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THE EFFECT OF EXPOSING DRIED OLIVE (*OLEA EUROPAEA*) LEAVES EXTRACT TO MICROWAVES IN THE SYNTHESIS OF IRON OXIDE NANOPARTICLES AND ITS EFFECT ON THE BIOLOGICAL ACTIVITY OF DIFFERENT EXTRACTS OF PATHOGENIC BACTERIA

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ABSTRACT : In this study, *Olea europaea* leaves extract was found to be capable in green synthesis of iron oxide nanoparticles (Fe_3O_4-NPs) as capping agent and their characteristics were studied byusing UV-visible spectrophotometer, SEM, TEM and AFM. The reaction was simple and easy to handle and was confirmed by using ultraviolet-visible spectroscopy (UV-Spectral analysis) to study and understand the specific properties such as optical, structural, morphological and particle size. The effect of the phytochemicals present in *Olea europaea* including saponins, phytosterols, phenolic compounds, unsaturated sterols, terpenes, sterol and steroid has a main roleas reducing agent that assists to the eco-friendly synthesis of Fe_3O_4-NPs with enhanced antioxidant property. Fourier-transform infrared spectroscopy (FTIR) indicated various biological compounds responsible for capping and stabilizing iron nanoparticles in suspension, while the presence of iron was presented by scanning electron microscopy (SEM) equipped with energy-dispersive X-ray. Jc-AgNps were confirmed to be uniform in shape, size and attitude through dynamic light scattering, transmission electron microscopy (TEM), X-ray diffraction, SEM and atomic force microscopy (AFM) analysis. It was found that the size of the nanoparticles varied within the range 10 nm to 55 nm. The antimicrobial property of synthesised Fe_3O_4-NPs was evaluated against Gram negative and Gram positive. Bacteria's pattern of sensitivity was observed in the order as *Staphylococcus aureus*> *Pseudomonas aeruginosa* > *Streptococcus aureus* > *Escherichia coli* > *Vibrio cholera* > *Bacillus subtilis*. Also, it was found *Olea europaea* leaves extract showed significantly (*P*>0.05) a high radical scavenging activity with 3 mM (IC_{50} = 12 µgml^{-1}) that exhibited more than 95% scavenging. Therefore, it can be recommended that iron oxide nanoparticles related to their fine nanoparticles size 32.5 nm and their uniform distribution with narrow range is valuable using in many scopes of science. *Olea europaea* biomass can serve as a highly economical, renewable and rich source of carbon. Therefore, naturally stabilized green synthesis of iron oxide nanoparticles with *Olea europaea* property can be usedin various biological applications.

Key words : *Olea europaea* leaves-Iron nano particle, antimicrobial activity, UV-Spectral analysis, Fourier Transform Infra-red Spectrophotometer.

INTRODUCTION

Nanoparticle is a microscopic particle that has at least one dimension less than 100 nanometers in size. Synthesized iron nanoparticles could have many biological applications in the areas of medicine, chemistry, environment, energy, agriculture information and communication, heavy industry,and consumer goods (Thirumurugan *et al*, 2011). Nanoparticles show unusual optical (Chandrase and Kamat, 2000), photoelectro-chemical (Ahmad *et al*, 2003) and electronic properties (Chandrase and Kamat, 2000). Greener chemistry approaches include assembly of nanoparticles from bacteria, fungi and plants (Roh *et al*, 2001). Many preliminary studies have used different plants extract in the manufacture of silver nanoparticle such as *Zea mays* (Bhattacharya and Rajinder, 2005), *Azadirachta indica* (Neem) used by Bansal *et al* (2004), *Medicago sativa* (Alfa alfa) used by Gardea-Torresdey *et al* (2003), Chandrase and Kamat (2000), *Aloe vera* used by Amkamwar *et al* (2005), *Emblica officinalis* (Amla) used by Shen *et al* (2007), *Capsicum annuum* used by Shankar *et al* (2003) and *Geranium* sp. used by Shankar *et al* (2004). The superparamagnetic inventions of iron nanoparticles with physical and chemical properties, their high thermal conductivity, their unique mechanical properties, as well as their various surface properties, gave it a wide range of possibilities, including biomedicine applications, such as tissue therapy and repair of cellular membranes (MRI) and hyperthermia (Sathyavathi *et al*, 2010). Green nanotechnology has been very popular in

