

In this study, iron oxide nanoparticles (Fe_2O_3 NPs) prepared by the green method were used using saffron extract as a reducing agent. Antifungal activities of the Fe_2O_3 NPs were examined on two pathogenic Candida sp (C. Albicans and C. glabrate) by the well diffusion method. the diameter of the growth inhibition zone of the fungal isolates increased with the increase in the concentration of iron oxide nanoparticles , where the growth inhibition zone of C. Albicans were, 30.1 ± 0.994 mm , 24.2 ± 0.789mm, 20.2 ± 0.788 mm, 18 ± 0.667 mm, and 14 ± 0.666 mm for concentrations 20mg/ml, 10mg/m, 4 mg/ml, 2 mg/ml and 1 mg/ml respectively furthermore, the diameter of the inhibition zones of C. glabrate were 37.8 ± 0.788mm, 35.2 ± 0.789mm, 28 ± 0.667mm, 23.7 ± 0.456mm and 17.9 ± 0.568 mm for Fe2O3 NPs
concentrations 20mg/ml, 10mg/m, 4 mg/ml, 2 mg/ml and 1 mg/ml
respectively.

Keywords: Candidiasis, Candida, C. Albicans, C. glabrate, Antifungal

1. Introduction

Candidiasis is caused by a fungus caused by Candida spp. It is a common disease, which Candida yeast is naturally present in the mucous membranes of the mouth, vagina and alimentary canal, which turns into an opportunistic pathogen under appropriate conditions, such as infection with some diseases such as HIV infection, diabetes and leukemia, take antibiotics or chemotherapy for cancer patients [1][2]. There are many types of Candida genus, but the pathogenic and causative types of candidiasis in humans are few, and

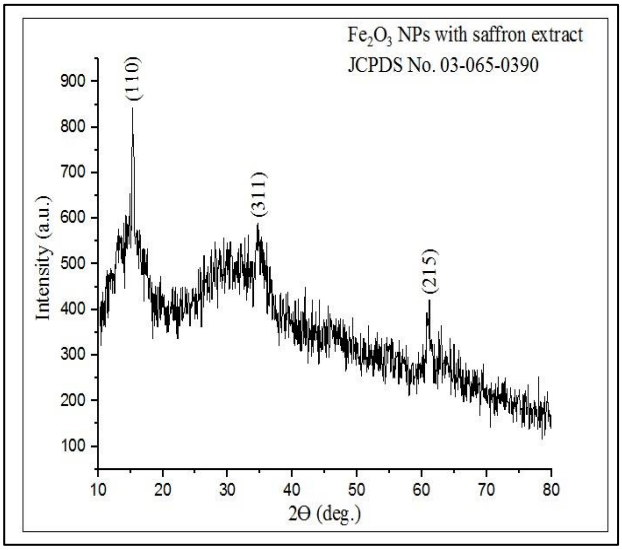
the yeast Candida albicans is the most prevalent species and is the leading cause of candidiasis[3], followed by other types, including C.glabrata and C.krusei and C.tropicalis and other species [4].

Due to the increased resistance to antifungals by the clinical isolates of Candida[5], it was necessary to find alternatives to antifungals that have high efficacy against pathogenic fungi in order to eliminate fungi resistant to antifungals and Nanoparticles have high efficacy against pathogenic fungi. The use of nanoparticles in biological systems constitutes an excellent opportunity for medical applications, as their small size contributes to overcoming vital barriers. As a result of the resistance of Candida to antifungals, the study aimed to use nanoparticles as an antifungal. The study was based on previous studies in which nanoparticles were used as antifungals, such as gold nanoparticles, titanium dioxide [6], silver nanoparticles [7] and other nanoparticles.

2. Experimental Part

2.1 Characterization of iron oxide nanoparticles

In this study, iron oxide nanoparticles prepared by the green method were used using saffron extract as a reducing agent. These particles were characterized by X-ray diffraction to determine the structural properties where the results obtained were following the standard (JCPD NO. 03-065-0390) as shown in Figure (1A) and using the Scherrer's Equation[8]; specified the crystal size that ranged between (27.2 - 15.6 nm), and by scanning electron microscopy; specified the size of nanoparticles that ranged between (24.27 - 46.27 nm) as shown in Figure (1B)



