

## SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF IRON CYCLOHEXANEDICARBOXYLIC ACID AND EXAMINATION OF pH EFFECT ON EXTRACTION IN WATER AND ORGANIC PHASES

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**Abstract:** In this study, iron cyclohexanedicarboxylic acid was synthesized under refluxing conditions. The structural characterization of the complex was performed by using physico-chemical and spectroscopic methods. The effect of pH on division constant ( $\infty$ ) and extraction percentages (% E) in water and organic phase was investigated with the extraction method. At the end of the experiment, most of the Fe<sup>2+</sup> ions in the water phase at pH=7.5 passed into the organic phase. Above this pH, the extraction percentage decreased because of the transition from water phase to organic phase. Iron cyclohexanedicarboxylic acid was extracted with ether, petroleum ether, benzole and carbon tetrachloride from aqueous solution and microbial activities of the complex were studied. The activity data showed that iron cyclohexanedicarboxylic acid has an important antibacterial and antifungal activities.

**Key words:** Fe (II), cyclohexanedicarboxylic acid, synthesis, extraction.

### Sikloheksandikarboksilli Asidin Sentezi ve pH Etkisi ile Su ve Organik Fazlardan Ekstraksiyonunun İncelenmesi

**Özet:** Bu çalışmada, reflux şartlarında demir sikloheksandikarboksilli asit sentezlenmiştir. Kompleksin yapısı fizikokimyasal ve spektroskopik metodlarla karakterize edilmiştir. Daha sonra reaksiyon sırasında pH'ın su ve organik fazlardaki paylaşma sabitleri ( $\infty$ ) ve ekstraksiyon yüzdelere (%E) etkisi ekstraksiyon metodu ile incelenmiştir. Deney sonucunda, su fazındaki Fe<sup>2+</sup> iyonlarının çoğu pH=7.5'ta organik faza geçmiştir. Bu pH'tan sonra ekstraksiyon yüzdesi su fazından organik faza geçiş yüzünden azalmıştır. Demir sikloheksandikarboksilli asit eter, petrol eteri, benzol ve karbon tetra klorür ile sulu çözeltilerinden ekstre edilmiş, ilave olarak kompleksin mikrobiyal aktiviteleri çalışılmıştır. Aktivite sonuçları, demir sikloheksandikarboksilli asitin önemli antibakteriyel ve antifungal aktiviteye sahip olduğunu göstermiştir.

**Anahtar kelimeler:** Fe (II), sikloheksandikarboksilli asit, sentez, ekstraksiyon.

### Introduction

Low molecular weight cyclic organic acids are liquids with disturbing odor and high viscosity, whereas high molecular weight cyclic acids are either liquids with light odor and low viscosity or solids. They are soluble in many organic solvents (Battersby *et al.* 1968, March 1985). Many studies on *cis* and *trans*-1,2-cyclohexanedicarboxylic acid have previously been published so far (Björkling *et al.* 1985, Reiznautt *et al.* 2009, Altun *et al.* 2010, Chen *et al.* 2014). For instance, the Hunsdiecker reaction of silver salts of *cis*- and *trans*-1,2-cyclohexanedicarboxylic acid was investigated by Abell (1957). Kamino *et al.* (1996) reported that the molecular packing was affected by the shape of polycarboxylate molecules in the case of phthalate, *cis*-1,2-cyclohexane dicarboxylate and 1,1-cyclohexanediacetate. Koster *et al.* (2001) examined hyperbranched synthetic polyesteramides synthesized by the polycondensation of trifunctional diisopropanolamine and dysfunctional

anhydrides of succinic acid, glutaric acid, *cis*-1,2-cyclohexanedicarboxylic acid and phthalic acid. We considered that if different metal salts of these compounds are synthesized, then they may be used in many different areas. For this purpose, we started a study with the aim of creation of iron salt of cyclohexanedicarboxylic acid to examine all aspects of this compound. We also investigated the extraction of the obtained iron cyclohexanedicarboxylic acid with pH effect between water and organic phase in addition to its antibacterial and antifungal activities.

