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Studying The Inhibition Activity Of Mentha Pulegiuml Extract Iron Nanoarticles For Human Pathogenic Bacteria.

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ABSTRACT

Biosynthesis of green iron nano particles by usingMentha pulegiumL aqueousextractWithiron sulfate. Characterization of green iron nano particles acting by using UV-Visible spectroscopy which showed higher peak at 425 nm, scattering (DLS) particle size analyser, ranges approximately between 1 nm to 35 nm with mean particle size of 17.5 nm. The X-ray power diffraction (XRD) analysis revealed the crystallographic structure of magnetic particles. The result of anti-inflammatory showed significantly inhibited albumin denaturation at concentration 300 µg/ml in compare with standard drug(Proven), also the anti bacterial activity of green iron nano particles showed significant results in inhibition pathogenic bacteria, and these results encouraged as to used green iron nano particles in pharmaceutical and biomedical industries.

Keywords: Green Iron Oxidenanoparticles; ; Antibacterial Activity, DLS, XRD.

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INTRODUCTION

Nanotechnolgy science supply environment with friendly materialsthat can be used in agricultural, medical and environmental sciences as well (1). The Physical properties of nanoparticles qualities of particulate in the size and composition and specifications (2).Many researcher found a high toxicity of iron nano particles against pathogenic micro organism but till now they didn't know the mechanism of killing those micro organism(3). There were many different methods for preparation iron nano particles such as chemical and physical methods and green iron nano particle by using different part of plants(4).The aim of our study was to biosynthesis of green nano particles by using Mentha pulegiumL Extract with ferrous sulfate in co-precipitation method and characterize it by X-ray diffraction (XRD), absorption spectrophotometer (UV- VIS), FTIR, X-ray diffraction (XRD), and studying the ability of green iron nano particle in inhibition human bacteria.

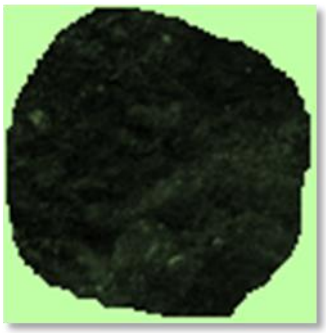
MATERIALS AND METHODS

Preparation of Mentha pulegiumL Extract

Preparing extract fromMentha pulegiumL extractleaves by sing 25 g powder in 250 ml deionized water, heated at 80°C to get extract, filtered solution kept at 4°C(5).

Synthesis of green iron nanoparticles

Synthesis nanoparticles by adding 1M Ferric sulphate to Mentha pulegiumL extractleaves in 1:1 in sterilized beaker, heating at 80°C black color, centrifuge the extract and discard the supernatant were discarded and the pellet was washed with deionised water and centrifuged again to remove any impurities(6).



Characterization Techniques

UV-VIS Spectra Analysis (UV-VIS)

MonitoringUV-VIS spectral analysis had done by using UV-VIS spectrophotometerat awave length 190-1100nm.

X-Ray Diffraction (XRD)

The green Iron nano particles crystallographic analysis crystallographic analysis had done by using Cu K-α (λ = 1.542000 Ao) with an accelerating voltage of 40 KV. Data were collected with a counting rate of 1°/min

Denaturation inhibition of albumin

1% human albumin incubated at 37 C^{o} for 20 minute, heated at 51 C^{o} cooling. Measured the turbidity at 660nm by UV Visible Spectrophotometer. ThePercentageof denaturation inhibition calculated by this equation = (Abs Control –Abs Sample) X 100/ Abs control

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Antimicrobial Activity

Evaluation the ability of green nano particles in inhibition pathogenic bacteria growth on seven species of bacteria such asStaphylococcus epidermidis, Staphylococcus aureus, Vibrio cholera, Bacillus spp., Streptococcus aureus, Shigella flexneri, Staphylococcus epidermidis. Method carried on depending (7).

