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Biomolecules in grape leaf extract involved in one-step synthesis of iron-based nanoparticles

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Abstract

Biomolecules in plant extracts are often used to reduce metal ions to nanoparticles in a single-step green synthesis process that is environment friendly and sustainable. However, our understanding of biomolecules as reducing and capping agents in plant extracts involved in green synthesis of metal nanoparticles is limited. In this paper, grape leaves which are the major waste generated in winemaking in Australia are utilized. Their components have an important environmental impact on waste reduction. Furthermore they permit the production of added value products such as iron-based nanoparticles (Fe NPs). To understand biomolecules involved in the synthesis of Fe NPs, the reactivity of Fe NPs synthesized using methanolic extract of grape leaves (~ 80.0%) was much higher than that of water extraction (~ 4.0%), where a high concentration of biomolecules in methanolic extract of grape leaves was monitored by UV-vis. Gas chromatography-mass spectrometry (GC-MS) analysis of before and after methanol extraction to synthesize Fe NP shows that the main biomolecules included phytols, terpenoids (α, and β amyrins, β and δ stiodterols), and antioxidants (δ-stan-3,5-diene, vitamin E) as reducing and capping agents. The potential biomolecules that can reduce Fe precursors were confirmed by Fourier Transform Infrared Spectroscope (FTIR). Well-dispersed and capped Fe NPs with an average size of 60 nm were observed by scanning electron microscopy (SEM), while the amorphous crystalline of Fe NPs was identified by X-ray diffraction (XRD). Finally, approximately 80.0% of acid Orange Ⅱ using Fe NPs was removed, while only 2.0% of acid Orange Ⅱ was removed by the extract, indicating the high reactivity of Fe NPs synthesized by methanolic extract of grape leaves. And such grape leaves extracts make Fe NPs be a potential low cost and environmentally friendly remediation technique.

Keywords: Green synthesis; Biomolecules; Grape leaves; Fe NPs, Azo dye, GC-MS.

1 Introduction

Grapes, one of the world's most widely harvested fruit crops, provide the main raw materials for winemaking, and exploiting their components most effectively is generating interest due to the expected economic profits and environmental concerns. For example, grape pomaces, the major wastes generated in the winemaking process, have a significant environmental impact in waste reduction and the production of added value products.[1,2] Grape pomaces contain large amounts of polyphenols, which are recognized as being beneficial to human health. More specifically, the pharmaceuticals and nutritional applications of some polyphenols have been reported.[1,2] However, the lack of new application areas is mainly associated with our lack of knowledge of the grape waste's chemical composition.

Recently, the application of iron-based nanoparticles (Fe NPs) for the remediation of chlorinated compounds and heavy metal ions has received significant attention due to their large surface area and rapid reactivity.[3] To date, a chemical method such as sodium borohydride ($NaBH_4$) as a reducing agent is often used in the production of Fe NPs, but its limitations include low production rates, high cost and the generation of hazardous by-products.[4] In contrast, the green synthesis of Fe NPs using plant extracts has been proposed as an alternative since the biomolecules in plant extracts act as capping and reduction agents that reduce the aggregation of Fe NPs and improve their stability. Consequently, green synthesis using plant extracts is generally cost-effective, biocompatible, non-toxic, and eco-friendly.[4] In addition, plant-based materials, including leaf, seed, root, and stem have been extracted for the green synthesis of metal nanoparticles. The rationale is that they are advantageous in terms of economic efficiency and provide a valuable alternative for large-scale production. Specifically the biomolecules in plant extracts serve as capping and reducing agents in the reduction of Fe^{2+} to Fe NPs.[4]

However, little is known about these biomolecules that exist in various plant extracts.[5] Plant extracts' potential to reduce metal ions depends on the presence of polyphenols, enzymes, and other chelating agents present in plants. This critically affects how many nanoparticles are produced. Understanding the process of bioreduction will enhance nanoparticle production. However, to date, only a few reports are available on the green synthesis of Fe NPs using plant extracts. Tea extracts on a polyphenol basis have been utilized mainly for the synthesis of Fe NPs.[6] Compared to chemically synthesized Fe NPs, green synthesized Fe NPs manifested greater removal efficiency as a result of polyphenols existing in tea extracts, which protected the Fe NPs from oxidation and aggregation. Other studies have reported the successful green synthesis of Fe NPs by plant extracts utilizing oolong tea extract, *Terminalia chebula* aqueous extract, and *Eucalyptus* leaf extracts.[7-9] However, despite these valuable scientific findings, much is still unclear, namely: (1) which biomolecules in plant extracts are involved in the bioreduction of Fe^{2+} to form Fe NPs, and how can these biomolecules be identified?; and (2) what functions do the biomolecules in plant extracts serve with regard to the stability and aggregation, morphology of Fe NPs as well as the reactivity of Fe NPs?