recent years because it does not produce any by-products or toxic substances that are thrown into the environment during synthesis that were proven by many investigators. "One of suchstudy was metal nanoparticles synthesis using inactivated plant tissue by Klaus *et al* (1999) and other one used parts of living plants by Alebel Abebe (2010) is a modern alternative for nanoparticles synthesis. Also, it was proved that green synthesis of nanoparticles eco-friendliness, non-toxic and safe reagents by (Pathania, and Siddiqui, 2009)". The possibility of iron nanoparticles synthesis as antibacterial activities is due to its small size, the structure of the cell wall, the modification of the surface (either coating or blocking agent), concentration of the extract and the method of purification as presented by Zhang *et al* (2015), Kasivelu *et al* (2008). It was synthesized many different nanoparticles from different minerals such as (gold, silver, Iron, etc.) with many different plants and with different concentrations. To date, there are little reviews of Iron nanoparticles using an aqueous leaf extract of olive leaf *Olea europaea*. In this study, *Olea europaea* extract with different concentrations was used for synthesis of iron nanoparticles as antibacterial substances. As the reducing agent and as biopolymer stabilizer is a non-toxic, low cost and easily available material in this study. "Microwave assisted synthesis using 'green' chemicals is getting more attention in recent times because of the eco-friendly nature, short reaction time, low energy utilization and improved product yield (Nora *et al*, 2005)". So that, it was useful work to fined green-synthesized nanoparticles which examined precisely using sophisticated techniques such ultraviolet-visible spectroscopy, transmission electron microscopy (TEM), atomic force microscopic analysis (AFM) and Fourier transform infrared (FTIR) spectroscopy to determine their particle size, functional group, optical, structure, morphological and shape, and then to see their effects on bacterial activities.

MATERIALS AND METHODS

Olea europaea leaves were collected from Ministry of Science and Technology gardens, Baghdad, Iraq. The leaves were washed with running tap water, air dried under shade, then ground to get the fine powder by lab. Grinder. *"Olea europaea* extract was prepared by mixing 10 g of dried powder with 100 mL deionized water in 500 mL Erlenmeyer flask and boiled for 10 min, for the reduction of Iron ions. Then the extract filtered, took 10 mL of leaf extract and mixed with 90 mL of 1 mM Fe_2So_4 and stirred at room temperature for one minute. The stirring bar was then removed and the solution was placed into the variable frequency microwave oven chamber to react for 1 min at 160°C. The center frequency of the microwave, the bandwidth and the sweeping time were 6.425 GHz, 1.15 GHz and 0.1 sec., respectively. The sample temperature should always be regulated by immersing the thermocouple of the device in a regulated solution. The ramping rate of the temperature in this study was set at 2°C/sec. and the color changing from pale green to black color was monitored precisely. Then, the obtainedblackish brown colloidal suspensionwas centrifuged at 10,000 rpm for about 10 minutes. Then nanoparticle were washed repeatedly withdeionized water to eliminate the uncoordinated biomolecules from the extract, thereafter washed with ethanol and dried at 40°C under vacuum. The yellow-colored NPs obtained were stored at room temperature until analysis. The obtained iron nanoparticles characteristic were examined and recorded with UV/ vis spectrophotometer and continuous scanning from 300nm to 600nm. Then carefully weighed quantity of the synthesized Iron nanoparticle powder was subjected to FTIR analysis, Scanning Electron Microscopic (SEM), Transmission electron microscopy (TEM) and Atomic Force Microscopic analysis (AFM) to study its characteristics were don by the method of (Kanagasubbulakshmi and Kadirvelu, 2015)".

Characterization

"UV-vis spectra were recorded as a function of reaction time on a Perkin Elmer- Lamda 25. After freeze drying of the purified Iron particles, the size and shape were analyzed by scanning electron microscopy (JOEL-Model 6390)".

