Biosynthesis of iron oxide nanoparticles from dates, characterisation, and investigation of anticarcinogenic and antimicrobial properties

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Abstract

Nanomaterial synthesis using natural biological systems, especially plant-based green synthesis, has displayed great excitement and interest. This study synthesised iron oxide nanomaterials from three different varieties of date palms obtained from Srivilliputhur, Tamil Nadu, India. The extract of date palms reduced ferric chloride in the solution and facilitated the formation of iron oxide nanomaterials. Phytochemical studies of the extracts obtained from all three varieties of date palms indicated the presence of phenols. The synthesised nanomaterials were characterised by UV-VIS spectroscopy, SEM, EDAX, XRD and FTIR methods. The ability of the synthesised nanomaterials to inhibit human microbial pathogens proliferation was tested in *Staphylococcus* sp. and *Bacillus* sp. by turbidity method and recorded the maximum antimicrobial property against *Bacillus* sp. in the iron nanomaterial synthesised from Sukhri date palm. The cytotoxic potential of the iron oxide nanomaterials against two human cancer cell lines (A-549 and MDA-MB-231) by MTT assay. The iron oxide nanomaterials from Sukhri date palm detected a substantial cytotoxicity response.

Keywords: Anticancer; antimicrobial; date palms; green synthesis; iron oxide nanomaterials.

1. Introduction

The demand for producing nontoxic, hygienic, eco-friendly solvents is growing. Hence, the synthesis of nanoparticles using biological systems and organisms has been given more importance (Das & Brar, 2013; Mahdavi *et al.,* 2013; David *et al.,* 2014; Kajani *et al.,* 2016; Muthu & Priya, 2017). The biological synthesis of nanoparticles is being exploited commercially to a more significant extent (Mahdavi *et al.,* 2013). Plant leaf and fruit extracts are used to synthesise iron oxide nanomaterials because of their nontoxic and ecofriendly nature and their benefits in pharma industries (Das *et al.,*2014). A plant's system has inorganic substances mostly found in nanoscale sizes (Raveendran *et al.,* 2003). The extracts from various plant parts produce biochemical nanoparticles of different sizes and compositions. Therefore, green synthesis of iron nanoparticles obtained using plant parts such as leaf and fruit extracts are most feasible, and they need not require cell culture maintenance. Besides, nanoparticles' size, shape and dispersity are controlled effectively in plant cell extracts. (Ghorbanpour, 2015; Pansare *et al.,* 2016; Yadav *et al.,* 2017; Rivera-Rangel *et al.,* 2018). Bioreduction of metal ions occurs during the synthesis of nanoparticles from various plant parts. The constituents in plant cell extracts are vital for the reduction of iron ions, while water-soluble heterocyclic compounds are involved in the stabilisation of nanoparticles generated. Ferric chloride is also used to remove plant extracts (Senthil & Ramesh, 2012). Iron nanomaterials have exceptional antibacterial activity (Oladipo *et al.,* 2017; Rajan *et al.,* 2017). Moreover, the size of these iron oxide nanomaterials played a vital role in controlling their effects (Karthik *et al.,* 2016; Asiabani *et al.,* 2017; Lebaschi *et al*., 2017). Iron nanocrystals have also exhibited added effectiveness when equated to cisplatin in lysing HeLa cells (Gao *et al.,* 2007; Nadagouda *et al.,* 2008; Song *et al.,* 2010).

Date fruits from varieties such as Ambera, Sukhri and Khudri possess high nutritional value and are effective for healing diseases. These three varieties of date palms contain the following nutrients, viz., 5.33 g of carbohydrates, 0.3 g of fat, 0.6 g of dietary fibre, 4.5 g of sugar, 0.17 g of protein, 0.012 mg of Vitamin B 6 or pyridoxine, 0.07 mg of iron, 47 mg of potassium and 3 mg of magnesium. Besides these nutrients, they also possess substantial amounts of calcium, sulphur, manganese, copper and phosphorus. They have a high nutritional profile offering several health benefits. Date palms are a major source of fiber, iron, numerous nutrients, and antioxidants, providing health benefits from better digestion and a reduced risk of diseases. They are essential in treating several diseases due to their low glycemic index, fiber content, and antioxidants. They also promote brain health and bone health.