To the best of our knowledge, to date, no study has been published on understanding of synthesized process of Fe NPs using plant extracts. The significance of this study will therefore provide new insights into the green synthesis of Fe NPs mediated by plant extracts. Specifically, using GC-MS will enable us to identify biomolecules in grape leaf extracts. Consequently, this study posits that large-scale production of Fe NPs is possible by improving production methods. It also aims to promote using grape leaves to: firstly, produce Fe NPs that can remediate the environment; and secondly, reduce the impact of grape leaf waste on the environment.

In this paper, the synthesis of Fe NPs using grape leaf extracts was addressed. To understand the green synthesis of Fe NPs, the biomolecules in methanolic extract of grape leaves were identified by UV and GC-MS. The formation and stabilization of Fe NPs was also confirmed by FTIR while the morphology of Fe NPs was characterized by SEM and XRD. Finally, the Fe NPs' reactivity was demonstrated in their removal of acid Orange Ⅱ.

2 Experimental

2.1 Chemicals and reagents

Ferrous chloride ($FeCl_2$, purity > 99%), acid Orange Ⅱ ($C_{16}H_{11}N_2NaO_4S$, purity > 99%) and methanol were all purchased from Sigma-Aldrich Co. (Australia), and they were of analytical grade. De-ionized water obtained from the Milli-Q Elga System was used in all experiments.

2.2 Synthesis of Fe NPs using grape leaf extracts

The grape leaf extract was prepared by extracting 1.0 g of finely ground grape leaf powder (collected in Adelaide, South Australia) in 50 ml of methanol or de-ionized water at room temperature for an hour. Then it was filtered through a 0.45 μm polytetrafluoroethylene (PTFE) filter. Subsequently, 10 ml of $FeCl_2$ solution (0.01 M) was added to the 10 ml methanolic or water extract of grape leaves in a 1:1 ratio, and mixed thoroughly using a magnetic stirring apparatus at room temperature. The formation of Fe NPs was indicated by the appearance of intense black precipitate.

2.3 GC-MS analysis of grape leaf extracts

1.0 g of finely ground grape leaf powder was extracted with methanol for 1 h, followed by filtering through a 0.45 μm PTFE filter. To understand the main components of in grape leaf

extracts that act as reducing and capping agents, the extracts before and after the green synthesis of Fe NPs were compared using GC-MS. The samples were stored at 4^{o}C prior to GC-MS analysis.

An Agilent 6890 N GC system (Agilent, Palo Alto, CA) with a split/splitless injector and interfaced with an Agilent 5973N mass spectrometer analysed the samples. The injector was set at 280 ^{0}C. An Agilent MSD ChemStation Software (E.02.00.493 version) was used to control the system. For separation, a DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) (USD306454) was used. Helium was the carrier gas (1.1 mL/min). One microlitre of the sample was injected. The GC conditions were as follows: initial temperature was 25 ℃ for 7.36 min. Then the temperature was increased up to 325 ℃ at 15 ℃/min and maintained for 32.4 min giving a total run time of 60 min. For the MS system, the temperatures of the transfer line electron impact mass spectra were recorded at 70 eV ionization voltages. The acquisitions were undertaken in scan mode (from 50 to 600 amu). Peak identification was carried out by analogy of mass spectra with those of the mass library (WILEY 6.0 and NIST 2.0).

