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APPLICATION OF METHYL FATTY HYDROXAMIC ACIDS BASED ON *JATROPHA CURCAS* SEED OIL AND THEIR METAL COMPLEXES AS ANTI MICROBIAL AGENTS

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Hydroxamic acids, fatty hydroxamic acids and their metal complexes are known as compounds that have biological activity. They have been investigated as antimicrobial compounds and were applied as antibacterial and antifungal agents in pharmacy andpharmaceutical compounds. In this research, the methyl fatty hydroxamic acids (MFHAs) based on *Jatrophacurcas* seed oil and their metal complexes include the copper (II) methyl fatty hydroxamate (Cu-MFHs) and iron (III) methyl fatty hydroxamate (Fe-MFHs) were prepared and applied as anti microbial agents against the *Escherichia coli (E. coli*), *Proteus vulgaris* (*P. vulgaris*) and*Proteus mirabilis* (*P. mirabilis*) as gram-negative bacteria; methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (*S. epidermidis*) as gram-positive bacteria ; *Candida parapsilosis* (*C. parapsilosis*) and *Candida Albicans*(*C. Albicans*) as yeast family of fungi. The results showed that the antimicrobial activity of MFHAs, Cu-MFHs and Fe-MFHs increase while their amounts increase. Also metal complexation of MFHAs caused the anti microbial activity arise and this activity is higher for complexation by Cu(II) compared to that of Fe(III). Comparing antimicrobial activity of MFHAs, Cu-MFHs andFe-MFHs based on *Jatrophacurcas* seed oil with several antibiotic drugs such as ampicillin, chloramphenicol, gentamicin streptomycin, tetracycline and nystatin against the mentioned microbial showed that the Cu-MFHs andFe-MFHs have very strong antimicrobial activity.

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1. Introduction

Hydroxamic acids and their metal complexes have been very attractive as a research area for scientists due to their widely applications such as food additives, growth factors, antibacterial

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agents, fungicides, cell division factor, tumor inhibitors, enzyme inhibitors, analytical spectroscopy agents, metal chelators, antiradical andantioxidant [1-12]. The hydroxamic acids with branch alkyl containing at least eight carbon atoms are usually named as fatty hydroxamic acids and generally have been synthesized through enzymatic reaction[13]. Jahangirian and his colleagues synthesized fatty hydroxamic acids from canola oil [14] and derivatives of fatty hydroxamic acids from canola and palm oils [15, 16] by a one-step lipase catalyzed reaction.

Hydroxamic acids have been investigated as antimicrobial agents. Dudman applied sorbichydroxamic acid as antifungal agent against the *Aspergillusniger*, *Penicilliumnotatum*, *Botrytis cinerea*, and an unidentified *Rhizopus* species [17]. He showed that sorbichydroxamic acid is an antifungal agent which is effective over a wide pH range (3.6-9.2) and can be used as an additive in foods instead sorbic acid. This is one of the early reports about antifungal properties of hydroxamic acids. Coutts and his colleagues prepared a number of quinoline N-oxides and quinoline hydroxamic acids and evaluated their antibacterial properties against *S. aureus* and *E. coli*[18]. They indicated that most of this compounds , the antimicrobial activity depends on the position of the Nitro-group on the quinoline nucleus. Rao et al. investigated antifungal properties of many hydroxamic acids such as N,2'-diphenylaceto hydroxamic acid, 2,2'-diphenylaceto hydroxamic acid, 2-phenylaceto hydroxamic acid and their Cu(II), Ni(II) and Co(II) chelates against *Alternariaalternata*, *Fusariumoxysporum* and *Aspergillusflavus*[19]. They showed that the toxicity was augmented to a greater extent and the fungi toxicities of these metal chelates were found to be in the following order: Cu(II) > Ni(II) > Co(II) which coincides with the order of the stability of the chelates. Bravo and Lazo synthesized hydroxamic acids from 2,4-dihydroxy-1,4-benzoxazin-3-one or 4-hydroxy-1,4-benzoxazin-3-one and then used them as antibacterial agents against the *S. aureus, E. coli* and *Candida albicans*. The results showed that the synthesized products had moderate antibacterial effect [20]. Agarwal et al. investigated antibacterial and antifungal properties of many of aryl alkyl hydroxamic acids [21]. They showed that N-*o*-tolylbenzohydroxamic acid, acetohydroxamic acid, benzohydroxamic acid and salicylhydroxamic acid were highly active against fungi *Candida albicans.* Recently Jahangirian and his colleagues investigated the antibacterial activity of phenyl fatty hydroxamic acids against gram-positive [i.e. *Staphylococcus aureus*] and gram-negative bacteria [i.e. *Escherichia coli*] by the disk and well diffusion methods using Mueller-Hinton Agar (MHA). Their resultsshowed that phenyl fatty hydroxamic acids have high antibacterial activity and their antibacterial property on *E. coli* is stronger than on *S.* aureus and furthermore the antibacterial property of phenyl fatty hydroxamic acids increased with the increase in their concentration [22].

