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Bio - Synthesis and Characterizations of Magnetic Iron Oxide Nanoparticles Mediated By Iraq Propolis Extract.

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Abstract
Background: *The present study report for the first time the most eco-friendly, safety, less toxic method for the synthesis of iron oxide nanoparticles using bees products ,the propolis. Biosynthesis of iron oxide nanoparticles (IONPs) using natural materials with less expensive and less harmful effects is of interest in the present time, therefore,*
Material and Method: *The formation of IONPs from two types of iron salts using propolis water extract was evaluated. The formed iron oxide nanoparticles was illustrated by changes in color from brown to black color due to bioreduction of iron salts by propolis extract that is rich of poly phenols. The synthesized magnetite nanoparticles were characterized by UV – Vis absorption spectroscopy, FTIR, XRD and SEM Techniques.*
Result: *The involvement of propolis phenol groups in iron oxide nanoparticles synthesis, is evident from the results of the FTIR. The XRD results depending on the latency of the particles –at 2θ revealed the average particle size of magnetic NP formed from mixture of $FeCl_3$ and $FeCl_2$ are found to be in the range 31,714 – 46,20nm, and with SEM of these nanoparticles showed an agglomeration*
Conclusion*, In conclusion the biosynthesis of iron oxide using propolis water extract consider as effective one step rout for reduction of iron salts to form iron oxide and it may be the future way for eco-friendly, safety, less toxic method for the synthesis of iron oxide nanoparticles.*
Keyword: *Biosynthesis, Iron Oxide Nanoparticles, Magnetite, Propolis*

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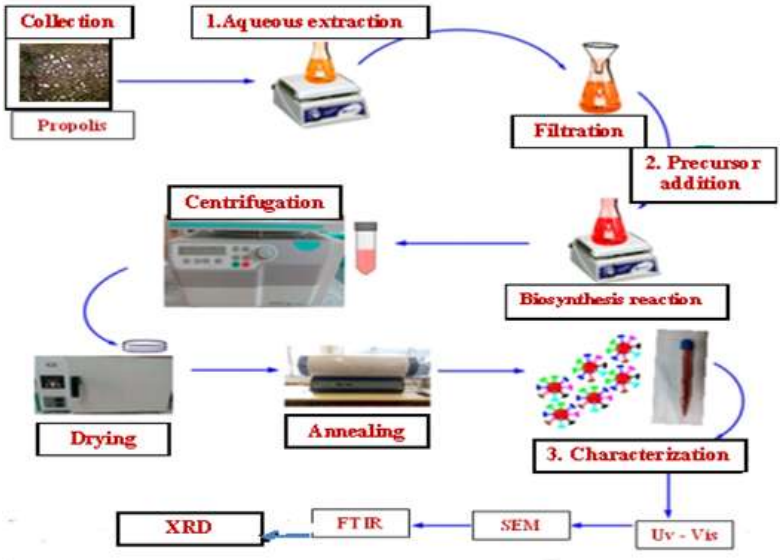

Figure-1: **Steps for biosynthesis of Iron oxide Nanoparticles using Iraq propolis**

I. Introduction

Nanotechnology is study of the synthesis, characterization, design and the application of structures and systems for the control of the shape and size of nanoparticles components. Nanoparticle can be defined as a microscopic particle that having dimension of the arrangement of 100 or less nanometers, they exhibit different properties' result of high surface area and volume ratio[Christian etal.,20081], Nanoparticles are find a