2.4 Characterization

The morphology, size and surface composition of Fe NPs are important because these properties' homogeneous nature leads to many practical applications. The following techniques were employed for characterizing Fe NPs in this study. Samples used in SEM and XRD were prepared, where small amount of freshly prepared Fe NPs solution was dropped on the surface of cupper substrate, followed by drying using vacuum desiccator within several minutes prior to use. For FTIR, the dried powder for methanolic extract and the corresponding Fe NPs were obtained using pressure blowing concentrator. Firstly, scanning electron

146 microscopy (SEM) was done employing a FEI Quanta 450 FEG SEM with an EDS Apollo
147 detector, using an accelerating voltage of 15 kV. Secondly, X-ray diffraction (XRD) patterns
148 of Fe NPs were obtained using XRD-6000 (Shimadzu Corporation, Japan) with Cu Kα
149 radiation (λ = 1.5418 Å). Sample was scanned from 10° to 80° (2θ) at a scanning rate of 3°
150 (2θ) per minute. Thirdly, methanolic extract of grape leaf and Fe NPs were determined by a
151 Fourier Transform Infrared Spectroscope (FTIR Nicolet 5700, Thermo Corp., USA). Samples
152 for FTIR measurement were prepared by mixing 1.0 % (w/w) specimen with 100 mg of KBr
153 powder and pressed into a sheer slice. Spectra over the 4000–400 cm^{-1} range were obtained
154 by the co-addition of 32 scans with a resolution of 4 cm^{-1}.
155
156 *2.5 Batch experiment*
157 The reactivity of Fe NPs was tested by having them remove azo dyes such as acid Orange Ⅱ.
158 To compare the reactivity of Fe NPs synthesized by methanolic extract and water extract from
159 grape leaf, the experiments for the degradation were carried out using a solution containing
160 10.0 mg/L acid Orange Ⅱ. High speed centrifuge was used to separate the Fe NPs from the
161 reaction mixture solution. The 8 mL upper solution of freshly synthesized Fe NPs was
162 discarded after centrifugation, followed by adding them into the dye solution. To compare the
163 removal efficiency using methanolic extract of grape leaf and Fe NPs, the same amount of Fe
164 NPs and methanolic extract were firstly dried using pressure blowing concentrator, which
165 subsequently reacted with dye solution. These were then placed on a rotary shaker at 298 K
166 and 250 r/min. The degraded solutions were then filtered through 0.80 μm membranes to
167 determine the concentration of acid Orange Ⅱ. This concentration was in turn measured using
168 a UV-Spectrophotometer (Lambda 18, Perkin–Elmer) at 485 nm. The efficiency of Fe NPs in
169 removing acid Orange Ⅱ was calculated using the following equation: [10]

$$R\ (\%) = (C_0 - C_t)/ C_0$$

where R (%) is the efficiency in degrading acid Orange Ⅱ, C_0 (mg/L) is the initial concentration of acid Orange Ⅱ in the solution, and C_t (mg/L) is the concentration of acid Orange Ⅱ at t min.

3 Results and Discussion

3.1 Biomolecules in grape leaf extract involved in the synthesis of Fe NPs.

Fig. 1(a) and (b) shows the difference of UV-vis spectra between methanolic and water extracts of grape leaves. Fig. 1(a) reveals that the peaks at 450, 475 and 670 nm in methanolic extract may correspond to the polyphenols and pigments, which were recently confirmed by analysis of the grape extracts.[11] It was observed that these biomolecules indicated high extract efficiency in the methanolic extract compared to the water extract. However, as shown in Fig.1(b), the peaks of these biomolecules disappeared in the synthesis of Fe NPs due to their involvement in the formation of Fe NPs as both reducing and capping agents, which led to the reaction mixture's color changing rapidly from brown to black. This indicates that the formation of Fe NPs as observed in broad absorption occurred at a higher wavelength (500 nm-700 nm).[12] More importantly, the absorption peak of the Fe NPs at 500-700 nm synthesized by methanolic extract was stronger than that of the water extract, which meant the reactivity of Fe NPs was superior.

Such an outcome could support what is shown in Fig. 2, which evaluates the reactivity of the Fe NPs synthesized using both methanolic and water extracts of grape leaves. These were

used to degrade acid Orange II in aqueous solution with an initial concentration of 10.0 mg/L. Fe NPs synthesized using methanolic extracts removed nearly 80.0 % of acid Orange II, but only 4.0 % was removed by Fe NPs using water extract. This indicates that: firstly, high reactivity of Fe NPs emerged when methanolic extracts were used; and secondly, a more efficient and higher degradation rate of acid Orange II was obtained. This is attributable to the fact that a high concentration of polyphenols and other biomolecules in methanolic grape leaf extracts not only served as capping agents that reduced the aggregation of Fe NPs, but also served as reducing agents involved in the synthesis of Fe NPs.[13] Consequently, to further analyse and confirm the enhanced stability and reactivity of Fe NPs and the existence of biomolecules in methanolic extracts, GC-MS was used and is expanded on below.