Jatropha, a crop native to North American region is now distributed in several regions (Africa, India, South East Asia and China) across the World [23]. Jatropha seeds contain about 30 to 40% of oil [24] on the other hand Jatropha oil contains toxic compounds[25]so cannot be used for nutritional and food purposes but its use as a source of energy or bio fuel source [26-31] and it has also recently investigated as catalyst, antioxidant, biomass, cellular sciences, bioactive compounds and a crop protector against insects and pests [32-38].

In this investigation, we carried out the synthesis of the methyl fatty hydroxamic acids (MFHAs) based on *Jatrophacurcas* seed oil and their metal complexes include the copper (II) methyl fatty hydroxamate (Cu-MFHs) and iron (III) methyl fatty hydroxamate (Fe-MFHs) then we applied them as antimicrobial agents against several of gram-negative and gram-positive bacteria and many of the yeast family of fungi. This is the first report about antimicrobial activity of the MFHAs based on *Jatrophacurcas* seed oil and their metal complexes

2. Material and method

2.1 Material and Microorganisms

2.1.1 Material

Jatrophacurcas seeds were obtained from the experimental plot in Universiti Kebangsaan Malaysia and itsoil was gained by the soxhlet extraction method. Hexane and absolute methanol were supplied by Systerm Co., Malaysia. Sodium hydroxide, sodium acetate, copper (II) nitrate,

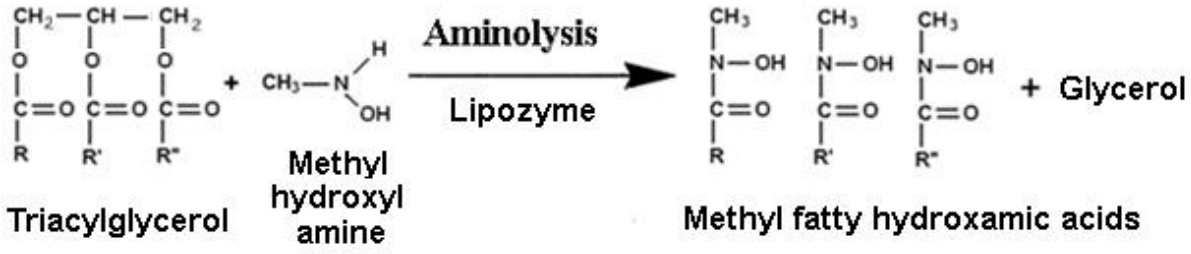
iron (III) chloride and methyl hydroxylamine hydrochloride were purchased from Merck Co., Germany. Commercial antibiotics of nystatin, gentamicin, streptomycin, chloramphenicol, tetracycline and ampicillin were purchased from Sigma-Aldrich Co., USA. Lipozyme TL IM and was obtained from Novo Nordisk, Denmark. Mueller-Hinton agar (MHA) Difco brand was obtained from Voigt Global Distribution, USA.

2.1.2Microorganisms

E. coli (ATCC 25922), methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 33591), *Staphylococcus epidermidis* (*S. epidermidis*) (ATCC 35984),*Proteus vulgaris* (*P. vulgaris*) (ATCC 12454), *Proteus mirabilis* (*P. mirabilis*) (ATCC 12453) and *Candida parapsilosis*(ATCC 22019), and *Candida albicans* (ATCC 10231) as fungal species of the yeast familywere from clinical isolates which conformed to the Clinical and Laboratory Standards Institute, (CLSI, 2000).

2.2 Synthesis of MFHAs

Methyl hydroxylaminolysis of Jatropha seed oil was carried out by shaking mixtures of the reactants, which contained 850 mg N-methyl hydroxylamine (*N*-MHA) dissolved in 20 ml distilled water and 2.6 g Jatropha seed oil dissolved in 30 ml hexane in the presence of the enzyme, Lipozyme TL IM (240 mg) in a 250 ml flask sealed using Teflon film. The mixture was shaken at 120 rpm and 39 °C in a water bath shaker for 72 h. The product was separated from the reaction mixture as follows. First the enzyme was removed by filtration. The filtrate that was as two phases liquid was then transferred into a separation funnel and aqueous phase was separated from organic phase. The organic phase in the funnel was mixed with distilled water (20 mL) and residual glycerol was removed from organic phase. For removing the unreacted *N*-MHA, 20 mL HCl solution (2 M) was added to the organic phase and after shaking, two phases were separated again. Hexane was then removed from organic phase by rotary evaporation to obtain mixture of the product and unreacted oil. Finally the product was separated from the unreacted oil by extraction using absolute methanol (20 mL) and then removing the methanol by rotary evaporation. Scheme 1 shows the methyl hydroxylaminolysis of Jatropha seed oil.