variety of applications in the felid of medicine, physics ,chemistry, environment, energy, agriculture, pharmaceuticals ,advance material, information, and communication and heavy industry (Nochehdehi et al.,2017; Herlekar et al.,2015; Thakur and Karak,2014). Although iron and iron oxide nanoparticles are extensively used in MRI, immunoassay, drug delivery system, catalysis, and magnetic material in biology and medicine, their application in today's life is more significant (Teje and Pei- Yoongk,2009). Synthesis of single or mixed iron oxide nanoparticles (Magnetite) has captured the attention of researchers because of the increasing need for alternatives to address iron deficiency and get rid of the health problem anemia (Rieznichenko et al.,2013). There are many methods that can been used to synthesize iron oxide nanoparticles including, chemical , physical and biological methods. Previous studies showed that chemical methods are commonly used for the synthesis of iron oxide nanoparticles (Woo et al.,2004; Jubb and Allen,2010; Hoaget al.,2009; Laurent et al.,2008; Machado et al 2013). Iron oxide nanoparticles are naturally formed in the nature, but without coating to this particles, they are very low soluble and precipitate. Thus to make these IONPs more effective for medical use and to improve their biocompatibility and distribution in the body , they coated with many types of biological compounds, dextran (Chengyin and Ravindra,2012), Vitamin C (Tassa et al.,2014), silica (Sreeja et al.,2015) and starch (Andrade et al.,2009). Synthesis of a mixed iron oxide compounds magnetite nanoparticles has great importance as a pharmacological compounds , since the mixed oxide ($FeO\text{-}Fe_2O_3 = Fe_3O_4$) from Fe^{+2} and Fe^{+3} will produce molecules with mostly octahedral and tetrahedral coordination , allowing to has six sites for O^{-2} rather than four O^{-2} atoms (Cole et al.,2011).

Propolis (bee glue) is a sticky dark coloured material that honeybees collect from living plants, mix with wax and use in construction and adaptation of their nests. From different botanical and geographical origins of world, more than 300 compounds including volatile organic compounds, flavonoid a glycanes, phenolic acids and their esters, phenolic aldehydes, alcohols and ketones, sesquiterpenes, quinones, coumarins, steroids, amino acids were reported to have been isolated from propolis (Bankova et al.,2000; alencar et al.,2007) Most of the compounds from isolated propolis include the phenolic Flavones, coumarines, and many other phenolic compounds have reducing activity, hydrogen-donors and metal chelating properties. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [Gulcin et al.,201019].All of these compounds are responsible for its biological and pharmacological activities. Therefore, propolis has been used in traditional medicine, cosmetics and food industry from the Europe to East Asia (Banskota et al.,2001; Banskota et al.,2002; Bachiega et al.,2012). Some other researches have been conducted to investigate the phenolic composition of propolis samples (alencar et al.,2007). The compounds reported by different authors include caffeic acid, P-Coumaric Acid, 3,4-Dimethoxycinnamic acid, Quercetin, Pinobanksin 5-Methyl Ether; Apigenin, Kaempferol , Pinobanksin; Cinnamylideneacetic Acid; Chrysin; Pinocembrin; Galangin; Pinobanksin 3-Acetate, Phenethyl Caffeate, Cinnamyl Caffeate, Tectochrysin and Artepillin C (Gulcin et al.,2010; Banskota et al.,2001). Reducing agent of propolis associated with present the reductones (Banskota et al.,2002; Bachiega et al.,2012; Bonvehi and Gutierrez,2011; Velazquez et al.,2007). The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reducers (i.e. antioxidants) causes the reduction of the Fe^{+3}/ ferricyanide complex to the ferrous form (Fe^{+2}) monitored at 700 nm (Sousa et al.,2008). The synthesis of nanoparticles by biological methods may be lead to development of clean, environmentally acceptable and nontoxic nanoparticles. The biosynthesis of safe, and easily distributed iron oxide nanoparticles using propolis extract from single and mixed iron salts was the aim of the present study.

II. Materials And Methods

2.1 Biosynthesis of Iron Oxide Nanoparticles(IONPs):

2.1.Preparation of Propolis Extract

Resins blocks of propolis obtain from local market /Baghdad-Iraq. Propolis extract was prepared by maceration method, briefly, after chopping and fragmentation of blocks, 50 gm of crude propolis putted into glass beaker added 500ml distal water and mixed by continuously stirrer in 35 Cº for 3 days until get a thick suspension, then this suspension filtrated with filter paper the supernatant was cooled to room temperature and stored at 4Cº until use.