### Materials and Methods

#### *Materials and Apparatus*

Cyclohexanedicarboxylic acid (Aldrich) and diethylether (Merck) were all organic reagent grade, and FeSO<sub>4</sub> . 7H<sub>2</sub>O (Merck) was analytical reagent grade.

Elemental analyses for C and H were obtained on dried samples using a Perkin Elmer 2400 elemental analyzer. FT-IR spectra were determined on a Perkin Elmer BXII spectrometer as KBr pellets in the frequency range 400-4000 $\text{cm}^{-1}$ . UV/Vis spectra were recorded at 25°C with a Shimadzu UV-1700 Pharma spectrophotometer in the wavelength range 200-800nm. XRD analyses were investigated in Shimadzu XRD-6000. A calibrated Metrohm 654 digital pH meter with a pH glass electrode mounting was employed for pH measurements. The pH meter was adjusted before use with pH 4 and 7 Metrohm AG CH 9100 Hersau Buffers.

#### Synthesis of iron cyclohexanedicarboxylic acid

##### Method I. Two step substitution reaction

Step I: 20g (0.117mol) of cyclohexanedicarboxylic acid was dissolved in 150ml ether and placed on a magnetically-stirred heater. The system was heated and stirred to reflux while 10g (0.25mol) NaOH in 40ml water solution was added dropwise within 20-24 minutes. The reaction mixture was poured into an extraction funnel. The pH was adjusted to 7 by adding NaOH solution. Aqueous phase was removed and washed with ether, evaporated slowly and a white powder, identified as sodium dicarboxylic acid, was obtained (1) [Color: White. Yield (%): 82 %. M.P.: 232.6°C. FT-IR bands ( $\text{cm}^{-1}$ ): 2878 $\text{cm}^{-1}$  ( $\nu_{\text{CH}}$ ), 1738-1550 $\text{cm}^{-1}$  ( $\nu_{\text{carboxyl anions}}$ ), 1475 $\text{cm}^{-1}$  ( $\nu_{\text{CO}}$ ). UV/Vis bands (nm): 302].

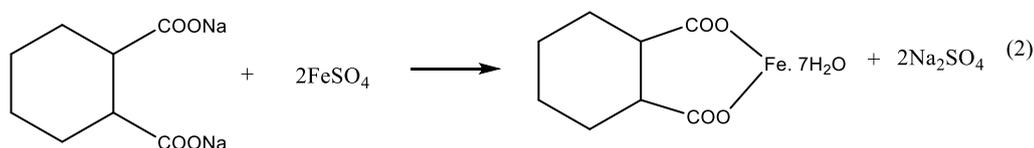
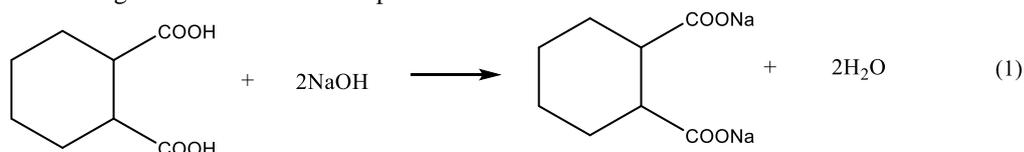
Step II: 5g (0.023mol) sodium dicarboxylic acid was dissolved in 20ml water and placed in a magnetically stirred 250ml three necked flask which was equipped with a thermometer, a water condenser and an additional funnel filled with 5g (0.018mol)  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  solution in 7ml water.  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  solution was added dropwise at 85-90°C within one hour. At the end of the reaction, solid brown iron dicarboxylic acid was filtered and washed with distilled water several times to remove water soluble material. The remaining solid residue was kept under

vacuum producing a brown solid identified as iron dicarboxylic acid (2) [Color: Brown. Yield (%): 84. M.P.: 265°C. Elemental Analyses (%): Calc. C 42.55, H 4.23. Found: C 42.6, H 4.4. FT-IR bands ( $\text{cm}^{-1}$ ): 2944 $\text{cm}^{-1}$  ( $\nu_{\text{CH}}$ ), 1593-1532 $\text{cm}^{-1}$  ( $\nu_{\text{carboxyl anions}}$ ), 1436-1398 $\text{cm}^{-1}$  ( $\nu_{\text{CO}}$ ). UV/Vis bands (nm): 290, 511. XRD ( $2\theta$ ): 5.2300, 14.9200 and 19.5600].