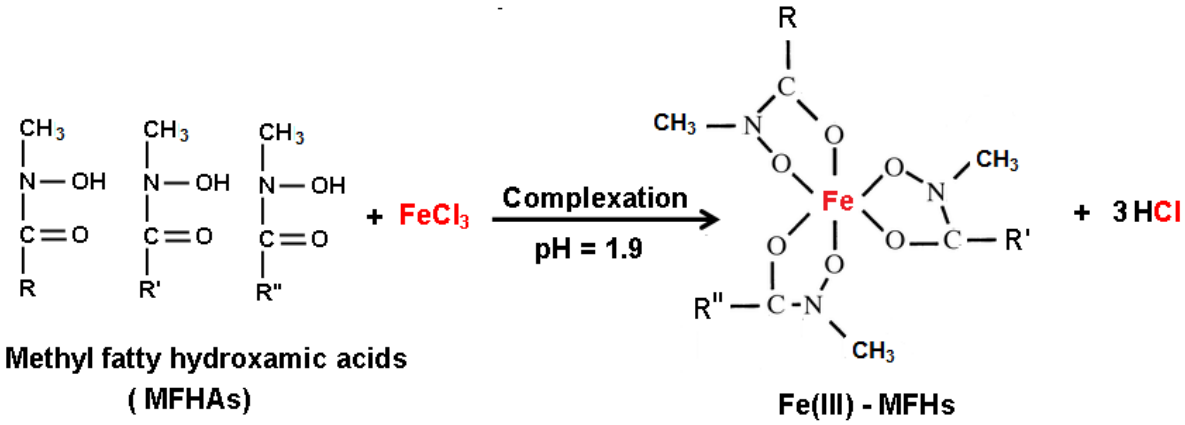


Scheme 1. Methyl hydroxylaminolysis of Jatrophacurcas seed oil

2.3 Iron (III) complexation of MFHAs based on Jatropha seed oil

For preparation of iron(III) methyl fatty hydroxamates (Fe(III)–MFHs), the iron(III) complexes of MFHAs, 50 ml of 0.016 M MFHAs hexane solution was mixed with 50 ml of 1.6 mM iron(III) chloride aqueous solution. The mixture was stirred at 500 rpm at 25 ± 1 °C for 10 minutes buffered by sodium acetate at the desired pH 1.9 [10]. Finally the Fe(III)–MFHs was obtained by the separation of organic phase from aqueous phase followed by removal of hexane from the organic phase using rotary evaporation. The structure and coordination number of iron(III) complexes of MFHAs based on Jatropha seed oil are the same of them based on palm kernel oil because they differ just in long of alkyl branches (R) and this difference is unaffected

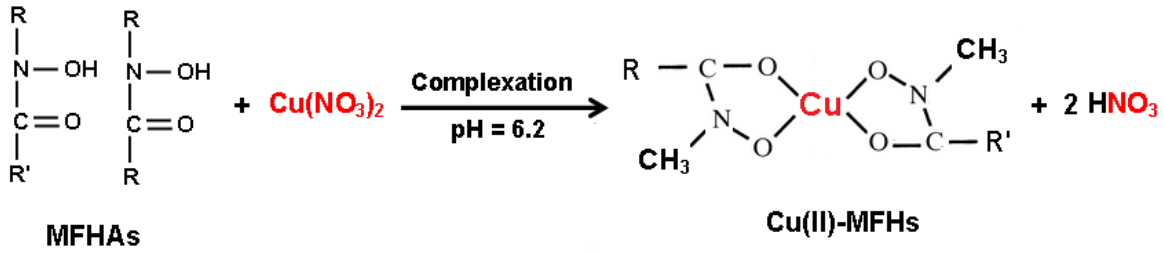
in structure and coordination number of complexes. However the structure and coordination number of iron(III) complexes of MFHAs based on palm kernel oil previously were described in our other papers [10].Scheme 2 shows the reaction equation of iron (III) complexation of methyl fatty hydroxamic acids based on Jatropha seed oil.