Phytochemical qualification of the *Olea europaea* extract

Alcoholic and aqueous extracts were prepared for phytochemical screening of *Olea europaea* leaves. The extracts were subjected to phytochemical tests for leaves secondary metabolites tannins, saponins, steroid, alkaloids, flavonoid, unsaturated sterol and terpen using the methodology described by Parekh and Chanda (2008). The qualitative results of these tests were expressed as positive (+) or negative (–).

Microorganisms

Six bacterial strains were selected for the experiments. *Escherichia coli*, *Vibrio cholera* and *Pseudomonas spp.* as Gram negative *Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus aureus*as Gram positive.

Antibacterial activity

The antibacterial activities of synthesis nanoparticle

were carried out by disc diffusion method (Murray *et al*, 1999). Antibacterial activity, *E. coli*, *Pseudomonas* spp, *Bacillus subtilis*, *Streptococcus aureus* and *Staphylococcus aureus* were grown in Mueller Hinton broth (Merck, Germany) at 37^{0}C for 24h. Final cell concentrations were 108 cfu/ml according to the McFarland turbidometry. 100 µl of the inoculum was added to each plate containing Mueller Hinton agar (Merck, Germany). Nutrient agar sterilized in medium plates and solidified. After solidification bacterial cultures were swabbed on these plates. The sterile discs were dipped in Iron nanoparticles solution (10 mg/ml) and placed in the nutrient agar plate and kept for incubation at 37^{0}C for 24 hours and the diameters of inhibitory zones were measured. The assay was carried out three times for each extract as presented by Cruickshank (1968) and Shankar *et al* (2004). Inhibition zones of for control, Iron oxide nanoparticle and Iron sulphate were measured.

Free radical scavenging ability

Free radical scavenging ability of *Olea europaea* was determined by using of stable free radicals: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical (Von Gadow *et al*, 1997).

RESULTS AND DISCUSSION

Results of this study showed the synthesis of Iron Nano particle Fe_3O_4 as in Fig. 1. The presence of effective groups of phytochemicals such as hydroxyl, carboxylic, and amino acids in olive leaves act as effective reducing agents of the metal, as well as capping agents and a strong coatings of the nanoparticles of the iron immediately after mixing. The color then changes from light-yellow to dark blackish green, thus indicating the synthesis of iron nanoparticle (Elavazhagan and Arunachalam, 2011 and Huang *et al*, 2014).

Phytochemical qualification

Olea europaea aqueous extract was investigated for the presence of various phytochemicals by carry out a series of qualitative chemical tests as in Table 1. Presences of these phytochemicals have contributed to its medicinal value as well as physiological activity (Lewis *et al*, 1977). The *Olea europaea* extract was found to have high Phytosterol and saponin content, then Flavonoids, Phenolic compounds, Unsaturated sterols and terpens with medium amount. While alkaloids and tannins were absence.

It was found that iron nanoparticles (Fe_3O_4) synthesized by co-precipitation of ferric sulphate was measured by UV-Visible spectroscopic analysis and their scanning absorbance vs. wave length (λ) has been established to study and understand the specific properties

Table 1 : Phytochemical qualification of *Olea europaea* aqueous extract.

Phytochemicals	Aqueous extract
Alkaloids	_
Flavonoids	+
Phytosterol	++
Saponin	++
Phenolic compounds	+
Tannin	_
Unsaturated sterols and terpens	+
Sterol and steroid	+

Notes: + = presence, _ = absence.

such as optical, structural, morphological and particle size as shown in (Figs. 1and 2). It was found that shape of Iron nanoparticles peak increasingly sharp at 450 nm. Also it was determined that particle size distribution of the iron oxide nanoparticles by laser diffraction method with a multiple scattering technique revealed that the particle size distribution in the region 10-55 nm with mean particle size of 32.5 nm shows the well size reduction by phytochemical of *Olea europaea* extract. And the distribution of oxide nanoparticle is more uniform with a narrow distribution with increasing volume percent as shown in Fig. 3. This finding is comparable to Elavazhagan and Arunachalam (2011).