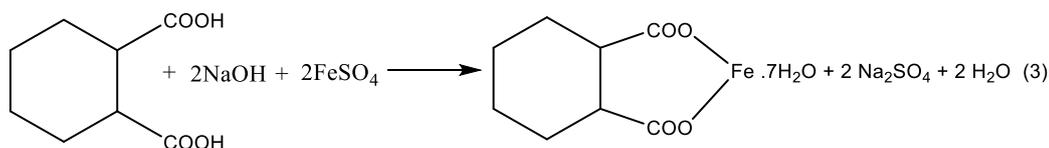
##### Method II: One step substitution reaction

A solution of 20g (0.11 mol) cyclohexanedicarboxylic acid in 150ml ether was placed in a magnetically stirred three necked flask on which a thermometer, a condenser and an additional funnel were attached. 10g (0.25mol) NaOH in 40ml water was placed in the additional funnel and dropwise addition was begun after internal temperature reached to 40-45°C with external heating. After NaOH addition was completed, 5g (0.018mol)  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  solution in 7ml water was placed in the additional funnel and the previous operation was repeated at 85-90°C. Mixture was stirred for about one hour. During the progress of the reaction ether was lost gradually from the system. Finally, the liquid phase was filtered giving brown residue which was washed with distilled water to get rid of the water soluble impurities. The brown solid was oven dried giving a compound with a spectrum consistent with the spectrum of the compound obtained in Method I.

We adjusted the pH value of sodium dicarboxylic acid solution to 7 before treating it with  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  solution in both methods to obtain the maximum yield. At pH values higher than 7 complications occur in the reaction as a result of a reaction between hydroxyl and iron ions (3) [Color: Brown. Yield (%): 89 %. M.P.: 265.2°C. Elemental Analyses (%): Calc. C 42.53, H 4.26. Found: C 42.6, H 4.4. FT-IR bands ( $\text{cm}^{-1}$ ): 2944 $\text{cm}^{-1}$  ( $\nu_{\text{CH}}$ ), 1605-1558 $\text{cm}^{-1}$  ( $\nu_{\text{carboxyl anions}}$ ), 1400 $\text{cm}^{-1}$  ( $\nu_{\text{CO}}$ ). UV/Vis bands (nm): 290, 511. XRD ( $2\theta$ ): 5.2300, 14.9200 and 19.5600].



Scheme 1



Scheme 2

The extraction of iron cyclohexanedicarboxylic acid in water and organic phases with pH effect

The aqueous solution of 5ml of 0.1N NaOH for pH=4 to 9.5 and solution of 1.3g (0.078mol) cyclohexanedicarboxylic acid in 10ml flask were stirred in ten separate flasks. Afterwards, 10ml of aqueous solution including 1mg/ml Fe<sup>2+</sup> was added to this mixture, and after stirring for one hour at ambient temperature, the mixture was left for 24 hours. Composed solutions were separately placed in an extraction flask, and the amount of Fe<sup>2+</sup> ions in organic and water phases was determined with atomic absorption with Unicam model 929, Aagrame (Yang *et al.* 1991, İnci 2002).

The percentage of the extraction was calculated with the following formula:

$$\% E = (\alpha + V_w / V_o) \cdot 100 \quad (1)$$

$$\alpha = C_o / C_w \quad (2)$$

Where % E= Extraction percentage, V<sub>org</sub> = Volume of the organic phase (ml), V<sub>w</sub> = volume of water phase,  $\alpha$  = sharing constant, C<sub>org</sub> = Fe<sup>2+</sup> concentration in organic phase (mg/ml), C<sub>w</sub> = Fe<sup>2+</sup> concentration in water phase (mg/ml).