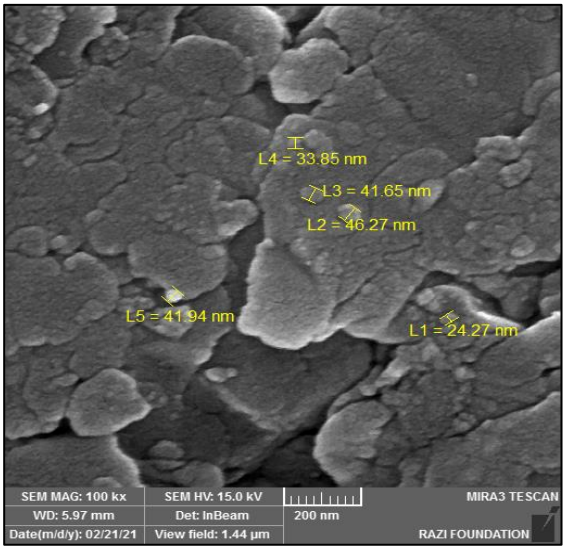


Figure (1): A) XRD analysis of Fe_2O_3 NPs, B) SEM images of Fe_2O_3 NPs.

2.2 Preparation of iron oxide nanoparticle concentrations

A concentration of 20 mg/ml of iron oxide nanoparticles, weighing 200 mg of it, is prepared in 10 ml of distilled water to form a stock solution that is diluted to different concentrations (10,4,2,1) mg/ml according to the dilution equation ($N_1V_1 = N_2V_2$)[9].

2.3 Antifungal Activity of Iron oxide nanoparticles

In this study, two types of isolated Candida (C. Albicans and C. Glabrata) were used, obtained from the fungi laboratory of the Department of Biology, College of Science, University of Baghdad.

The test was done using the Well diffusion method[10][11]. The isolated Candida fungus swab was taken with a cotton swab and spread in the culture medium. Before that, the culture medium did prepare according to the manufacturer's instructions. Etching was done using a cork borer, and 100 µl of concentrations (20,10,4,2,1) mg/ml prepared from iron oxide nanoparticles were placed in the pits and then incubated in the incubator at a temperature of 37°C for time 24 hours, record the diameter of inhibition produced by each concentration. This experiment was applied to ten replicates of Candida albicans and ten replicates of Candida glabrata, and a statistical process was performed for the results. Statistical analyses were done using Stat View version 0.5, social science statistics (SSS) and Statistical Package for Social Sciences version 20 computer software (SPSS)[12] associated with Microsoft Excel 2010.

3. Results and discussion

Antifungal activities of the Fe_2O_3 NPs were examined on two pathogenic Candida sp (C. Albicans and C. glabrate) by the excellent diffusion method, as shown in Table (1). Compared with the control group represented by deionized water, the concentrations of Fe_2O_3 NPs had an apparent inhibitory effect on both species of Candida, when comparing the average diameter of the inhibition zone for those concentrations with deionized water led to apparent significant differences (P-value) that ranged between 0.0001 for high Fe_2O_3 NPs concentrations to less than 0.05 for low concentration of Fe_2O_3 NPs.

Table (1): Compared effect of Fe_2O_3 NPs on studied Candida species

Fe_2O_3 NPs concentrations	**Zone of inhibition (mm)**		**95% confidence**	**T test**	**P value**
	C. albicans	**C. glabrata**			

	Mean ± SD	Mean ± SD			
20 mg/ml	30.1 ± 0.994**	37.8 ± 0.788**	-8.543 to -6.857	19.196	< 0.001 [HS]
10 mg/ml	24.2 ± 0.789**	35.2 ± 0.789**	-11.741 to -10.259	31.174	< 0.001 [HS]
4 mg/ml	20.2 ± 0.788**	28 ± 0.667**	-8.4859 to -7.1141	23.892	< 0.001 [HS]
2 mg/ml	18 ± 0.667*	23.7 ± 0.456**	-11.237 to -10.163	41.878	< 0.001 [HS]
1 mg/ml	14 ± 0.666*	17.9 ± 0.568*	-4.4815 to -3.3184	14.0896	< 0.001 [HS]
D.W (control)	8 ± 0.0	8 ± 0.0	---------	--------	1 [NS]
****significant differences (P<0.01) in compared with control, * significant differences in compared with control (P<0.05), HS refer to high statically significant (P<0.0001), NS refer to non-statically correlation (p>0.05), Standard deviation (SD)**					