SEM micrographs have a large depth of field yielding a characteristic three dimensional appearance useful for understanding the surface structure of a sample. Results of this study showed the formationof iron oxide nanoparticles and its morphological dimensions that were analyzed by SEM as in Fig. 4. The results of the SEM study showed that the iron nanoparticles are homogeneous, symmetrical and spherical in shape. The morphological study also showed the average size of the atoms was 32.5 nm for FeNPs. The spherical shaped nanoparticles, formation has encouraged by plant enzymes and iron sulphate compounds present in the sample have an influence in the morphology of the nanoparticles, may be due to the very narrow electron beam, SEM micrographs have a large depth of fieldyielding a characteristic three dimensional appearance useful for understanding the surface structure of a sample". Morphological shape with uniform average grain size of less than 30 nm was also previous reported by Abbas *et al* (2015) and Arumugam *et al* (2015). Also, it can be seen that particles have irregular spherical iron nanoparticles distribution that indicating the chain-like structure and the mean of particle size is about 32.5nm.

The AFM technique has been conducted to study and understand the surface properties of synthesized nanoparticles and the results of micrograph are presented

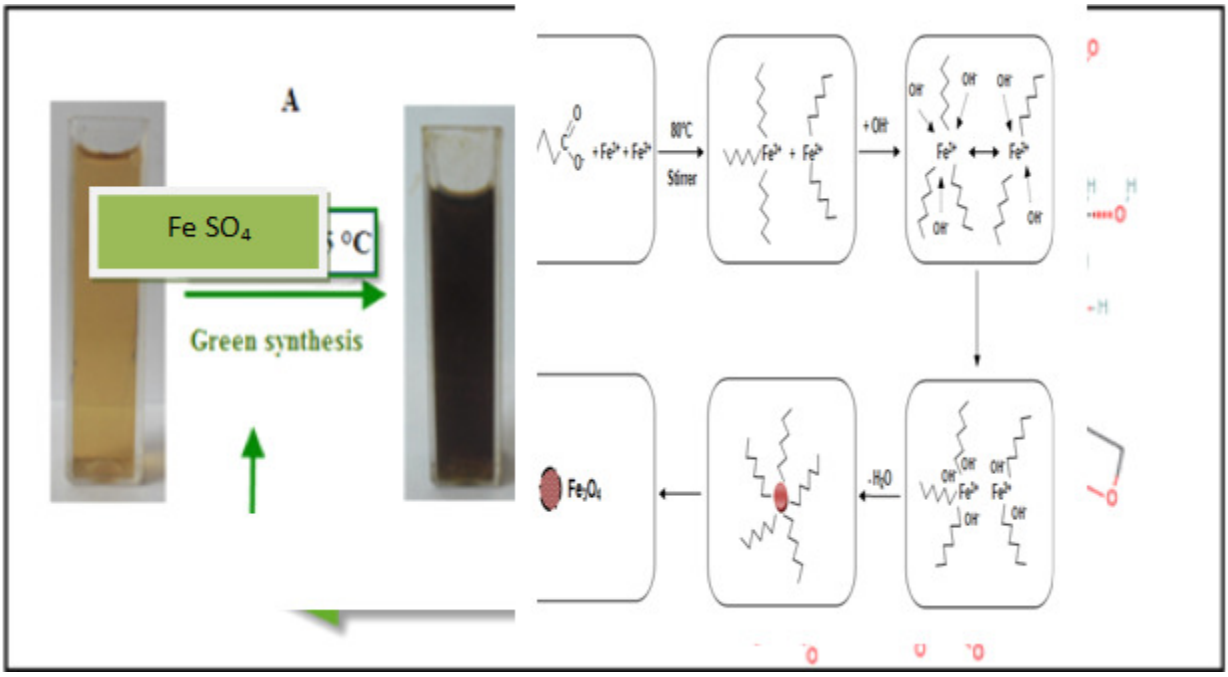

Fig. 1 : Synthesis of Iron nano particle Fe_3O_4.