In all samples, 5ml 0.1N NaOH solution, 10ml FeSO<sub>4</sub> .7 H<sub>2</sub>O (1mg/ml Fe<sup>2+</sup>) solution and 10ml ether solution in proportion 1.3g (3.7x10<sup>-3</sup>mol) iron dicarboxylic acid were used.

Biological activity procedure

The antibacterial and antifungal activities of the obtained complexes is tested by using agar well-diffusion susceptibility test (Shaukat *et al.* 1980) is carried out against Gram-positive bacteria (*Bacillus subtilis*

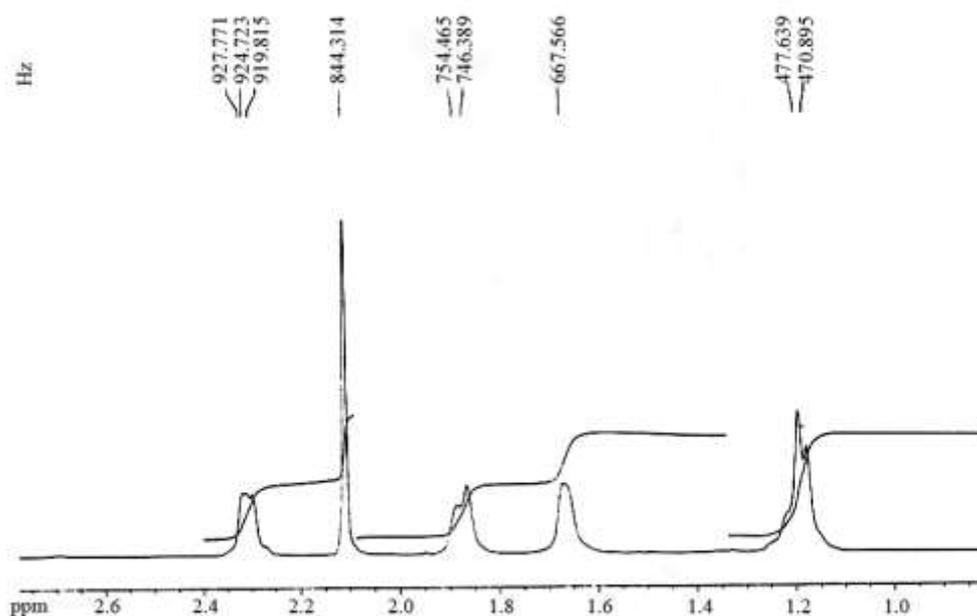
Ehrenberg, *Staphylococcus aureus* Rosenbach) and Gram-negative bacteria (*Escherichia coli* T. Escherich, *Pseudomonas aeruginosa* (Schroeter) Migula) and the agar tube dilution protocol method (Kazmi *et al.* 1991) is applied against *Candida albicans* (Berkhout), *Candida glabrata* (Anderson) Mey & Yarrow and *Fusarium solani* (Mart.) Sacc), respectively.

**Results and Discussion**

Synthesis and analyses

In the present study, the synthesis and the properties of a coordination compound of cyclohexanedicarboxylic acid and Fe (II) were studied. Two methods were employed for the synthesis and high yields were found to be fairly close with both methods. Spectroscopic methods showed the possible structure of iron dicarboxylic acid. Iron dicarboxylic acid is a brown solid. The solubility in different solvents and the results of the elemental analysis are given in Table 1 and Table 2. Elemental analysis determination is in good agreement with the general formula for the complex. The elemental analysis demonstrated that the obtained iron cyclohexanedicarboxylic acid has 1:1 stoichiometry. Complex is solid, insoluble in water but soluble in some organic solvents such as benzene, ether, Acetone, CCl<sub>4</sub> and CH<sub>3</sub>COOH.

The FT-IR spectra of the complex (Figure 1) demonstrates two bands centered at 1593 and 1532cm<sup>-1</sup> are attributed to the (ν<sub>carboxyl anions</sub>) vibrations. The band at about 2944cm<sup>-1</sup> is assigned to the absorption of the (ν<sub>CH</sub>) vibrations and the (ν<sub>CO</sub>) stretching of the complex are found in the frequency range about 1593 and 1532cm<sup>-1</sup>. FT-IR bands of cyclohexanedicarboxylic acid (2878cm<sup>-1</sup> (ν<sub>CH</sub>), 1738-1550cm<sup>-1</sup> (ν<sub>carboxyl anions</sub>), 1475cm<sup>-1</sup> (ν<sub>CO</sub>)) are shifted to lower frequencies in the complex.