2.2. Preparation of Iron Oxide Nanoparticles.

Biosynthesis of Iron oxide using propolis crude extract as reducing agent for ferric and ferrous iron salts according to the followings (fig-1):

1. PrIONPs1 synthesized by mixing of 0.1 M $FeSo_4$ (BDH company , England) with propolis extract, in volume ratio 1: 2 ratio with stirring at room temperature for three days.
2. PrIONPs2 : prepared by mixing of 0.1 M of $FeCl_3$ –$FeCl_2$ (1:2) (BDH company , England) with propolis extract in volume ratio 1:2 with stirring at room temperature for three days.

3. After 3 days the nanoparticles formed ware isolated by centrifuge at 6000 rpm for 25 min, then the precipitate washed by absolute ethanol in volume ratio 1:2 and dried at 60c and kept for the analysis

2.3. Charactrazation of iron oxide nanoparticles

There were continues observation for color changed in directing reduction of $FeSO_4$ and $FeCl_2$-Fe Cl_3 to Fe_3O_4.

2.3.1. UV-Visible Spectra Analysis:

UV- visible spectroscopy is important technique for the evaluation of formation and stability of IONP in aqueous solution, the determination of IONP synthesis of various nanoparticles from different method were analyzed by UV – visible spectroscopy Shemadzu UV – 1600 , japan ,wave rang (190.00 – 800.00 nm), scan speed fast , sampling interval 0.5 , single scan mod and the light source change wavelength was 340.8 nm . A progressive increase in the characteristic peak with increase in reaction time and concentration of biological extracts with salt ions is a clear indicator of nanoparticle formation. UV-Vis absorption spectrum shows peaks characteristics of the surface Plasmon resonance of nanosized particles, according to the procedure described by (Karkuzhali and Yogamoorthi ,2015).

2.3.2. Fourier Transform Infrared [FTIR] Spectroscopy: The prepared iron oxide nanoparticles (IONP) was analyzed by FTIR spectroscopy (ABB/Spectro-Lab/MB3000/UK), Laser phase and the F, D amplitude 35 and the rejected scan counter about 24. FTIR characterization was carried out under classic KBr pellet technique that measures infrared intensity *vs.* wavelength of light from 400 – 4000 (1/cm), it is used to determine the nature of associated functional groups and structural features of biological extracts with nanoparticles. Different functional groups in the nanoparticles have different characteristic absorption in the IR region. This gives the bonds of the compounds an energy to be more stretch and curvature peaks. The calculated spectra clearly reflect the well-known dependence of nanoparticle optical properties, i.e. the resonance wavelength, the extinction cross-section, and the ratio of scattering to absorption, on the nanoparticle dimensions.

2.3.3. The X-ray diffraction (XRD) Analysis :

The phase identification and crystalline of nanoparticles was characterized by X – Ray Diffraction, XRD patterns obtained for $PrFe_3O_4$NP synthesized. This technique is used to establish the metallic nature of particles by giving information on size and shape of the unit cell from peak positions and information on electron density inside the unit cell, namely where the atoms are located from peak intensities [7]. The prepared nanoparticles were examined using XRD 6000/Shemadzu Japan. XRD patterns were calculated using X'per Rota flex diffraction meter using Cu K radiation and λ =1.5406 A°,40.0 kv voltage , 30.0 mA x ray current, the measurement of XRD obtained in theta – 2, continues scan, in the rang 20.000 – 60.000 deg speed about 50000 deg/min in 0.60 sec. Crystallite size is calculated using Scherrer equation (CS) [28].

$$CS = K\lambda / \beta \cos \theta \qquad (1)$$

Where CS is the crystallite size Constant, [K] = 0.94 β is the Full Width at half maximum [FWHM).