Many reports have been published on the synthesis of metal nanoparticles using plant extracts. It is evident that various biomolecules in plant extracts such as proteins, amino acids, polysaccharides, alkaloids, alcoholic compounds, vitamins and polyphenols are involved in the bioreduction, formation and stabilization of metal nanoparticles.[4,5] However, note that few reports have identified what biomolecules are involved in the synthesis of Fe NPs. To address this problem, GC-MS was used to examine extract samples before and after synthesis occurred to understand which specific biomolecules were involved. Fig. 3(a) illustrates a methanolic extract of the chromatograms corresponding to the biomolecules identified in a methanolic extract of grape leaves. Here the main compounds include phytols (retention time: 23.521, 23.899), terpenoids (β and δ sitosterols: 34.621, 37.030; α or β amyrin, 37.442), and antioxidants (1,4 – eicosadiene: 24.071; δ-stan-3,5-diene: 34.921; vitamin E: 35.547). Similar chemical compositions were obtained in a recent report on the integrated utilization of grape skins derived from white grape pomace.[14]

However, these biomolecules disappeared after participating in the synthesis of Fe NPs (as shown in Fig. 3(b)), which can be explained by the reason that these biomolecules in grape leaf extracts served as reducing and capping agents.[4,5] However, on the basis of mass spectrometry of the main biomolecules summarized in Table 1, two important conclusions can be made. Firstly, phytols, β and δ sitosterols, amyrin and vitamin E were used as both reducing and capping agents due to their functional groups. For example, C=C, -OH, =O, where -OH, =O were oxidized to -COOH and Fe^{2+} was reduced to Fe NPs, while C=C was capped on the Fe NPs' surface to resist oxidation and enhance the stability of Fe NPs. Secondly, biomolecules such as 4-eicosadiene and δ-stan-3,5-diene only acted as capping agents in the synthesis of Fe NPs because they contain two double bonds, leading to resist the oxidation of Fe NPs and hence an improvement in their stability.

GC-MS could not detect the existence of non-volatile biomolecules in grape leaf extract, for example organic acids, alkaloids, alcoholic compounds, flavonoide and polyphenols.[15] Therefore, to confirm the role of capping agents on the surface of Fe NPs, FTIR characterized the methanolic extracts before and after synthesis to prove that organic functional groups such as carbonyls, hydroxyls and other surface chemical residues were attached to the surface of Fe NPs.[5] As shown in Fig. 4, it was observed that band intensities and shifts of the spectrum in the extracts occurred between before and after involving synthesis were observed. For example, the band shifts include 3396-3385, 2923-2921, 1615-1611, 1458-1441, and 1369-1372 cm^{-1}. The broad and intense absorption band at around 3396 cm^{-1} corresponds to the O-H stretching vibrations of polyphenols, phenolic acids, phytols, sitosterols, amyrin and vitamin E.[15] The shift from 3396 to 3385 cm^{-1} may indicate the involvement of OH functional group in the synthesis of Fe NPs.[16] The band at 2923 cm^{-1} can be attributed to the symmetric and asymmetric C-H stretching vibration of aliphatic acids,[16] and shifting to 2918 cm^{-1}

indicates this group's possible involvement in the synthesis of Fe NPs. The band at 1735 cm^{-1} is attributable to C=O stretching vibrations in aldehydes and ketones, indicating the presence of phenolic acids and terpenoids.[17] Stretching vibrations at 1615, 1369 cm^{-1} refer to C=C of aromatic ring, C-N in aromatic amines, respectively, in the grape leaf extract.[5,18] However, shifts of 1615 to 1611 cm^{-1}, and 1369 to 1375 cm^{-1} were observed, indicating that alkaloids could be involved the formation of Fe NPs.[15] The band at 1071 cm^{-1} may be due to COH of carboxylic acids,[19] where its intensity decreased and no shift was observed. This indicates that these compounds acted only as capping agents.