**Figure 1.** The FT-IR Spectrum of iron cyclohexanedicarboxylic acid

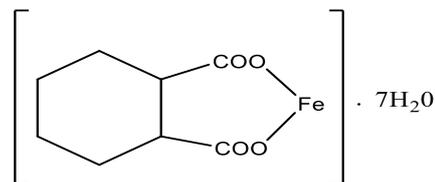
The UV/Visible spectrum of cyclohexanedicarboxylic acid molecule shows an absorption band at 302nm, whereas the complex demonstrates absorption bands at 290 and 511nm. The band at 290 which is attributed to  $n-\pi^*$  and  $\pi-\pi^*$  transitions, while the band at 511nm is caused by the electronic transitions both  $n-\pi^*$  and  $\pi-\pi^*$  and also charge transfer transition arising from  $\pi$  electron interactions between the metal and ligand that involves either a metal-to-ligand or ligand-to-metal electron transfer.

The XRD-Powder Pattern spectrum (Figure 2) of the complex is recorded by an X-Ray diffractometer and the unit cell parameters are calculated from  $2\theta$  values with the help of a computer. Powder XRD pattern of complex consist of three important peaks in the range  $5-50^\circ(2\theta)$ . The inter planar spacing (d) is calculated from the positions of intense peaks using Bragg's relationship. The  $2\theta$  values with maximum intensity of the peaks for Fe (II) complex were found to be 5.2300, 14.9200 and 19.5600 ( $2\theta$ ) which corresponds to d:16.97784, 5.93296 and 4.53476, respectively.

Thermogravimetric analysis determined the localization of the central cation in the complex molecule. Thermogravimetric analysis of iron cyclohexanedicarboxylic acid indicated two steps for decomposition and the amount of water in the composition of the molecule. Experimental thermolysis is given in Table 1 and Figure 3.

The decomposition of two carboxyl groups can be monitored from the thermogram. Up to  $181^\circ\text{C}$ , a water molecule leaves the compound, then between  $306-724^\circ\text{C}$ ,

the organic component decomposes. The remaining compound over  $724^\circ\text{C}$  was determined as FeO. After these observations we concluded that the structure of iron cyclohexanedicarboxylic acid is  $[\text{Fe}(\text{C}_8\text{H}_{10}\text{O}_4)] \cdot 7\text{H}_2\text{O}$ . The structural formula of the compound is shown below.