Wells of 6 mm diameter were punched over the agar plates using (cork borer) which swabbed with pathogenic bacteria. 100 µl (50 mg/ml) of nanoparticle powder in sterile distilled water were poured into the wells. The plates were incubated at 37°C for 24 h. The inhibition zone appeared around each well after the incubation , refers to antibacterial activity of the green iron nanoparticle . Amoxicillin (50 mg/ml) was taken as standard

RESULTS AND DISCUSSION

The green iron nanoparticles was characterized by UV-Visible spectroscopic Fig (2) the peak appeared at wave length 425 nm, which is due to charge transfer spectra

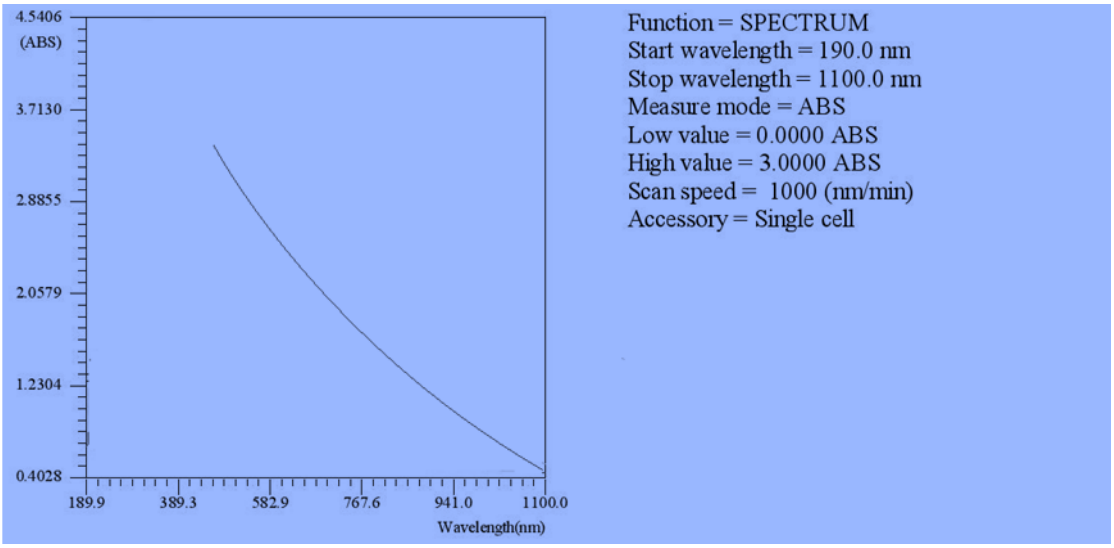

Fig 2: The UV-VIS spectrum of green iron naoparticles.

Fig (3) showed the results of green iron nanoparticles XRD analysis, the diffraction angles at: 45.2° and 78° which refers to the crystal of nanoparticle diffraction surfaces, The X-ray power diffraction (XRD) results confirmed that nano-particles product was a magnetite (Fe3O4) (7).

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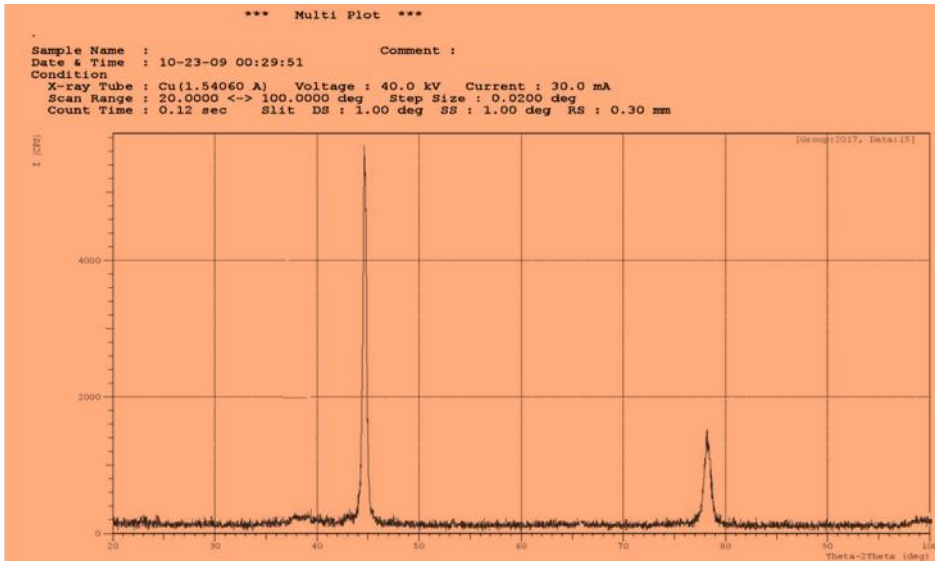

Fig (3): XRD of gree iron nanoparticles.