The FTIR spectra indicate that the functional groups (CHO, C=O, COOH, and OH) are involved in the reduction and stabilizing of Fe NPs. The typical grape leaf extract contains polyphenols, flavonoid, phytols, terpenoids and antioxidants,[15] and some of these were confirmed by GC-MS in the previous section. Consequently the formation of Fe NPs using grape leaf extract requires Fe^{2+} to be complexed with biomolecules containing carboxyl and hydroxyl to form complex ions. The aldehydes and ketones existing in biomolecules were oxidized to carboxyl and Fe^{2+} was reduced to Fe NPs. When Fe NPs are being formed, carboxyl and hydroxyl form capping agents on their surface. This capping process may cause steric hindrance around the particles and thereby stabilize them.[19]

3.2 Characterization

Nanoparticles are generally characterized by their morphology, size, shape and dispersity since these criteria relate to many applications.[5] In this study, Fe NPs synthesized by methanolic extract of grape leaves were characterized by scanning electron microscopy (SEM), energy-dispersive spectrometer (EDS) and powder X-ray diffraction (XRD).

To understand the morphology and size of Fe NPs, an SEM image of their synthesis by methanolic extract of grape leaves is presented in Fig. 5, where the Fe NPs' diameter was quasi-spherical shape, and ranged in size from 15-100 nm. It is interesting to note that almost all the Fe NPs are equally distributed and surrounded by a thin layer of biomolecules, indicating that Fe NPs were capped and dispersed by the biomolecules existing in grape leaf extract.[13,14] This may prevent them from largely aggregating and could logically explain the extract's higher stability and reactivity.

EDS analysis was carried out to better understand the elemental composition of the Fe NPs' surface as shown in Fig. 6, where Fe (20.06%), O (15.56%), C (39.91%), and Cl (24.48%). These percentages were obtained in Fe NPs that capped the grape leaf extract. The Cl signals must have originated from the $FeCl_2$ precursor used in the synthesis of Fe NPs. The C and O signals are attributed mainly to the biomolecules in the grape leaf extract. However, three Fe peaks were observed, demonstrating the Fe NPs exist in the form of iron oxide and iron since the O element is observed. These values could be helpful in reflecting the atomic content of the surface and near surface regions of the Fe NPs.[13, 14]

Fig. 7 shows a typical XRD pattern of Fe NPs synthesized by grape leaf extract, where no obvious peaks referring to iron oxide (Fe_3O_4) and iron oxohydroxide (FeOOH) were found.[13, 20] A broad hump appearing at about 2θ of 20° was observed, which could be biomolecules forming a capping layer on the Fe NPs' surface resulting from the methanolic extract of grape leaves. This can be interpreted by the fact that a thin layer of biomolecules is capping on the Fe NPs surface in order to stabilize Fe NPs resistance to oxidation, which was observed in the SEM section.[5, 6] However, a characteristic peak of zero-valent iron (α-Fe) was not observed

since the generated Fe^0 is in an amorphous state in nature. Recent reports indicate that Fe^0 was produced by reduction using green tea.[6]

3.3 The reactivity of Fe NPs and their functions

To assess the reactivity of the Fe NPs synthesized by methanolic extract of grape leaves and their functions, an experiment was conducted comparing the efficiency in removing acid Orange II using Fe^{2+} (both in water and methanol), methanolic extract and Fe NPs with an initial concentration of 10.0 mg/L under common conditions. As shown in Fig. 8, the best removal efficiency (approx.. 80.0 %) occurred when Fe NPs were used, while only around 2.0 % of acid Orange II was removed by methanolic extract. This marked difference indicates that Fe NPs used to remove acid Orange II could be based on adsorption and reduction. Furthermore this could also because that acid Orange II interacts with the functional groups of biomolecules in capping layer, since 2.0% of acid Orange Ⅱ was removed in Fig. 8.[21] This was followed by the reduction of acid Orange Ⅱ by Fe^0.[22] However, no removal of acid Orange Ⅱ by Fe^{2+} was observed, indicating that the discolorization of acid Orange II did not occur in the presence of Fe^{2+}.[20] The results show that functional Fe NPs synthesized by grape leaf extract have the potential to remove acid Orange II.