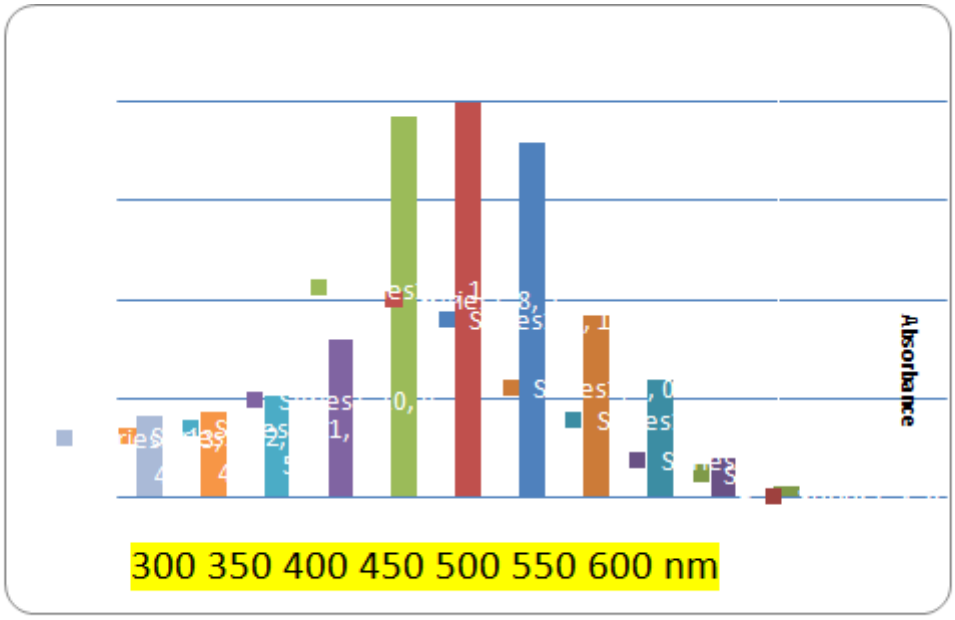

Fig. 2 : The UV-VIS spectrum of Fe_3O_4 nanoparticles.

in Fig. 5, with an excellent luminescence properties greatly enhance its uses in biological applications and other scientific fields. ‘‘FT-IR Spectroscopy was used to resemble the biomolecules present in the iron-nanoparticles after its synthesis,different types of vibrations represent the different functional groups of iron-nanoparticles. It was found that the strong bands for all the iron particles at 530.9 cm^{-1}, 536.36 cm^{-1}, 535.87 cm^{-1} correspond to iron, iron oxide nanoparticles and iron sulphied micro particles.

Fig. 6 showed the result of FTIR spectra of *Olea europaea* leave extract iron nano particle. The spectra that were between 420-610cm^{-1} were referring to Fe_3O_4, and 545 cm^{-1} characteristic band of 545 cm^{-1} refer to metal–oxygen. While the spectra 3100 to 3400 cm^{-1} spectra of FT-IR results indicate the presence of polyphenols and hence showed that Fe_3O_4 can be obtained by eco-friendly method (Venkateswarlu *et al*, 2014).