Scheme 2. The reaction equation of iron (III) complexation of Methyl fatty hydroxamic acids. R, R′, R″ = alkyl branches of different acyl groups obtained from Jatrophacurcas seed oil.

2.4 Copper (II) complexation of MFHAs based on Jatropha seed oil

For preparing of copper(II) methyl fatty hydroxamates (Cu(II)–MFHs), the copper(II) complexes of MFHAs, 50 ml of 0.008 M MFHAs hexane solution was mixed with 50 ml of 1.6 mMcopper(II) nitrate aqueous solution. The mixture was stirred at 500 rpm at 25 ± 1 °C for 10 minutes buffered by sodium acetate at desired pH 6.2 [11]. Finally the Cu(II)–MFHs complexes were obtained through separation of organic phase from aqueous phase and followed by removal hexane from the organic phase using rotary evaporation. The structure and coordination number of Cu(II) complexes of MFHAs based on Jatropha seed oil are the same of them based on palm kernel oil because they differ just in long of alkyl branches (R) and this difference is unaffected in structure and coordination number of complexes. However the structure and coordination number of Cu(II) complexes of MFHAs based on palm kernel oil previously were described in our other papers [11]. Scheme 3 shows the reaction equation of copper (II) complexation of methyl fatty hydroxamic acids based on Jatropha seed oil.



Scheme 3. The reaction equation of copper(II) complexation of Methyl fatty hydroxamic acids. R, R′ = alkyl branches of different acyl groups obtained from Jatropha seed oil

2.5 Evaluation of antimicrobial properties

In the vitro the antimicrobial activities of MFHAs, Fe(III)-MFHs and Cu(II)-MFHs were evaluated by the disc diffusion method using Mueller-Hinton agar (MHA) with determination of inhibition zones in millimeter (mm). The *E. coli*, *P. vulgaris* and*P. mirabilis* as gram-negative bacteria; MRSA and *S. epidermidis* as gram-positive bacteria; *C. parapsilosis* and *C. Albicans* as fungi were used for the antimicrobial assay. In the disc diffusion method, the sterile paper discs (6 mm in diameter) were impregnated with 20, 30, 40 and 50 µl of each MFHAs, Fe(III)-MFHs and Cu(II)-MFHs solutions (concentration, 20% in hexane) separately and left to dry at 37°C for 24 hours under sterile condition. The final amounts for each of the MFHAs, Fe(III)-MFHs in the discs were 4, 6, 8 and 10 µg/disc. The surface of MHA in the each of the test plates were completely inoculated by the equal amount of each one of the bacteria or fungi using a sterile swab, which were steeped in the prepared suspension of the microbes. Finally the impregnated paper discs were put on the surface of agar plates and were incubated at 37°C for 48 hrs. After the end of incubation period, the diameter of the growth inhibition zones was measured. The bacteria and fungi suspensions were prepared by making a saline suspension of isolated colonies selected from the 18 to 24 hours of agar plate. The microbe's suspension was adjusted to match the tube of 0.5 McFarland turbidity standard using the spectrophotometer of 600 nm, which equals to 1.5×10^8 colony-forming units (CFU)/ml. Jatropha seed oil, copper (II) and iron (III) solution and hexane were used as the control negative standards while antibiotic drugs ampicillin, chloramphenicol, gentamicin streptomycin, tetracycline and nystatin were used as the control positive standards in order to control the sensitivity of the antimicrobial activity. The copper (II) and iron(III) solution for negative control was prepared similarly to complexation condition of MFHAs. For these purposes, for the preparing of copper(II) solution, the amount of 100 ml hexane (without MFHAs) was mixed with 100 ml of copper nitrate aqueous solution buffered by sodium acetate at pH 6.2 ($[Cu^{2+}]$ ~ 500 mg/lit). The mixture was stirred at 300 rpm and 30°C for 20 minutes then organic phase was separated from aqueous and was used as negative control. For preparation of iron (III) solution, the amount of 100 ml hexane (without MFHAs) was mixed with 100 ml of iron (III) chloride aqueous solution buffered by sodium acetate at pH 1.9 ($[Fe^{3+}]$ ~ 500 mg/lit). The mixture was stirred at 300 rpm and 30°C for 20 minutes then organic phase was separated from aqueous phase and used as negative control. All above mention tests were done in three replicates.

2.6 Characterization

Visual observation of color change was used for qualitative identification of hydroxamic acids by observing the color of their metal complexes. For this purpose solution of MFHAs in hexane was mixed with 0.01 M of copper (II), iron (III) and vanadium (V) solutions and agitated for about 5 min [14, 15]. The amounts of MFHAs, Fe (III)-MFHs and Cu(II)-MFHs were estimated based on nitrogen content, determined by elemental analyzer (model 932 LECO, USA). Perkin Elmer GX Spectrophotometer (USA) Infrared Fourier Transform Spectrometer FTIR was used for recording FTIR spectra.