4 Conclusion

In this study, grape leaf extract can be used for the green synthesis of Fe NPs, which in turn remove acid Orange II. Biomolecules in methanolic extract of grape leaves involved in the synthesis of Fe NPs were identified by GC-MS. Two new and major findings emerge. Firstly, phytols, β and δ sitosterols, amyrin and vitamin E were used as both reducing and capping agents due to their functional groups: C=C, -OH, =O, where -OH, =O were oxidized to -

317 COOH and Fe^{2+} was reduced to Fe NPs; while C=C was capped on the Fe NPs' surface.
318 These processes enhanced the Fe NPs' stability and ability to resist oxidation. Secondly,
319 biomolecules such as 4-eicosadiene and δ-stan-3,5-diene only acted as capping agents in the
320 synthesis of Fe NPs since they contained two double bonds, thereby preventing the oxidation
321 of Fe NPs and improving the stability of Fe NPs. Furthermore, the potential biomolecules that
322 could function as reducing and capping agents include polypholes, alkaloids and terpenoids,
323 which was confirmed by FTIR. However, it required further confirmation. Finally, 80.0% of
324 acid Orange Ⅱ using Fe NPs was removed, indicating two things: firstly, Fe NPs were highly
325 reactive when synthesized by methanolic extract of grape leaves; and secondly, Fe NPs could
326 represent a potentially cost-effective and environmentally friendly remediation technique.
327

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332

333 ## References

334 1 G. Spigno, T. Pizzorno and D. M. De Faveri, *Bioresour. Technol.*, 2008, **99**, 4329-4337.
335 2 S. O. Prozil, D. V. Evtuguin and L. P. C. Lopes, *Ind. Crops Prod.*, 2012, **35**, 178–184.
336 3 D. O'Carroll, B. Sleep, M. Krol, H. Boparai and C. Kocur, *Adv. Water Resour.,* 2013, **51**,
337 104–122.
338 4 S. Iravani, *Green Chem.*, 2011, **13**, 2638–2650.
339 5 A. K. Mittal, Y. Chisti and U. C. Banerjee, *Biotechnol. Adv.*, 2013, **31**, 346-356.

340 6 G. E. Hoag, J. B. Collins, J. L. Holcomb, J. R. Hoag, M. N. Nadagouda and R. S. Varma,
341 2009, *J. Mater. Chem.*, 2009, **19**, 8671-8677.
342 7 L. L. Huang, X. L. Weng, Z. L. Chen, M. Megharaj and R. Naidu, *Spectrochim. Acta A.*,
343 2014, **117**, 801-804.
344 8 K. M. Kumar, B. K. Manda, K. S. Kumar, P. S. Reddy and B. Sreedhar, *Spectrochim. Acta*
345 *A.*, 2013, **102**, 128-133.
346 9 T. Wang, X. Y. Jin, Z. L. Chen, M. Megharaj and R. Naidu, *Sci. Total Environ.* 2014, **466**,
347 210–213.
348 10 X. Zhang, S. Lin, Z. L. Chen, M. Megharaj and R. Naidu, Water Res. 2011, **45**, 3481-3488.
349 11 L. Rustioni, D. R. Bedgood, O. Failla, P. D. Prenzler and Kevin Robards, *Food Chem.*
350 2012, **13**, 22194–2201.
351 12 L. L. Huang, X. L. Weng, Z. L. Chen, M. Megharaj and R. Naidu, *Spectrochim. Acta A*
352 *Mol. Biomol. Spectrosc.,* 2014, **130**, 295-301.
353 13 T. Shahwan, S. AbuSirriah, M. Nairat, E. Boyac, A. E. Eroğlu, T. B. Scott and K. R.
354 Hallam, *Chem. Eng. J.* 2011, **172**, 258-266.
355 14 J. A. S. Mendes, A. M. R. B. Xavier, D. V. Evtuguin and L. P. C. Lopes, *Ind. Crops*
356 *Prod.*, 2013, **49**, 286-291.
357 15 J. Garrido and F. Borges, *Food Res. Int.,* 2013, **54**, 1844-1858
358 16 N. Yang and W. H. Li, *Ind. Crops Prod.*, 2013, **48**, 81-88.
359 17 P. Lu and Y. L. Hsieh, *Carbohydr. Polym.*, 2012, **87**, 2546-2553.
360 18 N. V. Farinella, G. D. Matos and M. A. Z. Arruda, *Bioresour. Technol.* 2007, **98**, 1940-
361 946.