2.3.4. Scanning Electron Microscopy(SEM) analysis:

Scanning Electron Microscopy (SEM) image is very important to identify the shapes of the nanoparticles formed. SEM is able to provide images of three dimensional objects because in its normal mode of operation it records not the electrons passing thorough the specimen but the secondary electrons that are released from the sample by the electron beam impinging on it. The images created without light waves are rendered white and black. SEM (Tescan Vega lll /Czech) was used to study the morphology of samples. To get the best view under SEM, samples were slightly pressed into pellets at 0.5 ton-load. Magnitude of 30.0 kX, SEM HV 10.0 kV and the particles were examined at 2 -10µm scale (Wang et al., 2014).

III. Result

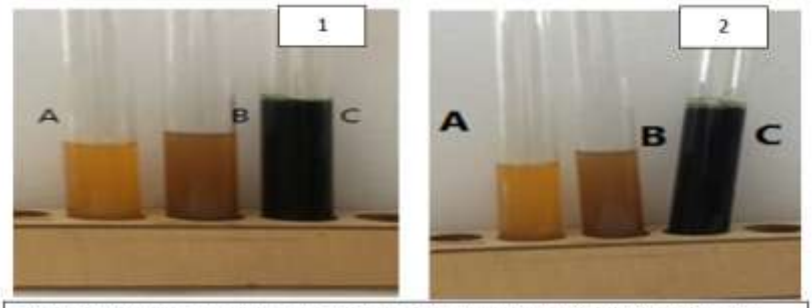

Figure-2: changes in color and density of precipitate formed immediately after mixing of 1-- iron sulfate and 2- iron salts mixture to propolis extract in: 1:2 ratio. A: Iron salt mixture B: Propolis extract C: the iron salt: propolis mixture.

Results of the present study regarding color changes of the prepared nanoparticles using propolis extract are shown in figure-2. There were changing in color in min from yellow to black and the density and intensity of precipitate increased gradually to reach the highest darkness and density of precipitate after 24 hours of mixing propolis extracts with ferric and ferrous mixture. The best appearance of nanoparticles biosynthesis was in the tube of mixing ferric and ferrous mixture with propolis extract in 1:2 a v: v ratio.The produced particles were crystallized and precipitate clearly at the bottom of the tube (Figure-2)

3.1 Ultraviolet – Visible spectroscopy analysis: Optical density of the formed IONPs were examined using UV-Vis spectrum with a wave light 190 – 800 nm. Results shown in Figure 1 reveal that the prepared compounds from propolis and different mixed and single ferric salts strongly absorbed radiation at wide wave length of uv-vis spectra ranged from 190 to 800. The absorbance at certain waves considered as indicators to the formed iron oxide, are shown in the Table 1. Iron oxide nanoparticles that are synthesized using iron chloride mixture (PrIOPs2) showed the highest absorbance at both UV and visible light than iron oxide nanoparticles synthesized from ferric sulfate (PrIOPNs1).

Table - 1: The ultraviolet- visible spectroscopy analysis of biosynthesis of iron oxide nanoparticles prepared using propolis extract.

Symbol of the IOPs	**iron salts**	**UV**		**Visible**	
		wave	**abs**	**wave**	**abs**
PrIONPs 1	**$Feso_4$**	**299**	**2.8**	**463**	**3.9**
PrIONPs 2	**$Fecl_3$:$Fecl_2$ (1:2)**	**242**	**3.7**	**404**	**3.6**