3. Results and discussion

3.1 Antimicrobial activity

The inhibition zone amounts of the MFHAs, Fe(III)-MFHs and Cu(II)-MFHs against the *E. coli*, *P. vulgaris* and*P. mirabilis* as gram-negative bacteria; MRSA and *S. epidermidis* as gram-positive bacteria; *C. parapsilosis* and *C. albicans* as fungi were determined by disc diffusion methods. The results of the inhibition zones are presented in Table 1 and 2.The significance of antimicrobial activities of MFHAs and their metal complexes were compared to that of commercial antibiotics with similar dosage (10 µg) using t-test. The results show that the antimicrobial activity of the MFHAs, Fe(III)-MFHs and Cu(II)-MFHs against all the tested microbes increase while the amount of test products increase. Also the evaluations indicated that antimicrobial activity of MFHAs increases with metal complexation and the antimicrobial activity

of Cu(II) complex is higher than that of Fe(III). This phenomenon occurred due to the synergistic combination of coordinated copper and iron ions with MFHAs. This issue was reported by Rao et al on the antifungal activity of a group of hydroxamic acids and their Cu (II) chelates against the *Alternariaalternata*, *Fusariumoxysporum* and *Aspergillusflavus*[19]. Also recently Haron et al reported similar behavior for antifungal activity evaluation of phenyl fatty hydroxamic acids and their copper complexes against the *Aspergillus fumigatus, C. parapsilosis* and *C. albicans*[39]. The results of Table 1 and 2 also show that the lowest antimicrobial activity was occurred on the*P. vulgaris* meanwhile the highest antimicrobial activity was occurred on the *S. epidermidis*.Furthermore the results show that the antimicrobial activity of the MFHAs, Fe(III)-MFHs and Cu(II)-MFHs against gram positive bacteria and both yeasts are higher than gram negative bacteria. This phenomenon may be occurred due to the difference at layer cell of the tested microbes groups. The details significant of data evaluation comparing the antimicrobial activities between MFHAs, Fe(III)-MFHs and Cu(II)-MFHs are as follows:

1 - The antimicrobial activity of Cu (II)-MFHs are significantly higher than Fe(III)-MFHs against the *E. coli* ($P < 0.002$), MRSA ($P < 0.001$), *S. epidermidis* ($P < 0.02$) and *P. mirabilis* ($P < 0.002$) and also the antimicrobial activity of Cu (II)-MFHs are higher than Fe(III)-MFHs against the *C. parapsilosis* ($P < 0.2$) *P. vulgaris* ($P < 0.2$) and *C. albicans* ($P < 0.2$).

2 - The antimicrobial activity of Cu(II)-MFHs are significantly higher than MFHAs against the *E. coli* ($P < 0.001$), MRSA ($P < 0.001$), *S. epidermidis* ($P < 0.001$), *P. vulgaris* ($P < 0.001$), *P. mirabilis* ($P < 0.001$), *C. parapsilosis* ($P < 0.001$) and *C. albicans* ($P < 0.001$).

3 - The antimicrobial activity of Fe (III) -MFHs are significantly higher than MFHAs against the *E. coli* ($P < 0.002$), MRSA ($P < 0.01$), *S. epidermidis* ($P < 0.001$), *P. vulgaris* ($P < 0.02$), *C. parapsilosis* ($P < 0.001$) and *C. albicans* ($P < 0.001$) and also the antimicrobial activity of Fe (III) -MFHs is higher than MFHAs against. *P. mirabilis* ($P < 0.2$).

The results in Table 1 and 2 also show that the antimicrobial activities of the MFHAs, Fe(III)-MFHs and Cu(II)-MFHs against the tested microbes are higher than activities of commercial antibiotics such as ampicillin, chloramphenicol,gentamicin, streptomycin, nystatin and tetracycline except gentamicin and also antimicrobial activities of Fe(III)-MFHs and MFHAs are higher than activities some of the tested commercial antibiotic. The details significant of data evaluation comparing the antimicrobial activities of MFHAs, Fe(III)-MFHs and Cu(II)-MFHs with commercial antibiotics are as follows:

1 - The antimicrobial activity of Cu(II)-MFHs are significantly higher thanampicillin against the *E. coli* ($P < 0.01$), MRSA ($P < 0.001$), *S. epidermidis* ($P < 0.001$), *P. vulgaris* ($P < 0.002$) and *P. mirabilis* ($P < 0.001$).