Antioxidant activity

Fig. 7 showed the antioxidant activity revealed in

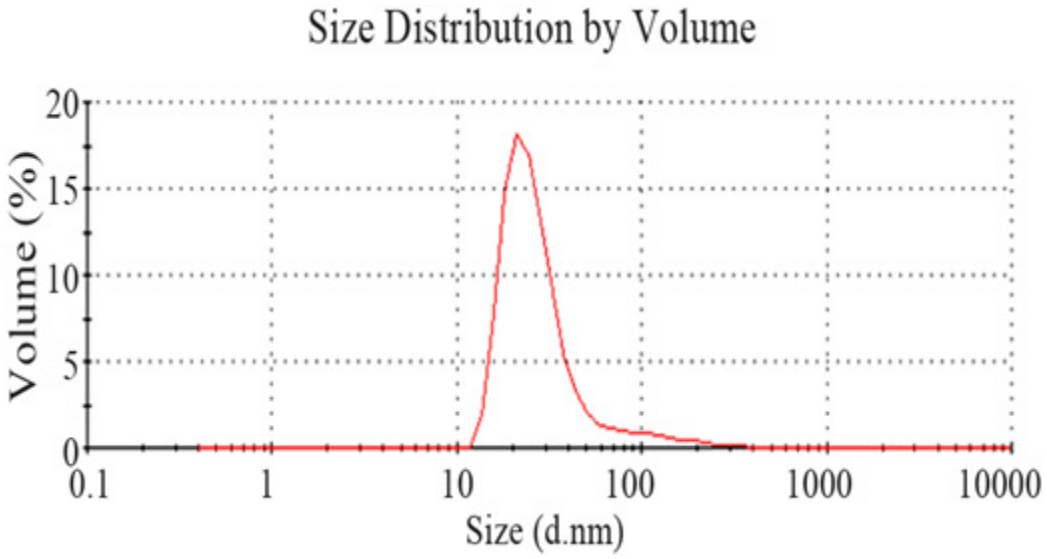

Fig. 3 : Size distribution size of Nano particle.

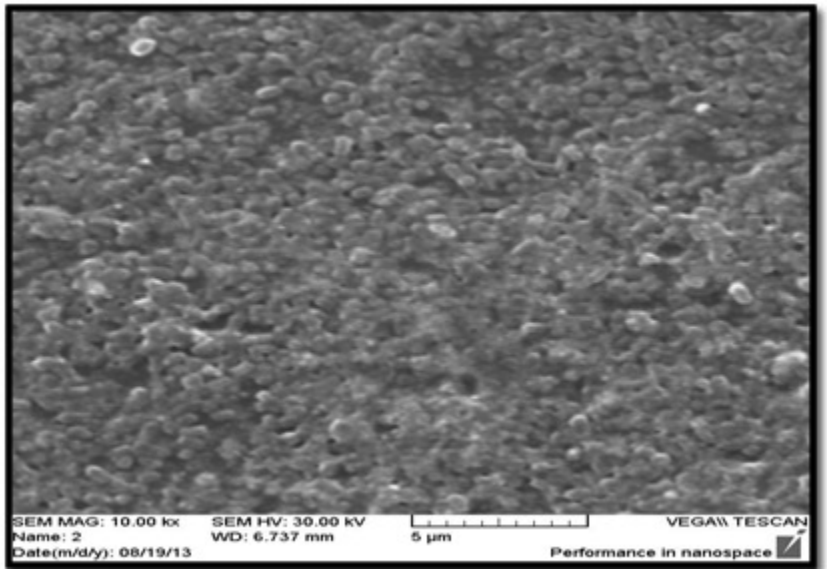

Fig. 4 : SEM image of *Olea europaea* leaves FeNPs (nanoparticles synthesized) at 50.000 amplification.

Table 2 : Inhibition zones of iron oxide nanoparticle and standard antibiotics.

Strains	Iron oxide nanoparticles (25 mg/ml)	Standard antibiotics neomycin (30 µg/disc)
Escherichia coli	22 ± 0.73	12 ± 0.35
Staphylococcus aureus	30 ± 0.0	0 ± 0.0
Vibrio cholera	21 ± 1.4	22 ± 0.70
Pseudomonas aeruginosa	27 ± 0.35	29 ± 0.15
Bacillus subtilis	18 ± 0.70	9 ± 0.0
Streptococcus aureus	24 ± 1.4	0 ± 0.0

scavenging DPPH stable free radical by Iron nanoparticle extract and biosynthesis in different concentrations 1, 2 and 3Mm we found that the 3Mm highest scavenging activity followed by extract, 2Mm and 1Mm. IC_{50} of *Olea europaea* leaf extract and Iron nanoparticle in different concentrations 3, 2 and 1Mm were the highest radical scavenging activity was observed by 3mM (IC_{50} = 12 µgml^{-1}) significantly higher than extract and other Nano particle concentration (P>0.05). The 3Mm scavenging activity exhibited more than 95% scavenging followed by 2Mm 81%, extract 66% and 1Mm 58% may be the Nano particle accumulate the active component of *Olea europaea*. But it was found that highest concentration of *Olea europaea* nanoparticle give the highest scavenging activity as shown in Table 1. A similar method was also presented by Ahmad *et al* (2003) and Lin *et al* (2000), in which Fe+2 reacted with oxygen to create hydrogen peroxide (H_2O_2). This H_2O_2 consequently reacted with ferrous irons via the Fenton reaction and produced hydroxyl radicals which are known to damage biological macro- molecules (Makhluf *et al*, 2005). The