In the present study, the diameter of the growth inhibition zone of the fungal isolates increased with the increase in the concentration of iron oxide nanoparticles As shown in the figure (2), where the growth inhibition zone of C. albicans were, 30.1 ± 0.994 mm , 24.2 ± 0.789mm, 20.2 ± 0.788 mm, 18 ± 0.667 mm, and 14 ± 0.666 mm for concentrations 20mg/ml, 10mg/m, 4 mg/ml, 2 mg/ml and 1 mg/ml respectively Figure (3) furthermore, the diameter of the inhibition zones of C. galabrata were 37.8 ± 0.788mm, 35.2 ± 0.789mm, 28 ± 0.667mm, 23.7 ± 0.456mm and 17.9 ± 0.568 mm for Fe_2O_3 NPs concentrations 20mg/ml, 10mg/m, 4 mg/ml, 2 mg/ml and 1 mg/ml respectively Figure (4).

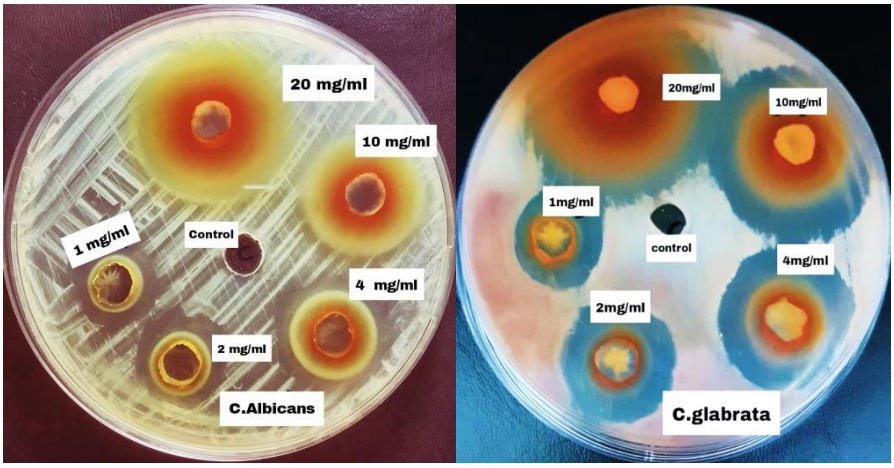


Figure (2): Antifungal activities of the Fe_2O_3 NPs

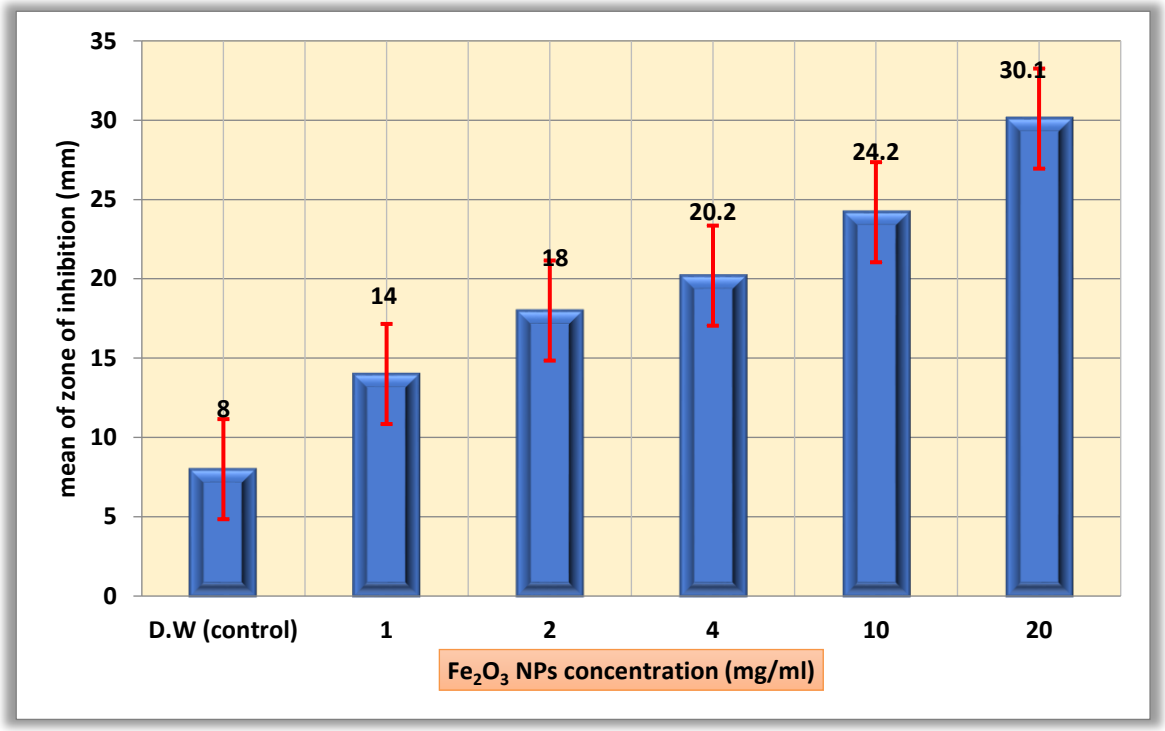


Figure (3): The Effect of different concentrations of Fe_2O_3 NPs on the growth of C. albicans

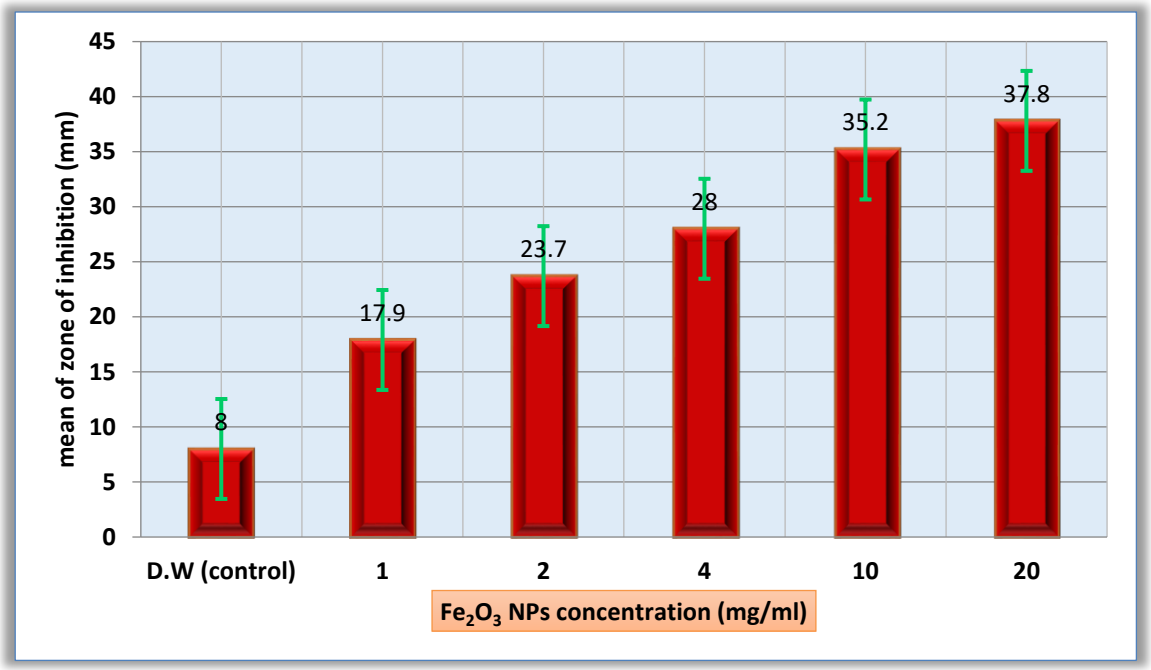


Figure (4):The Effect of different concentrations of Fe_2O_3 NPs on the growth of C. galabrata

The results of the current research in Figure (5) showed that theC. galabrata was more affected by Fe_2O_3 nanoparticles compared to the C. albicans fungus, which led to clear significant differences ($P<0.0001$) recorded in Table(1). Also, the median diameter of the inhibition zone appeared for 10isolates of C. galabrata ranging from 18 mm to 38 mm as in Table (2) while the median diameter of the inhibition zone for the same number of isolates of C. albicans ranged between 14 mm and 30 mm as in Table (3).

In descriptive statistics, the interquartile range, also called the mean range, mean 50%, or H prevalence, is a measure of statistical dispersal, equal to the difference between the 75^{th} and 25^{th} percentiles, or between the upper and lower quartiles of measures of inhibition zone that resulting from the effect of nanoparticles concentrations, in another sense, is a measure of the change in the inhibition area of fungal growth as a result of nanoparticle action.