2 - The antimicrobial activity of Cu(II)-MFHs are significantly higher thanchloramphenicol against the MRSA ($P < 0.002$) and also the antimicrobial activity of Cu(II)-MFHs are higher thanchloramphenicol against *E. coli* ($P < 0.2$)

3 - The antimicrobial activity of Cu(II)-MFHs are significantly higher thanstreptomycin against the *E. coli* ($P < 0.002$) and MRSA ($P < 0.001$).

4 - The antimicrobial activity of Cu(II)-MFHs are significantly higher thantetracycline against the *P. mirabilis* ($P < 0.001$).

5 - The antimicrobial activity of Cu(II)-MFHs are significantly higher thannystatin against *C. parapsilosis* ($P < 0.001$) and *C. albicans* ($P < 0.001$).

6 - The antimicrobial activity of Fe(III)-MFHs are significantly higher thanampicillin against the MRSA ($P < 0.02$), *S. epidermidis* ($P < 0.001$), *P. vulgaris* ($P < 0.01$) and *P. mirabilis* ($P < 0.001$).

7 - The antimicrobial activity of Fe(III)-MFHs are significantly higher thanstreptomycin against the MRSA ($P < 0.005$) and also the antimicrobial activity of Fe(III)-MFHs are significantly higher thanstreptomycin against the *E. coli* ($P < 0.1$).

8 - The antimicrobial activity of Fe(III)-MFHs are significantly higher thantetracycline against the *P. mirabilis* ($P < 0.01$).

9 - The antimicrobial activity of Fe(III)-MFHs are significantly higher thannystatin against *C. parapsilosis* ($P < 0.001$) and *C. albicans* ($P < 0.001$).

10 - The antimicrobial activity of MFHAs are significantly higher thanampicillin against the *S. epidermidis* ($P < 0.05$) and *P. mirabilis* ($P < 0.001$).

11 - The antimicrobial activity of MFHAs are higher thantetracycline against the *P. mirabilis* ($P < 0.2$).
12 - The antimicrobial activity of MFHAs is significantly higher thannystatin against *C. parapsilosis* ($P < 0.001$) and *C. albicans* ($P < 0.002$).

Finally the results showed that (data were not shown) hexane, copper and iron solution, MHA and Jatropha seed oil were not active as antimicrobial against the *E. coli*, MRSA, *S. epidermidis*, *P. vulgaris*, *P. mirabilis*, *C. parapsilosis* and *C. albicans* when they were tested at dosage of 10µg / disc as control negative.

Table 1. Disc diffusion method inhibition zone values (Mean ± SD, mm , n = 3) of E. coli, MRSA, S. epidermidis and P. vulgarisby MFHAs, Fe(III)-MFHs, Cu(II)-MFHs, ampicillin, chloramphenicol,gentamicin and streptomycin.

Antimicrobial Agents	Amount per disc	Inhibition zone, mm (Mean ± SD)			
		E. coli (-)	MRSA (+)	Staphylococcus epidermidis (+)	Proteus vulgaris (-)
MFHAs	4 µg	7.9 ± 0.9	7.2 ± 0.4	11.6 ± 0.6	6.2 ± 0.3
	6 µg	9.1 ± 0.9	8.5 ± 0.8	15.5 ± 0.7	6.5 ± 0.4
	8 µg	10.4 ± 0.8	10.1 ± 0.8	17.1 ± 0.5	9.8 ± 0.5
	10 µg	11.2 ± 0.6	13.6 ± 0.7	18.0 ± 0.4	12.5 ± 0.7
Fe(III)-MFHs	4 µg	10.2 ± 0.5	9.5 ± 0.5	13.1 ± 0.7	7.4 ± 0.4
	6 µg	11.5 ± 0.6	11.6 ± 0.4	16.3 ± 0.5	9.9 ± 0.3
	8 µg	13.3 ± 0.6	13.9 ± 0.7	18.3 ± 0.6	12.5 ± 0.4
	10 µg	16.6 ± 0.5	17.5 ± 0.8	22.8 ± 0.8	16.3 ± 0.5
Cu(II)-MFHs	4 µg	12.6 ± 0.7	16.5 ± 0.6	17.1 ± 0.6	8.0 ± 0.4
	6 µg	14.2 ± 0.4	17.4 ± 0.3	19.1 ± 0.7	10.7 ± 0.3
	8 µg	16.1 ± 0.8	18.9 ± 0.5	22.4 ± 0.8	14.9 ± 0.5
	10 µg	19.2 ± 0.6	20.5 ± 0.5	25.4 ± 0.7	17.1 ± 0.5
Control positive					
Ampicillin	10µg	16.9 ± 0.6	15.2 ± 0.4	16.9 ± 0.5	13.7 ± 0.5
Chloramphenicol	10µg	18.6 ± 0.5	17.1 ± 0.6	NA	NA
Gentamicin	10µg	22.7 ± 0.7	NA	NA	NA
Streptomycin	10µg	15.6 ± 0.5	13.4 ± 0.7	NA	NA