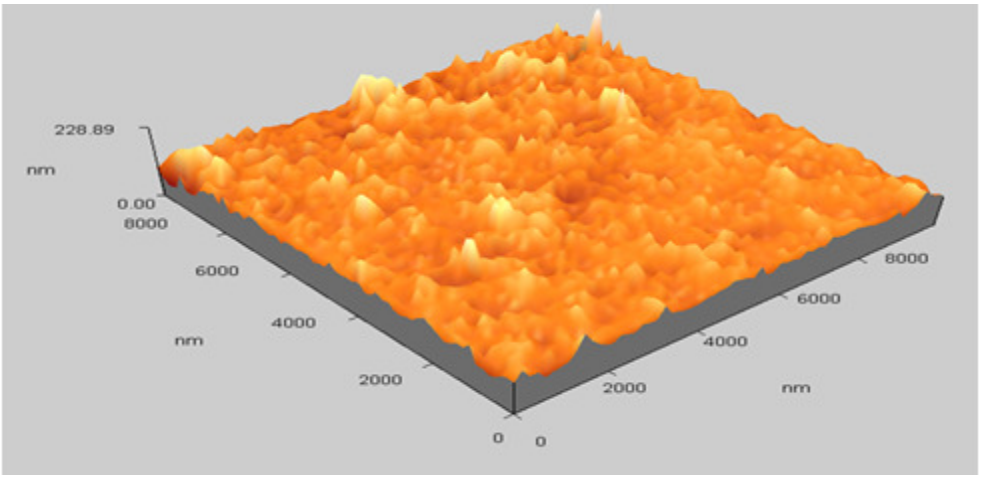

Fig. 5 : AFM photographs of synthesized Fe_3O_4 nanoparticles synthesized by *Olea europaea* leaves extract.

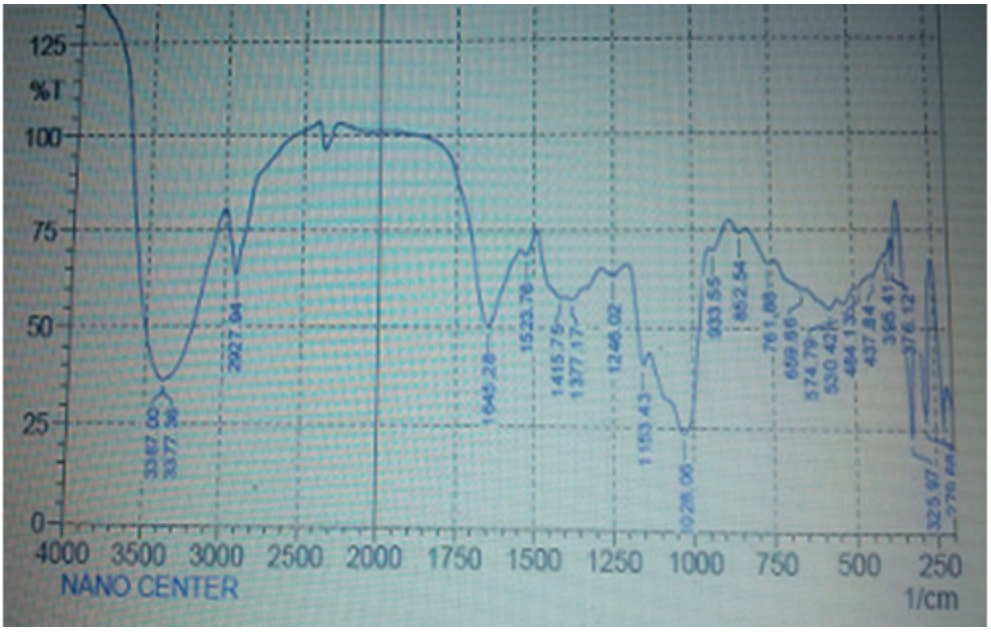

Fig. 6 : FTIR of Synthesis Iron nano particle.

