green synthesis and characterizaton of iron-oxide nanoparticles by guava aqueous leaves extract for doxorubicin drug loading
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Abstract
A green biosynthesis of iron oxide nanoparticles (Fe3O4 Nps) was carried out in one step. An aqueous extract of orange peels, green tea, and guava leaves were utilized as precipitating agent for metal precursors. The guava leaves extract was the most powerful one. The shape and size of (Fe3O4 Nps) were monitored by transmission electron microscopy. The existence of iron in the yield was studied by UV-visible spectroscopy. The stability of the particles was estimated by hydrogen peroxide reaction. The (Fe3O4 Nps) were incubated with human red blood cells (RBCs). The osmotic fragility test for (RBCs) showed no significant shifting from the control. The loading of doxorubicin cytotoxic drug was primitively monitored by scanning electron microscopy for further study plan.

Keywords: iron-oxide nanoparticles, green biosynthesis, doxorubicin

1 Introduction
Iron-oxide nanoparticles (Fe3O4NPs) have became the strong candidates for many biomedical application due to, their small sizes beside the magnetic properties (Monalisa P et al., 2013)1,2. It is important to choose the row material for (Fe NPs) preparation otherwise the methods for the adjustable physical and chemical properties of interest. Among the methods of preparation for these (Fe NPs) coprecipitation, thermal decomposition sonochemical methods are the most. In addition electrochemical and green syntheses are introduced by many researches (Akl M et al., 2012).3

Entrapping nanoparticles with drugs is a great challenge nowadays. FDA approved doxorubicin hydrochloride drug as liposome based has been used for treatment of cancer (XU et al., 2013)4.In the current work, we utilize the aqueous Guava leaves extract to produce iron-oxide oxide nanoparticles. The shape and size of these Fe NPs are observed by Transmission electron microscopy. The Doxorubicine drug was loaded on the prepared MNPs and the rate of drug loading efficiency was evaluated.

2 Materials and Methods
Preparation of plant extract
Fresh Leaves of guava, green tea, and orange plants were collected from the local markets in Alexandria city, Egypt. The leaves were washed twice with distilled water after that they were left to dry. 200 mg of dried leaves were squashed and incubated in 100
ml double distilled water in 250 ml beaker overnight. The aqueous leaf extract taken and the leaves debris were discarded. The leaves extract was centrifuged then filtered by Whatman filter paper twice to exclude any remnant debris. The clean aqueous extract was preserved in -20 °C for further use.

Characterization of iron-oxide nanoparticles

Ultraviolet-Visible absorption (UV-Vis)

Ultraviolet-visible spectroscopy (UV-Vis) refers to absorption spectroscopy in the UV-Visible region. This means it uses light in the visible and adjacent (near-UV and near-infrared) ranges. The absorption in the visible region directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions.

Fourier transformer Infrared analysis (FT–IR)

FTIR was used to identify the peak value of the functional groups of the active components by infrared irradiation. The sulfate group is responsible of the reduction of metal ions via oxidation of aldehyde groups in the molecules to carboxylic acids. The UV Visible spectrum of Fe3O4-NPs in the aqueous guava extract is shown in. The two absorption peaks at wavelengths of 402 nm and 415 nm indicate the formation of iron nanoparticles.

3 Results and discussion

Determination of total antioxidants

The total antioxidant activity (TAA) of plant leaves aqueous extract were determined by β-carotene bleaching method adopted from Kaur C et al. Oxidation of the β-carotene emulsion was assessed by spectrophotometer at 10-min interval at 470 nm. TAA was expressed as per cent β-carotene inhibition relative to control according to Equation 1

\[ \text{TAA} = \frac{\text{Absorbance (control} - \text{sample})}{\text{Absorbance of control}} \]

The preparation procedure was adopted from the previous work of (Aki M. Awwad et al., 2012 and Harajyoti M et al). Ferrous sulphate solution of 2 m mann 

Osmotic fragility test

Fragility of red blood cells (RBCs) was estimated after incubation of the cells with iron-oxide
nanoparticles. The osmotic fragility was adopted from H. A. Massaldi, et al incubated with BSR20 ul of Fe3O4 different concentration of Fe3O4 nanoparticles for about 6 hours at 37 °C was added to a serial dilution of normal physiological saline solution with different osmolality % from the following Equation 2:

\[
\text{Hemolysis\%} = \frac{\text{absorbance of the test}}{\text{absorbance of complete hemolysis}} \times 100
\]

Frability of red blood cells (RBCs) was estimated after incubation of the cells with iron-oxide nanoparticles. The osmotic fragility was adopted from H. A. Massaldi, et al ). 20 ul of RBS incubated with different concentration of Fe3O4 nanoparticles for about 6 hours at 37 °C was added to a serial dilution of normal physiological saline solution with different osmolality % from the following Equation 2:

**DOXO drug loading to MNPs**

Loading procedure was adopted from (Davaran et al., 2012). 2 ml of DOXO was added to 1 gm of dried MNPS. The mixture was stirring magnetically for 24 hours at room temperature. The Doxo-loaded MNPs was separated with centrifugation for further analysis. The percent of Doxo-loading were deduced by the relation Equation 3

\[
\text{Doxo loading efficiency} = \frac{\text{weight of Doxo after incubation}}{\text{weight initial Doxo}}
\]

The peaks at 1540 and 1105 cm\(^{-1}\) are attributed to the asymmetric and symmetric stretching vibration of COO\(^{-}\). The band at 1105.99 cm\(^{-1}\) can be assigned to the symmetric C–O vibration associated with aC–O–SO\(_3\) group. In addition, signals at 3698 cm\(^{-1}\) (OH stretching) and 2358 ad 23269 cm\(^{-1}\) (CH stretching) were also observed.

The presence of magnetite nanoparticles can be seen by two strong absorption bands at around 398 , 380, and 362 cm\(^{-1}\) which Figure 2, corresponding to the Fe-O stretching band of bulk magnetite (Fe3O4). These results revealed that the C=O groups were bonded on the magnetite particle surface. Overall the observation confirms the presence of organic compounds in guava leaf extract, which acts as a reducing agent and stabilizer for magnetite nanoparticles. These results are in co-ordination with the results of Nahmaz M et al.

The osmotic fragility results are represented in Figure 4, there is no significant skewness of the graph and control graph without Fe3O4 NPs this finding may support that Fe3O4 NPs have no osmotic stress on the RBCs integrity under these conditions.

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5 **References**