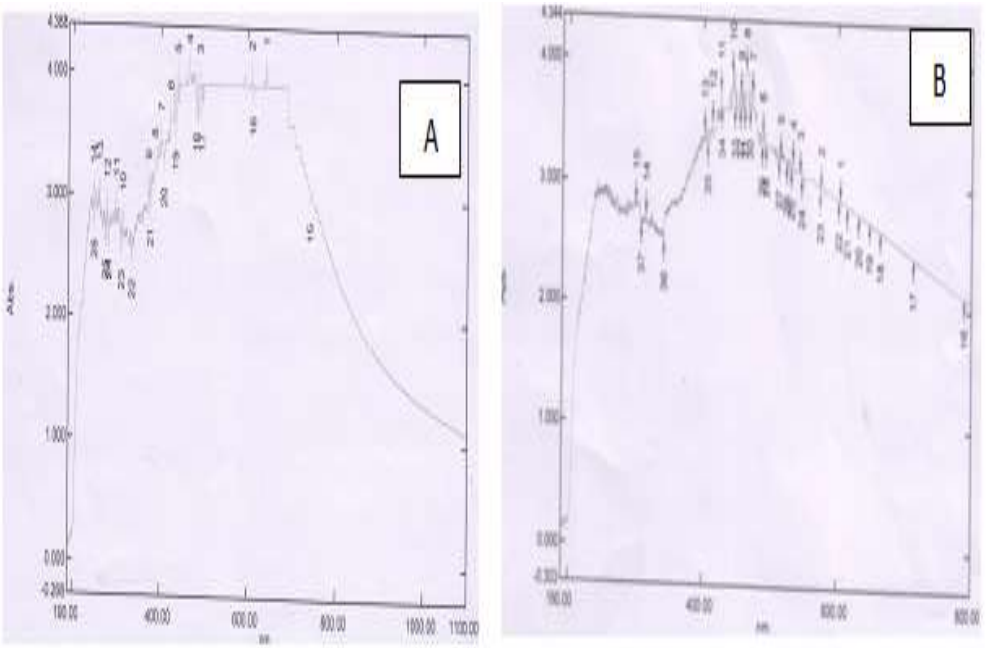

Fig -3 : UV – visible of iron oxide nanoparticles synthesis by propolis extract A: PrIONP1 , B:PrIONP2 ,

3.2. Fourier Transform Infrared [FTIR] Spectroscopy :

FTIR Analysis was performed in order to determine the functional group of propolis extract involved in the synthesis of iron oxide nanoparticles. The FTIR showed a different vibration according to different functional groups which have a characteristic absorption in the IR region this give the bonds of the compounds an energy to be more stretch and curvature the peaks . The analysis of the chromatographic images of the FTIR of the present prepared compounds (figures -3), showed vibrations in different IR absorption regions

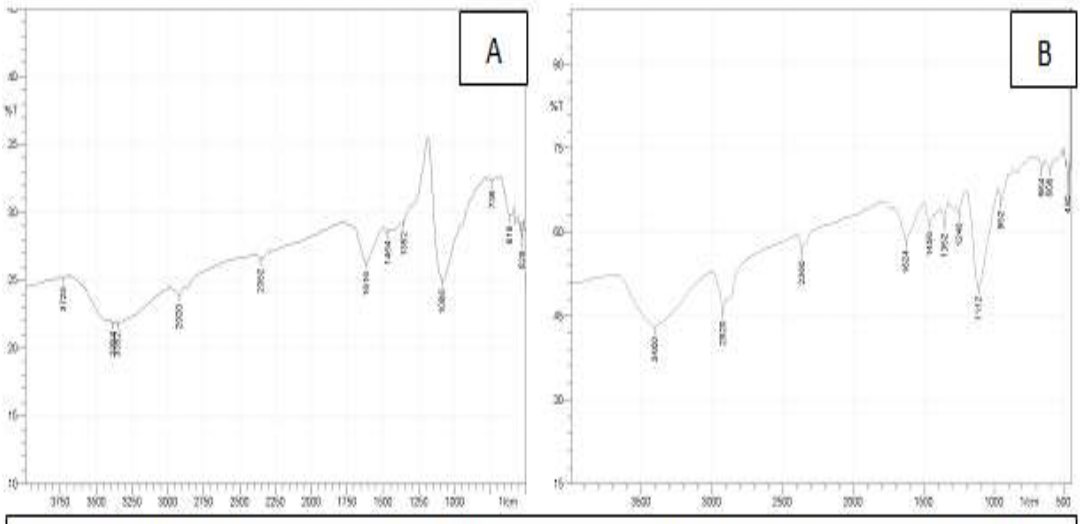

Fig -4: FTIR spectrum of Fe_3o_4 nanoparticles using iron salts and propolis extract. A:PrIONP1 , B: PrIONP2