Fig (4) showed size of greeniron nanoparticles . The particle size distribution of the iron nanoparticles determined by laser diffraction method with a multiple scattering technique revealed that the particle size distribution of iron nanoparticles ranges approximately from 1 nm to 35nm with mean particle size of 17.5nm, the distribution of nanoparticle was a uniform with a tight distribution range .

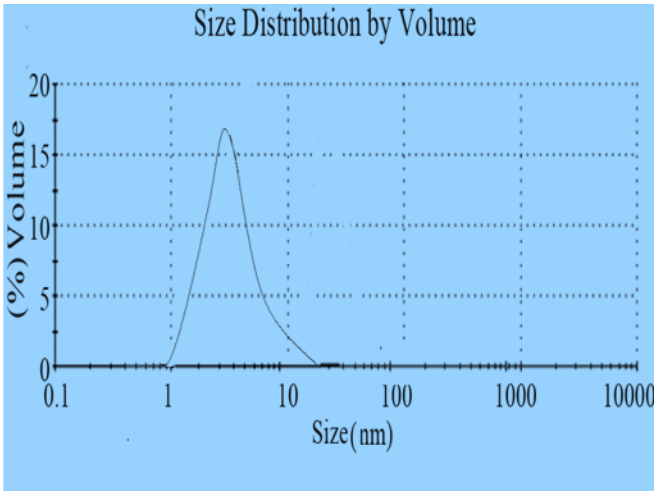

Fig 4: Size of green iron nanoparticles

Table (1) showed albumin denaturation inhibition by green iron nano particles. The result showed that higher value of albumin inhibition was 85.7% observed at 300 µg/mlof nano particles in compared with standard anti-inflammatory drug (proven).

Each value represents the mean ± SD. The results gave asignignificant values in compare with the control $p<0.01$. Proteins denaturation refers to cause of inflammation (8). We found that nano particles inhibited protein denaturation, so for this mechanism nanopaticle had anti-inflammatory activity (9).

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Table (1): Showed the inhibition of protein denaturation.

Sample	Concentration (µg/ml)	Absorbance (nm)	% inhibition of denaturation
CONTROL	-	0.62±0.01	-
1	50	0.53±0.03	20
2	100	0.45±0.02	39
3	200	0. 30±0,06	54
4	300	0.25±0.03	78
PROVEN	300	0.28±0.02	85

Table (2) showed the result of pathogenic bacteria inhibition by iron nano particles.We found that green nano particles have anti bacterial activity against pathogenic bacteria Vibrio cholera,gave the highest inhibition zone in compare with other bacteria and standard antibiotic . The antibacterial activities of the iron oxide nanopar-ticle evaluated against ten pathogenic bacteria (six Gram positive and four Gram negative) are presented in (10,11). The result of antibacterial activity of nanoparticle showed moderate antimicrobial activity against eight pathogenic strains (six gram positive and two gram negative) with zone of inhibition ranging from 9 mm to 22 mm (**Table 2**).

Table 2: Antibacterial activity of iron oxide nanoparticle and standard antibiotics.

Bacterial Species	Green iron nanoparticles(50mg/ml)	Standard antibiotic (50mg/ml)
Streptococcus aureus	± 0.30 **17.5**	19.2 ± 0.35
Vibrio cholerae	22 ± 0.70	14 ± 0.40
Pseudomonas Aeruginosa	18 ± 0.03	22 ± 0.21
Staphylococcus Epidermidis	± 0.2414.5	± 0.1418.4
Streptococcus aureus	±0.3321.2	±0.615
Bacillus subtilis	±0.2717.6	±0.1318.4
Escherichia coli	±0.2420.16	±0.2217.5

May be the inhibition activity of green iron nanoparticles due to oxidative reaction by releasing free radicals such as –OH, H2O2, O2, and may be Iron nanoparticles had iron oxide could be source of ROS which responsible about bacterial inhibition (12,13,14),while other researcher explane that the nanoparticle which had small size from 1-50 nm can introduced into bacterial membrane and react with oxygen intra cellular causing oxidative stress which damaged the bacteria(14). We dependent in our study on other article such as(15,16,17) to confirmed concentration of iron nano particles. It is also important to note that ironnano-particles do not negatively influence all cells and thus it can be said that with an appropriate external magnetic field, FeO nanoparticles may be directed to inhibition bacterial growth as needed throughout the bod

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