NA = Not applicable

Table 2. Disc diffusion method inhibition zone values (Mean ± SD, mm , n = 3) of P. mirabilis, C. parapsilosis and C. albicans by MFHAs, Fe(III)-MFHs, Cu(II)-MFHs, ampicillin, tetracycline and nystatin.

Antimicrobial Agents	Amount per disc	Inhibition zone, mm (Mean ± SD)		
		Proteus mirabilis (-)	Candida parapsilosis	Candida Albicans
MFHAs	4 µg	6.5 ± 0.2	8.5 ± 0.5	8.9 ± 0.7
	6 µg	7.1 ± 0.5	10.3 ± 0.6	10.9 ± 0.5
	8 µg	10.5 ± 0.7	11.9 ± 0.7	12.3 ± 0.4
	10 µg	14.5 ± 0.4	15.7 ± 0.5	13.9 ± 0.7

Antimicrobial Agents	Amount per disc	Inhibition zone, mm (Mean ± SD)		
		Proteus mirabilis (-)	Candida parapsilosis	Candida Albicans
Fe(III)-MFHs	4 µg	7.1 ± 0.4	10.2 ± 0.4	11.3 ± 0.5
	6 µg	10.3 ± 0.7	13.9 ± 0.7	14.4 ± 0.4
	8 µg	12.4 ± 0.3	16.6 ± 0.6	18.0 ± 0.8
	10 µg	15.0 ± 0.5	20.7 ± 0.8	22.8 ± 0.7
Cu(II)-MFHs	4 µg	11.3 ± 0.5	12.7 ± 0.4	13.5 ± 0.3
	6 µg	13.7 ± 0.7	14.5 ± 0.6	15.1 ± 0.5
	8 µg	15.4 ± 0.4	17.1 ± 0.8	18.9 ± 0.4
	10 µg	17.9 ± 0.4	21.5 ± 0.5	23.1 ± 0.8
Control positive				
Ampicillin	10µg	11.1 ± 0.4	NA	NA
Tetracycline	10µg	14.3 ± 0.6	NA	NA
Nystatin	10µg	NA	11.4 ± 0.5	9.9 ± 0.6
	20µg	NA	13.5 ± 0.7	12.1 ± 0.7
	80µg	NA	NA	15.3 ± 0.7

NA = Not applicable

3.2 Quantification and characterization of MFHAs, Fe(III)-MFHs and Cu(II)-MFHs

3.2.1Elemental Analysis

The data of elemental analysis by the CHN analyzer showed that the nitrogen content in the synthesized MFHAs, Cu(II)-MFHs and Fe(III)-MFHs from the Jatropha seed oil were 4.35% , 3.88% and 4.05% respectively. Results indicated that there were 3.11 mmol of methyl fatty hydroxamic acid groups, 1.39 mmol of copper methyl fatty hydroxamate groups and 0.96 mmol of iron methyl fatty hydroxamate groups in one gram of each of the products.

3.2.2 Qualitative identification of hydroxamic acids

One of the important physical properties of hydroxamic acids is their ability to form colored and very stable chelates with many metal ions. This property has been used for qualitative identification of the product. For this purpose a solution of MFHAs in hexane was mixed with 0.01 M of copper (II), iron (III) and vanadium (V) aqueous solutions and mixed for about 5 minutes. The colored complexes of the MFHAs were formed in the organic phase (hexane) were green, brownish red and purple for copper (II), iron (III) and vanadium (V) complexes of MFHAs, respectively.

3.2.3 Fourier Transform Infrared Spectroscopy (FT-IR)

The FTIR spectra main data of MFHAs, Fe(III)-MFHs and Cu(II)-MFHs based on Jatropha seed oil are shown in Table 3. In the MFHAs FT-IR spectra, the broad peaks that spread between 2700 and 3200 cm^{-1} correspond to O–H stretching, the weak peak at 3007 cm^{-1} belong to =C–H stretching, the peaks at 2921 and 2855 cm^{-1} refer to –C–H stretching of the long chain of alkyl and the peak at 1710 cm^{-1} belongs to C=O stretching that in accordance with the carbonyl peak of phenyl fatty hydroxamic acids based on canola oil which appeared at 1708 cm^{-1} that reported by Jahangirian et al.[15]. In addition the peaks at 1546 and 1455 cm^{-1} refer to C=C stretching, the peak at 1271 cm^{-1} corresponds to –C–N stretching and finally the peak at 718 cm^{-1} belongs to =C–H out of plan (OOP) bending.