indicating formation of different molecules some of them containing many bonds as in regions ranged 4000-1500, and others are appeared as a one molecules those with vibration below 1500 as shown in table-2 . In the present study the IR absorption (cm) divided in to four regions, 1st for vibrations appear in 4000-2500, 2nd for2500-200,3rd 200-1500, and the 4th for 1500-400. Results revealed that both prepared IONPs have formed a functional molecule with one bonds of Fe-O.

Table- 2: The FTIR analysis of Fe_3o_4 nanoparticles by propolis extract

	iron salts	**Wave number cm⁻¹ e number cm**			
		4000-2500 O-H, COOH N-H	**2500-2000 C-N C-C**	**2000-1500 C=C C=O**	**1500-400 Fe-O**
PrIONP1	**$Feso_4$**	**2900, 3352,3384,3728**	**2352**	**1616**	**1464 – 528**
PrIONP2	**$Fecl_2$:$Fecl_3$**	**2920**	**2360**	**1736,1625**	**480 - 1426**

3.3.X – Ray Diffraction (XRD) analysis : X-ray diffraction patterns have been widely used to characterize critical features such as types and nature of crystalline phases present in the compound, The diffraction of the X-ray beam caused by the sample in XRD indicated by the position (angle) and intensities can provide information about the sample . The analysis of the XRD included the theat-2 values of the differentiated peaks and the crystallite sizes using Schrerre's formula for the different types of iron oxide nanoparticles prepared using propolis extract are shown ,the phase identification and crystalline structure of nanoparticles and determination the strong diffraction peak with 2 theta value of 26.618°, 33.8515° ,35.05°,39.07°,46.375°, and 55.7° corresponding to the ,hkl values from (205), (109), (119), (222) and (422) crystal planes respectively PCPDFWIN – PDF #251402 Wavelength and PCPDFWIN – PDF #160653 Wavelength equal 1.54056. the average particles size of magnetic NP formed from mixture of $Fecl_3$ and $Fecl_2$ are found to be range 31,714 – 46,20 to the IONP synthesis from $FeSO_4$ about 153.45 – 245.17 nm . The IONP synthesis from mixture is more crystalline from Fig.5.