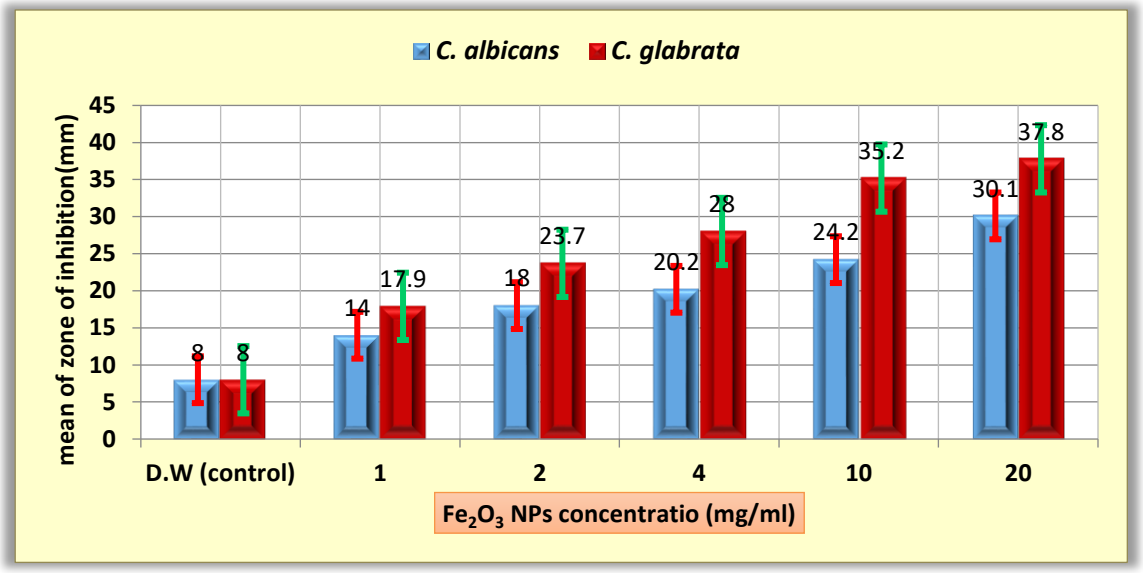


Figure (5): Compared effect of by Fe_2O_3 nanoparticles on studied Candida

Table (2): Descriptive statistics of inhibition zones of Fe_2O_3 NPs and de-ionized water on C. glabrata

Fe2O3 NPs concentration	Samples number	Zone of inhibition (mm)		
		Median	Mean ± SD	Interquartile range
20 mg/ml	10	38	37.8 ± 0.788	1.00
10 mg/ml	10	35	35.2 ± 0.789	1.00
4 mg/ml	10	28	28 ± 0.667	0.00
2 mg/ml	10	24	23.7 ± 0.456	1.00
1 mg/ml	10	18	17.9 ± 0.568	0.00
D.W (control)	10	8	8 ± 0.0	0.00
Deionized Water (D.W), Standard deviation (SD)				

Table (3): Descriptive statistics of inhibition zones of Fe_2O_3 NPs and de-ionized water on C. albicans

Fe2O3 NPs concentration	Samples number	Zone of inhibition (mm)		
		Median	Mean+ SD	Interquartile range
20 mg/ml	10	30	30.1 ± 0.994	2.00
10 mg/ml	10	24	24.2 ± 0.789	1.00
4 mg/ml	10	20	20.2 ± 0.788	1.00
2 mg/ml	10	18	18 ± 0.667	0.00
1 mg/ml	10	14	14 ± 0.666	0.00
D.W (control)	10	8	8 ± 0.0	0.00
Deionized Water (D.W), Standard deviation (SD)				

Comparing the effect of increasing or decreasing nanoparticles on fungal species growth is useful in determining the appropriate dose to inhibit or kill pathogenic fungi in the medical field. In order to obtain that, the significant differences between each two concentrations were measured, as shown in Table (4), based on the calculation of the mean area of inhibition. Statistically, p value was used through F stat value, which is more accurate to clarify the differences for such experiments.