Almost similar to MFHAs, the FTIR spectra of Fe(III)-MFHs contain peak at 3007 refers to =C—H stretching, the peaks at 2924 and 2855 cm^{-1} correspond to —C—H stretchings for the long chain alkyl, the peak at 1709 cm^{-1} belongs to C=O stretching, the peaks at 1542 and 1455 cm^{-1} correspond to C=C stretchings and the peak at 720 cm^{-1} belongs to =C–H out of plan (OOP) bending. Also the data Table 3 shows that the peak of C-N band has shifted from 1271 to 1282 cm^{-1}due to complexation. In addition the Fe(III)-MFHs spectra also contain new peak at 1180 cm^{-1} corresponds to —C—O stretching that was appeared due to connecting of Fe(III) to oxygen of C=O and this is supported by the appearance of other two new peaks at 605 cm^{-1} and 312 cm^{-1} which could be assigned to symmetrical and asymmetrical stretchings of oxygen iron metal bond, respectively. This issue confirmed by Brown et al for some of metalmonohydroxamic acid complexes such as copper, iron and nickel[40]. The results in Table 3 also show that FTIR spectra of Cu(II)-MFHs are similar to that of Fe(III)-MFHs. The details of FT-IR, HNMR and CNMR spectra and elemental analysis results of ligands (MFHAs based on Jatropha) were described in other our papers that recently was published [41].

Table 3.FTIR Spectrum analysis of MFHAs, Fe(III)-MFHs and Cu(II)-MFHs

Compound	Wavelength (cm^{-1})	Chemical bond assignment
MFHAs	2700 to 3200 3007 2921, 2855 1710 1546, 1455 1271 718	O—H stretching =C—H stretching —C—H stretching for long chain alkyl C=O stretching for hydroxamic acid C=C stretching —C—N stretching =C–H out of plan (OOP) bending
Fe(III)-MFHs	3007 2924, 2855 1709 1542, 1455 1283 1180 720 605 312	=C—H stretching —C—H stretching for long chain alkyl C=O stretching for hydroxamic acid C=C stretching —C—N stretching —C—O stretching =C–H out of plan (OOP) bending O—Fe symmetric stretching O—Fe asymmetric stretching
Cu(II)-MFHs	3006 2923, 2855 1709 1542, 1454 1282 1180 721 606 312	=C—H stretching —C—H stretching for long chain alkyl C=O stretching for hydroxamic acid C=C stretching —C—N stretching —C—O stretching =C–H out of plan (OOP) bending O—Cu symmetric stretching O—Cu asymmetric stretching

4. Conclusions

Elemental analysis and FTIR spectroscopy showed that MFHAs, Fe(III)-MFHs and Cu(II)-MFHs based on *Jatrophacurcas* seed oil were successfully prepared.Some important advantages such as simple preparation of substrates from *Jatrophacurcas* seed oil which is cheap and easily available, moderate conditions of reaction with enzyme in the synthesis which is environmental friendly according to green chemistry principles are the highlighted gained aspects of this investigation.

This investigation showed that the MFHAs, Fe(III)-MFHs and Cu(II)-MFHs are strong antimicrobial agents and antimicrobial activities of the compounds against the *E. coli*, MRSA, *S. epidermidis*, *P. vulgaris*, *P. mirabilis*, *C. parapsilosis* and *C. albicans* increase while the used amounts of products increase. Also antimicrobial activity of MFHAs increase with metal complexation and this activity is higher for complexation by Cu(II) compared to that of Fe(III). Furthermore, comparing the antimicrobial activities of MFHAs, Fe(III)-MFHs and Cu(II)-MFHs with commercial antibiotics such as ampicillin, chloramphenicol,gentamicin, streptomycin, nystatin and tetracycline against the test microbes showed that the antimicrobial activity of Cu(II)-MFHs is higher than all tested commercial antibiotic except gentamicin. Also the antimicrobial activities of Fe(III)-MFHs and MFHAs were higher than some of tested commercial antibiotics. Finally, this investigation is the first report which describes antimicrobial properties of MFHAs, Fe(III)-MFHs and Cu(II)-MFHs based on *Jatrophacurcas* seed oil.

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