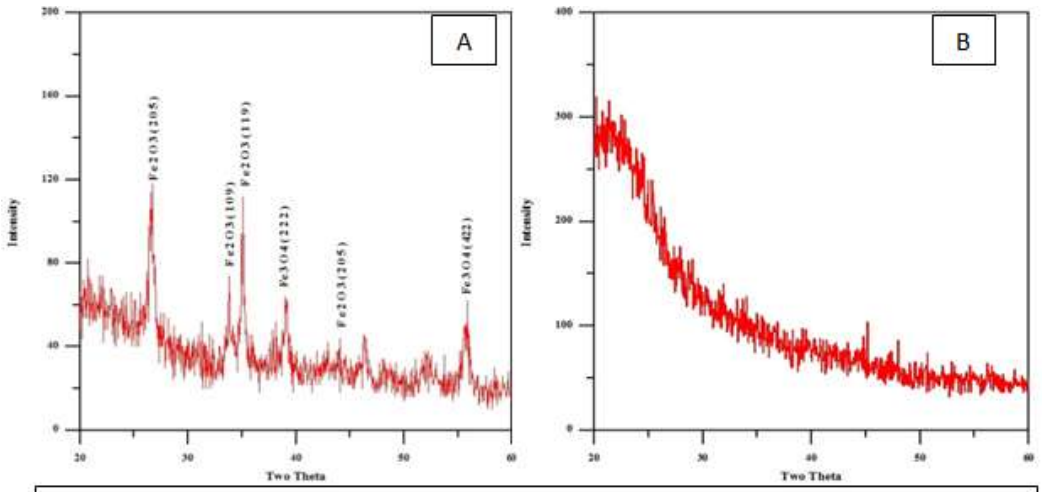

Fig-5: XRD spectrum of Fe_3o_4 nanoparticles using mixture of $FeSO_4$(A) $Fecl_2$, $Fecl_3$(B), and propolis extract volume ratio 1:2.

3.4. Scanning Electron Microscopy (SEM): The present results of the analysis of the SEM micrographs represented in Figure 6, showed the size and morphology of magnetic nanoparticles that was synthesized by propolis extract. Figure 6, clearly shows the agglomeration of iron oxide nanoparticles produced, with slight aggregation. The average of particle size in IONPs formed from mixture ferric and ferrous chlorides are about 31.714 – 46.20nm and the IONP synthesis from $FeSO_4$ about 153.45 – 245.17 nm in room temperature.

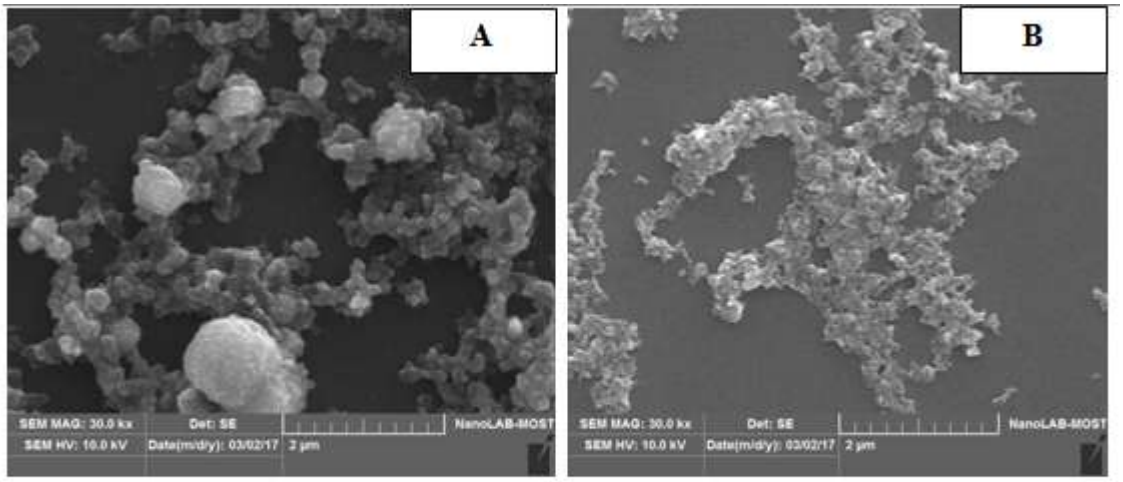

Fig-6: SEM image of propolis Iron Oxide nanoparticles A. PrIONP1 synthesis from $FeSO_4$, B. PrIONP2 synthesis from mixture ferric and ferrous .