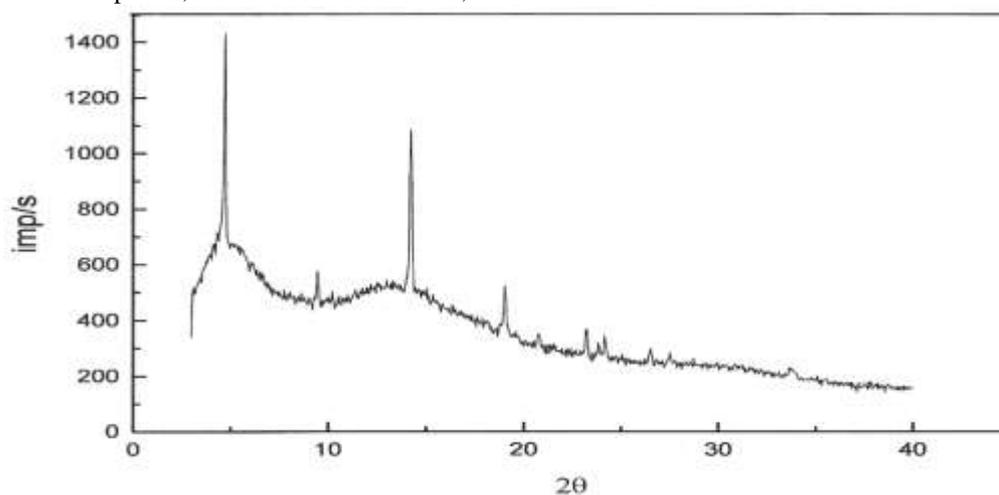


**Scheme 3**

### Extraction results

Table 2 shows the extraction results. The graphics which show transition related to pH of the sharing constant ( $\alpha$ ) and extraction percentages (% E) of iron dicarboxylic acid for these values are given in Figure 4 and Figure 5. At the end of the experiment, most  $\text{Fe}^{2+}$  ions in the water phase at  $\text{pH}=7.5$  passed into the organic phase, and the extraction percentage at this pH became  $E = 96.5\%$ . Extraction percentages decreased at pH values above 7.5 because of the transition from water phase to organic phase, (Figure 4).

Extraction experiments were repeated with benzene, benzole and carbon tetrachloride, apart from ether, and extraction yields were found to be close to 100 % between  $\text{pH} = 7.5-8$  (Table 2). In this way, it was proven that in addition to ether, petroleum ether, benzole and  $\text{CCl}_4$  solvents are also suitable extraction substances for iron cyclohexanedicarboxylic acid.



**Figure 2.** The XRD Spectrum of iron cyclohexanedicarboxylic acid

**Table 1.** Thermogravimetric analysis data of iron dicarboxylic acid

Compound	First step decomposition		Second step decomposition		FeO (%)
	T <sub>b</sub> -T <sub>e</sub> (°C)	Weight loss (%)	T <sub>b</sub> -T <sub>e</sub> (°C)	Weight loss (%)	
Iron Dicarboxylic acid	0-181	4.0	306-724	56.5	39.5

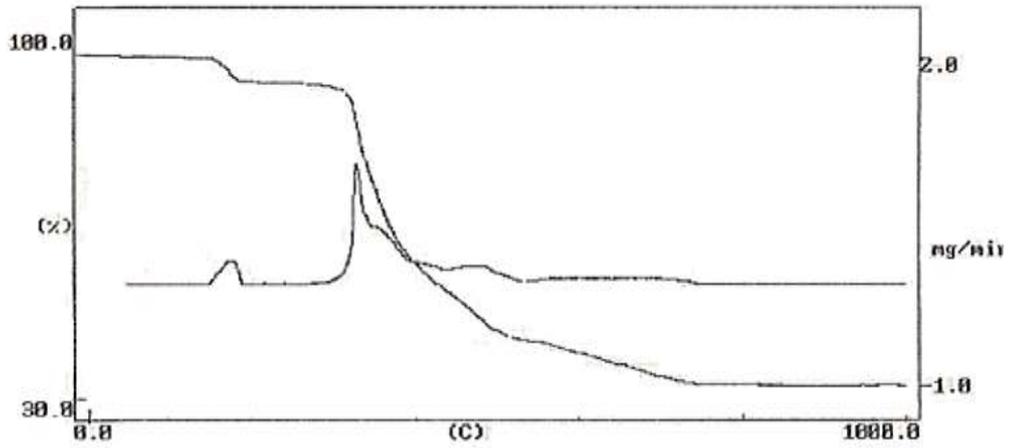


Figure 3. TG-DTA of iron cyclohexanedicarboxylic acid

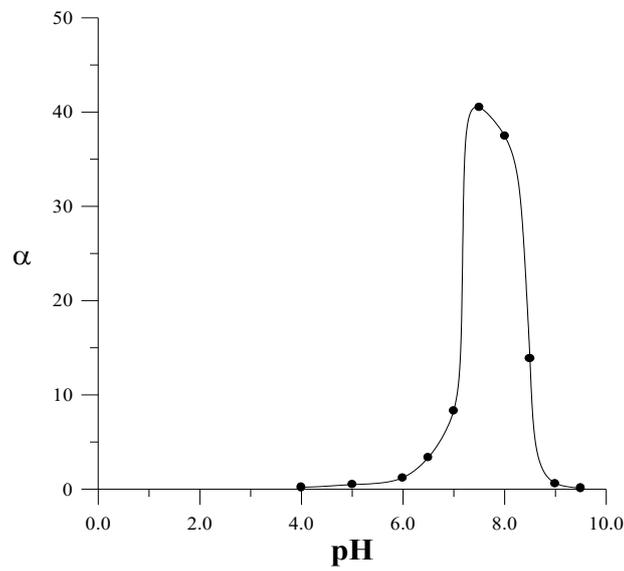


Figure 4. Transition of sharing constants of iron cyclohexanedicarboxylic acid with pH