19 T. C. Prarthna, N. Chandrasekaran, M. A. Raichur and A. Mukherjee, *Colloids Surf. B.*, 2011, **82**, 152-159.

20 Y. Kuang, Q. P. Wang, Z. L. Chen, M. Megharaj and R. Naidu, *J Colloid Interface Sci.,* 2013, **410**, 67-73.

21 A. Saeed, M. Sharif and M. Iqbal, *J Hazard Mater.*, 2010, **179**, 564–572

22 Z. X. Chen, X. Y. Jin, Z. L. Chen, M. Megharaj and R. Naidu, *J Colloid Interface Sci.,* 2011, **363**, 601-607.

371

372 **Figure captions**

373 Fig. 1 UV-vis spectra between methanolic and water (a) extracts of grape leaf (b) Fe NPs

374 Fig. 2 The removal efficiency of Fe NPs mediated by different extractants

375 Fig. 3 GC-MS spectra for (a) methanolic extract; (b) methanolic extract after reacting with

376 Fe^{2+} solution

377 Fig. 4 FT-IR spectra (a) methanolic extract; (b) methanolic extract after reacting with Fe^{2+}

378 solution

379 Fig. 5 SEM image of Fe NPs

380 Fig. 6 EDS spectrum for the Fe NPs

381 Fig. 7 XRD pattern for the Fe NPs

382 Fig. 8 Comparative removal efficiency of Orange Ⅱ (average of three times) using various

383 materials

384 Table 1 The classification for mass spectrometry of main biomolecules

385

386

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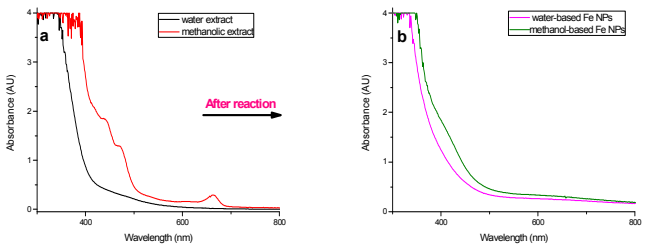