IV. Discussion

In present study the change in color from brown to black tack placed indicating formation of magnetite particles, the most predominant step in formation of magnetite is reduction of Ferric or ferrous to oxide to form Fe_2O_3 or Fe_3O_4,. Propolis water extract containing a phytochemical components with high reducing capability such as flavonoids, caffeine acid, and other phenolic compounds (Gulcin et al.,2010; Sousa et al.,2008). Results illustrated that propolis was efficient in synthesis of stabilized magnetite iron oxide. The synthesis of iron oxide nanoparticles using propolis water extract indicated no significant differences in color and density and duration for the two iron salt used after three days of preparations. However, the particles prepared from a mixture of 0.1 M of $FeCl_3$ –$FeCl_2$ showed colloidal precipitation more than that of $FeSO_4$. Furthermore the decreased pH as more H^+ ion released from the phenolic compound of the propolis replaced by Fe^{3+}which become free in the colloid of the reaction indicating the high reducing efficacy of propolis . The UV-visible results showed variable in the absorbance of compound in wide range of waves, indicating formation of conjugated compounds. The highest absorption at lower UV light was by nanoparticles of mixed ferric and ferrous more specific than from FeSO4, under visible light there were no differences between absorption at any given wave length. This analysis differentiate between organic molecules according to the differences in their conjugated or unconjugated molecules contents since conjugated multiple bonds compounds absorb strongly ultraviolet – visible spectroscopy radiation while the unconjugated does not absorb. The optical absorption spectrum of iron salts depended on particles size , shape, state of aggregation and surrounding dielectric medium (Velazquez et al.,2007). The present result confirmed that IONPs1 has absorption peak and intensity result of reduction reaction of propolis extract rich of polyphenol as reducing agent and formation magnetite nanoparticles increased of wavelength of absorption due to higher production of nanoparticles (Latha and Gowri, 2012). In this study FTIR analysis was conformed in order to evaluate the functional group on propolis extract and predict role in the formation IONPs, the strong absorption peaks were assigned to functional bands. Results revealed that both prepared iron oxide nanoparticles using propolis extract containing compounds rich with O-H , C-H , N-H and COOH at IR absorption 3400 -1500cm- , in addition they showed an absorption in the fingerprint region below 750-480 which specifically for FeO compounds (Shojaee et al.,2016). The FTIR analysis of magnetite oxide nanoparticles synthesized using propolis extract in present study , confirmed that the bioreduction of ferric chloride into IONPs by capping material of propolis extract because the propolis extract rich in poly phenol compound which conceders reducing agent (Karkuzhali and Yogamoorthi,2015). XRD analysis was an effective characterization to confirmed the crystal structure of the synthesis magnetite nanoparticles by propolis extract which there exist a strong peak with 2theta values according to type of iron oxide formed, in the XRD demonstrated that the nanoparticles are crystalline especially in IONP produced from mixture of iron salts with cubic shape which these Fe_3O_4 and Fe_2O_3 (Balamurgan et al., 2014). In present study the crystalline size of synthesized nanoparticles were calculated from Debye – Scherrer equation, the average crystalline size 31.714 – 46.20nm and 153.45 – 245.17 nm that nanoparticles were synthesized by bioreduction methods are crystalline (Mahdavi et al., 2013). The crystalline plane in this study similar kind of result have been found with PCPDFWIN – PDF #251402 and PCPDFWIN – PDF #160653 Wave-length equal 1.54056 in biosynthesis of iron oxide nanoparticles of magnetite nanoparticles including Fe_3O_4 and Fe_2O_3. These kinds of

results are similar the green synthesis of magnetite nanoparticles (Shojaee and Shahri ,2016). Scanning electron microscopic images were employed to analysis the morphology and size of nanoparticles that formed in presented study. The examined particles were agglomerated, the size morphology is irregular, the magnetite nanoparticles shape showed cubic shape particles and different particles size due to bioreduction of iron salts by propolis extract .this outcome can be explained by the fact that the polyphenol concentration in propolis extract plays roles in formation final structure and particles size of these green synthesis magnetite nanoparticles (Mahdavi et al.,2013). in present study the particles size in magnetite nanoparticles synthesis les from mixture ferric and ferrous is smaller than particle size which synthesis from $FeSO_4$ that its indicator to the propolis extract reducing the mixture more than $FeSO_4$, the iron oxide NPs formed by mixture is high activity from $FeSO_4$NP because decrease in particles size and increase in surface area.

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