Accordingly, we found clear significant differences ($p<0.05$) when comparing the effect of different concentrations of Fe_2O_3 NPson C. albicans in particular between the highest concentration of 20 mg/ml and the lowest concentration of 1mg/ml where the probability value is 0.0011 and on the same line, a clear statistical difference appeared between the concentrations 20 /2mg/ml ($p =0.0013$) and 10 /1mg/ml ($p = 0.0016$), while the effect of concentrations 4 /2 mg/ml were less exchange , so the probability appeared equal to 0.0491 as shown in Table (4) . On the same procedure, we conducted the same statistical test to find out the significant differences in the growth of C. galabrata resulting from the use of different concentrations of Fe_2O_3 NPs, where the results showed clear significant differences, especially between the highest concentration 20 mg/ml and the lowest concentration 1 mg/ml, where the statistical probability was 0.0013 as well as significant differences appeared between 10/1 mg/ml ($p = 0.0014$) followed by 20/2 mg/ml ($p=0.0024$). On the other hand, the effect of high concentrations 20 mg/ml and 10 mg/ml were less

significant, therefore the p value was equal to 0.0491 as in Table (5). It is clear from the above results that the Fe_2O_3 NPsconcentration 20 mg/ml is the best drug as a treatment to inhibit the growth or reproduction of those pathogenic fungi.

Table (4): Determining the significant differences in C. albicans growth that resulting from different concentrations of Fe_2O_3 NPs

Fe_2O_3 NPs concentration	**Zone of inhibition (mm)**				**F-Stat value**	**P value**
	Median	**Mean**	**SE**	**DF**		
20 mg/ml	30	30.1	0.314	1	216.07204	0.0299*
10 mg/ml	24	24.2	0.250			
20 mg/ml	30	30.1	0.314	1	608.36599	0.0163*
4 mg/ml	20	20.2	0.249			
20 mg/ml	30	30.1	0.314	1	1021.47444	0.0013*
2 mg/ml	18	18	0.211			
20 mg/ml	30	30.1	0.314	1	1808.45837	0.0011*
1 mg/ml	14	14	0.210			
10 mg/ml	24	24.2	0.250	1	128.5749	0.0462*
4 mg/ml	20	20.2	0.249			
10 mg/ml	24	24.2	0.250	1	360.36565	0.0031*
2 mg/ml	18	18	0.211			
10 mg/ml	24	24.2	0.250	1	975.3497	0.0016*
1 mg/ml	14	14	0.210			
4 mg/ml	20	20.2	0.249	1	45.37382	0.0491*
2 mg/ml	18	18	0.211			
4 mg/ml	20	20.2	0.249	1	360.36565	0.0461*
1 mg/ml	14	14	0.210			
2 mg/ml	18	18	0.211	1	179.982	0.0477*

1 mg/ml	14	14	0.210			
***statically significant (P<0.05), Standard Deviation (SD), Standard Error (SE), Degree of freedom (DF)**						

Table (5): Determining the significant differences in C. galabrata growth that resulting from different concentrations of Fe_2O_3 NPs

Fe_2O_3 NPs concentration	**Zone of inhibition (mm)**				**F-Stat value**	**P value**
	Median	**Mean**	**SE**	**DF**		
20 mg/ml	38	37.8	0.2494	1	54.32289	0.0491*
10 mg/ml	35	35.2	0.250			
20 mg/ml	38	37.8	0.2494	1	900.35165	0.0381*
4 mg/ml	28	28	0.211			
20 mg/ml	38	37.8	0.2494	1	1844.76978	0.0024*
2 mg/ml	24	23.7	0.2134			
20 mg/ml	38	37.8	0.2494	1	4193.35451	0.0013*
1 mg/ml	18	17.9	0.180			
10 mg/ml	35	35.2	0.250	1	485.9874	0.0389*
4 mg/ml	28	28	0.211			
10 mg/ml	35	35.2	0.250	1	1227.15559	0.0029*
2 mg/ml	24	23.7	0.2134			
10 mg/ml	35	35.2	0.250	1	3169.1853	0.0014*
1 mg/ml	18	17.9	0.180			
4 mg/ml	28	28	0.211	1	205.44926	0.0337*
2 mg/ml	24	23.7	0.2134			
4 mg/ml	28	28	0.211	1	1330.57913	0.003*
1 mg/ml	18	17.9	0.180			

2 mg/ml	24	23.7	0.2134	1	66.45394	0.0411*
1 mg/ml	18	17.9	0.180			
***statically significant (P<0.05), Standard Deviation (SD), Standard Error (SE), Degree of freedom (D**						

4. Conclusions

1. The synthesis of iron oxide nanoparticles had antifungal activity against two types of Candida (C. Albicans and C. glabrate)
2. The diameter of the growth inhibition zone of the fungal isolates increased with the increase in the concentration of iron oxide nanoparticles.
3. The study results showed that C. Glabrata was more sensitive to iron oxide nanoparticle concentrations than C. Albicans.

5. References

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