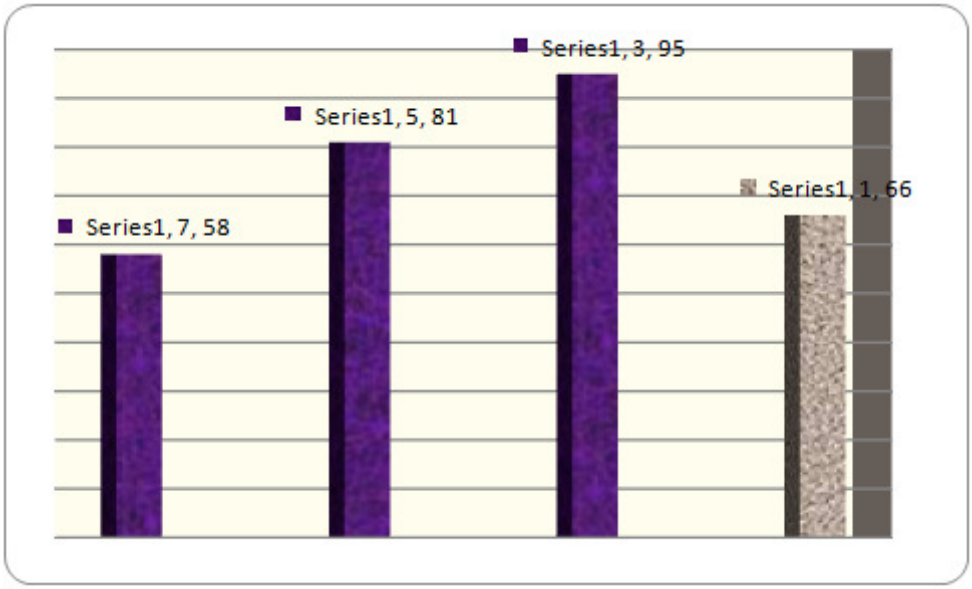

Fig. 7 : Antioxidant activity of *Olea europaea* extract and synthesis Nanoparticle in three different concentration (1, 2 and 3 Mm).

antibacterial activities of Iron nanoparticles are related to their size, the smaller particles having higher activities on the basis by comparing with equivalent iron mass content.

Antimicrobial Activity of Iron nanoparticles

The antimicrobial activity of iron nanoparticle measured in terms of inhibition zone is shown in Table 2. It showed that the IZ increased in dose-dependent manner and followed the same trend with respect to different bacterial strains. The highest inhibitory zone (30±0>0 mm) was observed in *Staphylococcus aureus* with 25 mg/ mlvolume, whereas the lowest inhibitory zone (18 ± 0.70mm) was found with *Bacillus subtilis*. Based on the overall results obtained from the IZ data, the pattern of sensitivity was observed in the order as *Staphylococcus aureus > Pseudomonas aeruginosa > Streptococcus aureus > Escherichia coli > Vibrio cholera > Bacillus subtilis*.

CONCLUSION

In this study, iron nanoparticles with an average size of 10 nm to 55 nm were synthesized using *Olea europaea* extract. The synthesized iron nanoparticles wasinvestigated by UV-Vis, TEM, SEM, AFM and FTIR Spectroscopy techniques. Iron nanoparticles synthesis was used instead of chemical method, and othersbecause it is cheap, short time, pollutant free, eco-friendly, and stabilizing agent's properties. Also, it was used in microwave process to play an important role in the production of reduced iron oxide in this study. The result showed the *Olea europaea* plays an important role in the reduction and stabilization of iron to iron nanoparticles. Further, these synthesized iron nanoparticles from *Olea europaea* shows antibacterial activity on both Gram positive and Gram negative bacteria. So, this eco-friendly technique is very important and interesting to synthesize inorganic materials (nanoparticle) on a wide range of scientific applications, such as bacterial sterilization in hospitals, healing of wounds and burns and many other medical fields, electronics, environment and water sterilization etc.

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