Moreover, they possess excellent antimicrobial activities (Al-orf *et al.,* 2012; Bouhlali *et al.,* 2015; Saafi *et al.,* 2011). Date palm varieties contain vitamins and numerous essential amino acids (Dash *et al.,* 2013). Phytochemicals found in the fruit of the date palm serve as potential metal reducing and stabilising agents (Philip, 2010). Fruits of date palms were chosen for the study because they are edible, most preferred dry fruits, and have better nutritive and therapeutic values. The synthesis of metal nanoparticles using plant extracts, inactivated plant parts, and fruit extracts is a modern alternative for their production. Recent research indicates that the major components of the dates exhibit antioxidant, antidiabetic, antimicrobial and anticancer activity. The present investigation aimed to develop a simple process to produce iron nanomaterials from date palms, their characterisation and evaluation of the potential of the nanomaterial in terms of antimicrobial and anticancer activity.

2. Materials and methods

2.1 Collection of date palm fruits and preparation of Extract

Three varieties of date palms, such as Ambera, Sukhri and Khudri, were obtained from various supermarkets such as RGN Nanayam supermarket, VRN Babu supermarket, and Alagapuri farm in Srivilliputhur, Tamil Nadu, India. These three varieties of date palms have been imported from Egypt and Saudi Arabia. First, the three varieties of date palms were dried in a hot air oven to remove the moisture content. Then, 10 g of the three varieties of date palms, such as Ambera, Sukhri and Khudri, were boiled in 200 ml of distilled water until the volume was reduced to half, and thereby the extracts of date palms were prepared.

Fig. 1. Three varieties of date palms used in the study, A. Ambera, B. Sukhri. C. Khudri

2.2 Chemicals

The chemical Ferric chloride hexahydrate ($FeCl_3.6H_2O$, 98%) and the solvents used in the investigation were pure analytical grades purchased from Sigma Aldrich.

2.3 Synthesis of Iron oxide nanomaterials

It was synthesised using a modified protocol obtained from previous studies (Pattanayak *et al.,* 2013; Ramya *et al*., 2012; Li., 2007). First, 20 g of ferric chloride was added to 200 ml of ethanol, mixed well, and 200 ml of ether was added to the mixture. The mixture was then transferred to the extracts obtained from three varieties of date palms and placed on a magnetic stirrer, Lanphan ZNCL-BS model, at 150 rpm for 90 min. to ensure proper mixing of the particles. Reduction of ferric chloride to ferric oxide occurred, and the modification showed the synthesis of iron oxide nanomaterials in colour to reddish-brown. The solution was then filtered, and the filtrate was kept in a hot air oven at 60◦C to form a powder.

2.4 Characterisation of Iron oxide nanomaterials

The iron oxide nanomaterials synthesised were characterised by performing different spectroscopic and analytical techniques (Jahja *et al.,* 2018). After 24 hours of incubation, the nanoparticle reaction medium was centrifuged at 10,000 rpm for 15 min. Then, the supernatant was subjected to UV-VIS Perkin-Elmer Lambda 25 spectrophotometer studies. The size and shape of the nanomaterials were observed with a Scanning Electron Microscope (SEM)with a very thin layer of platinum (Pt) (BAL-TECSCD 005 sputter coater) to increase the sample surface contrast and avert charge buildup. SEM pictures were attained using a Zeiss LEO instrument (Model 1455VP).

The microstructure of iron oxide nanomaterials was analysed by X-ray diffraction (XRD), Philips PW 11/90 diffractometer with nickel filtered CuKα (l = 1.5405 Å). The X-ray diffractometer was fitted with a nickel foil filter having a thickness between 15 – 25 µm. Using the Debye-Scherrer equation, the iron oxide nanocrystals' average diameter (D) was measured from the broadening of the XRD peak intensity after $K\alpha_2$corrections.

Fourier Transformation Infra-Red (FTIR) spectroscopy examined the samples' dissimilarities in chemical groups. FTIR spectroscopy has been used as a technique for the investigation of the characteristic functional groups of iron oxides. The wavelength of light absorbed is characteristic of the chemical bond reflected in the annotated spectrum. Therefore, the chemical bonds in a molecule were calculated by interpreting the infrared absorption spectrum. The FTIR spectra of the samples were recorded with a Perkin Elmer Spectrophotometer (Model Perkin Elmer-1600 series) in the range of 400 – 4000 cm^{-1} using the KBr pellet technique. FTIR studies were conducted on compressed pills prepared by mixing iron oxide powder with potassium bromide.