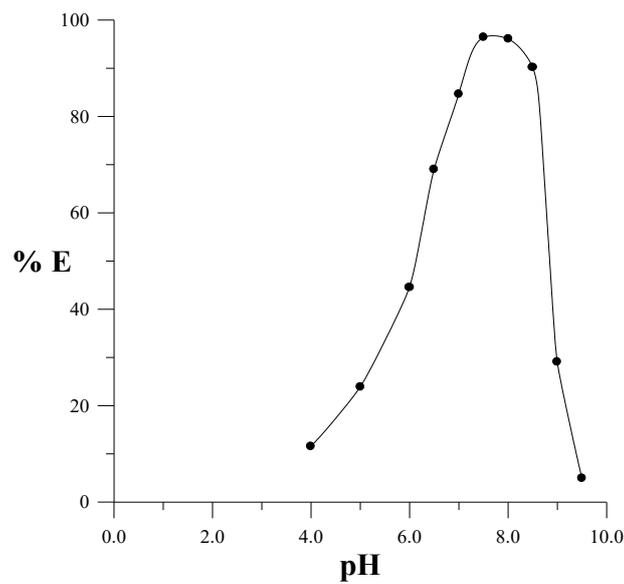


Figure 5. Transition of extraction percentages of iron cyclohexanedicarboxylic acid with pH

**Table 2.** Extraction of Fe<sup>2+</sup> in different solvents from the aqueous solution

Sample	Organic Solvents (10ml)	pH	Water phase Cw (mg/ml) at the end of the extraction (1mg/ml Fe <sup>2+</sup> )	Organic phase Co (mg/ml) at the end of the extraction (1mg/ml Fe <sup>2+</sup> )	α	E (%)
1	Petrol. ether	7.9	0.003	0.095	31.60	95.40
2	Benzole	7.6	0.005	0.156	38.8	96.2
3	CCl <sub>4</sub>	7.7	0.002	0.173	86.5	98.3

### Biological activity results

The results for the agar well-diffusion susceptibility tests showed that the iron complex potent antibacterial activity against Gram-positive bacteria (*Bacillus subtilis* Ehrenberg, *Staphylococcus aureus* Rosenbach) and Gram-negative (*Escherichia coli* T. Escherich, *Pseudomonas aeruginosa* (Schroeter) Migula) (Table 3).

**Table 3.** The antibacterial activities of the iron complex and standard

Bacteria <sup>a</sup>	Zone of inhibition (mm)	
	Iron Complex	Standard Drug <sup>b</sup>
<i>B. subtilis</i>	25	30
<i>S. aureus</i>	25	33
<i>E. coli</i>	20	32
<i>P. aeruginosa</i>	25	30

<sup>a</sup>Concentration of sample is 3mg/ml DMSO, <sup>b</sup>Inipenum, 10µg/disc

The results of the agar tube dilution protocol method showed that the iron complex has significant antifungal activity against *Candida albicans* (Berkhout), *Candida glabrata* (Anderson) Mey & Yarrow and *Fusarium solani* (Mart.) Sacc. (Table 4).

**Table 4.** The antifungal activities of the iron complex and standard.

Fungus	Inhibition (%)		
	Iron Complex	Standard Drug <sup>a</sup>	MIC <sup>b</sup> (10µg/mL)
<i>C. albicans</i>	75	Miconazole	110
<i>C. glabrata</i>	40	Miconazole	73
<i>F. solani</i>	70	Miconazole	110

<sup>a</sup>Incubation temperature: 27°C, <sup>b</sup>Concentration of sample 200µg/mL of DMSO.

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