Fig. 1

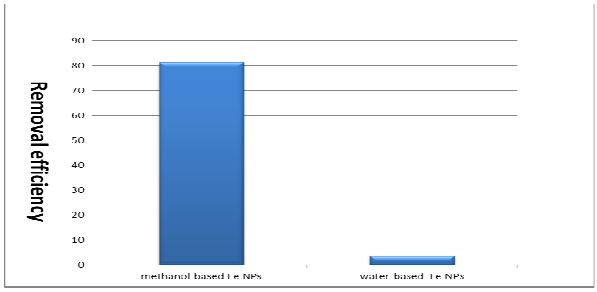


Fig. 2

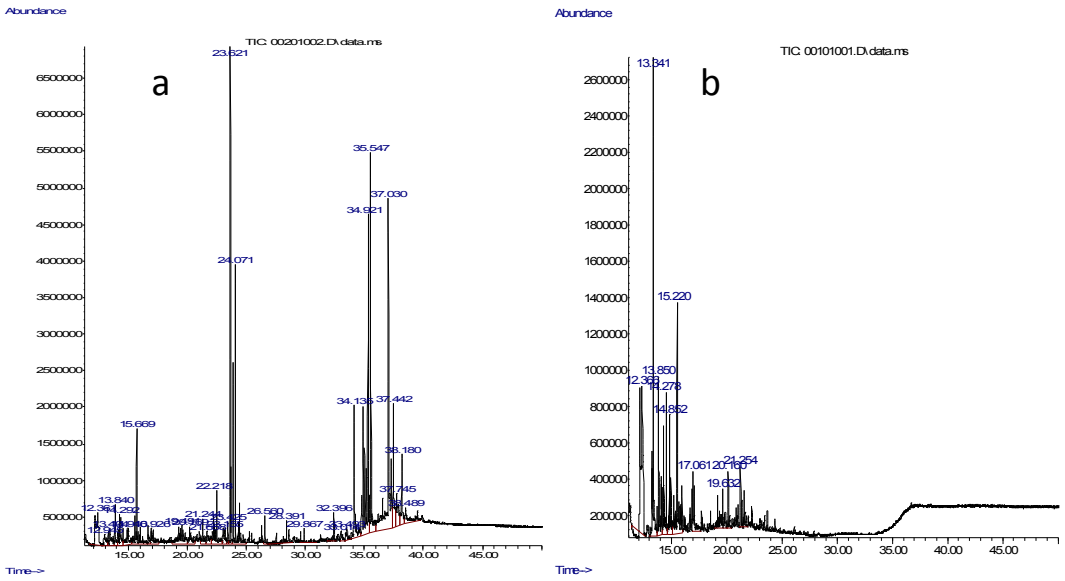


Fig. 3

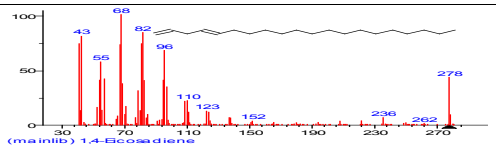
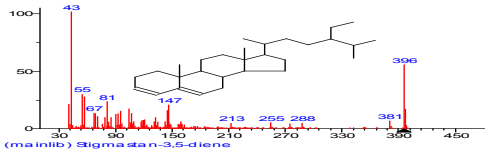
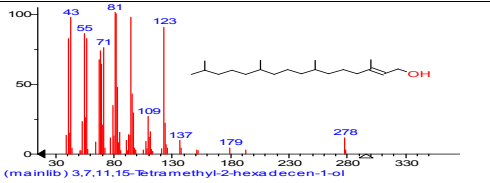
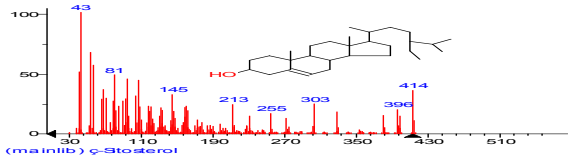
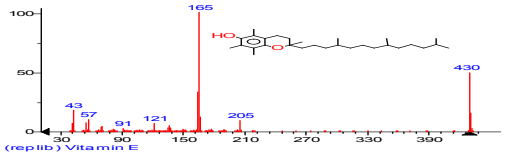
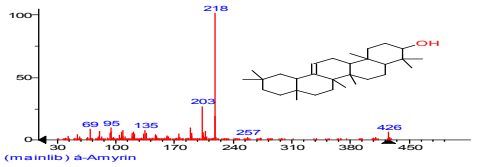
Function	Name/Retention time (min)	Information details
Capping agent	**1,4-Eicosadiene** Rt=24.071	
	δ-stan -3,5-diene Rt=34.921	
Capping and Reducing agent	**Phytols** Rt=23.521/23.899	
	β, δ -Sitosterol Rt=34.621/37.030	
	Vitamin E Rt=35.547	
	α-Amyrin Rt=37.442	

Table 1

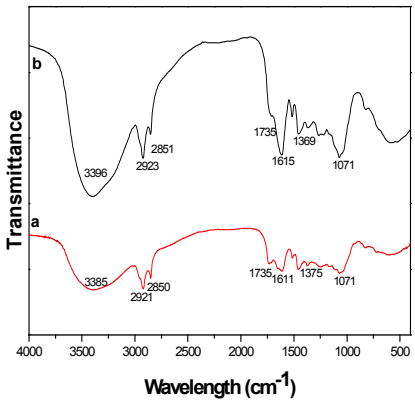


Fig. 4

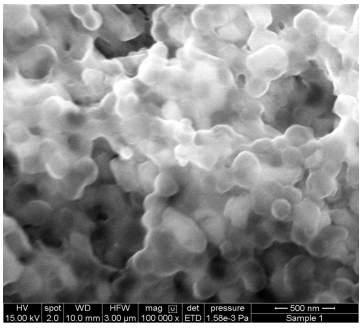


Fig. 5

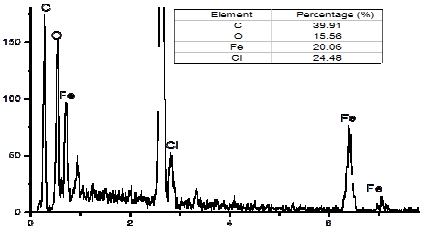


Fig. 6

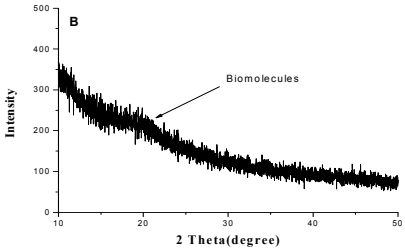


Fig. 7

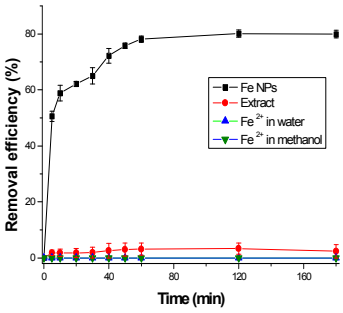


Fig. 8

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