2.5 Phytochemical analysis of date palms

Phytochemical analysis of the three varieties of date palms was carried out. The procedure is as follows:

a) Terpenoids: sample extract (1 ml) + chloroform (2 ml) + concentrated H2 SO4. Reddish Brown colouration was found at the interface layer.

b) Flavonoids ($AlCl_3$): 1ml of extract + 1ml of 1% $AlCl_3$. The appearance of a light-yellow colour.

c) Phenols: Folin-Ciocalteau reagent + Sodium Carbonate + extract. The appearance of dark green colour.

d) Saponins (Lead Acetate test): 1ml of extract + 1% lead acetate. Formation of white precipitate.

e) Cardioglycans: 1ml of extract + 1ml of glacial acetic acid + $FeCl_3$ + Concentrated H_2SO_4.

f) Tannins: 1ml extract + 5ml distilled water + few drops of 5% neutral solution (5% $FeCl_3$ in 90% alcohol).

g) Alkaloids (Mayer’s test): 2ml of extract + 2drops of Mayer’s reagent. Formation of a creamy white precipitate.

2.6 Determination of the antimicrobial activity of Iron oxide nanomaterials

Turbidity examined the antimicrobial action of the synthesised nanomaterials against bacteria such as *Staphylococcus* and *Bacillus*. Broth cultures of *Staphylococcus* and *Bacillus* were transferred to the extracts of the three different samples of date palms containing iron oxide nanomaterials at different concentrations such as100µl, 250µl and 500µland. The turbidity was determined using a colourimeter. The positive control contained the culture with an antibiotic, whereas the negative control contained only the culture (Sudhama & M, 2019).

2.7 Determination of the anticancer activity of iron oxide nanomaterials

The synthesised iron oxide nanomaterials' anticancer properties were tested against two cell lines such as A-549 and MDA-MB-231 by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. In addition, the anticancer potential of the iron oxide nanomaterials was compared with the standard drug, cisplatin (Sudhama, V. N. & M. Ramakrishnan, 2020).

3. Results and discussion

3.1 Biosynthesis of iron oxide nanomaterials

Biomaterials and biochemical compounds present in the date palm extract caused the conversion of iron precursor salts into iron oxide nanomaterials and served as stabilising agents. The appearance of reddish-brown colour in the extracts of date palms showed the formation and presence of iron oxide nanomaterials (Figure 2). The colour arises due to surface plasmon vibrations in the iron oxide nanomaterials.

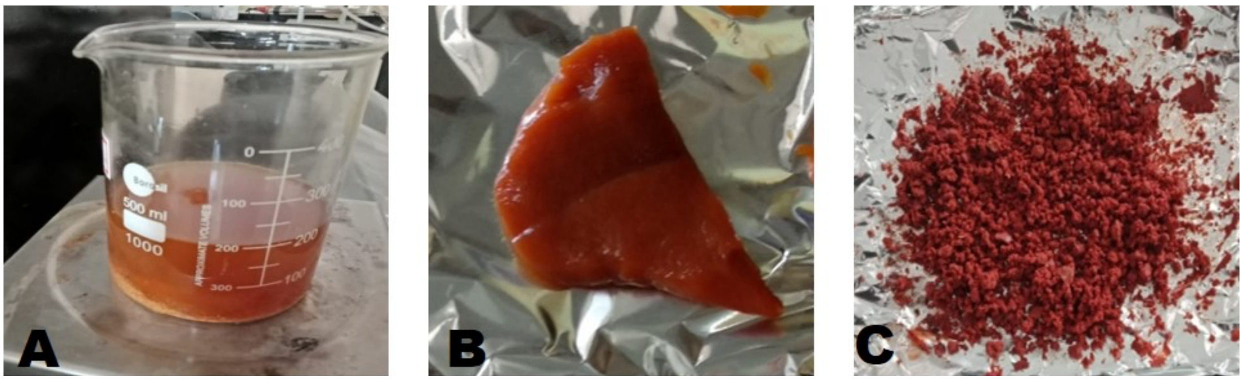


Fig.2. Process of synthesis of nanomaterials from date palms. A. Fe nanoparticle extraction. B. The paste obtained after filtration C. The powder obtained after drying

3.2 Characterisation of Iron oxide nanomaterials

The iron oxide nanomaterials have been subjected to UV-VIS spectrophotometric analysis. Absorption peaks were spotted at 270 nm and 390nm due to the iron metallic core hydrolysis products. Synthesis of nanomaterials from plant sources exhibits strong captivation of electromagnetic waves in the visible range due to their visual resonant stuff, called Surface Plasmon Resonance (SPR) due to excitation of surface plasmon vibrations. The frequency and width of the surface plasmon absorption depend on the size and shape of the metal nanomaterials and the dielectric constant of the metal itself and the surrounding medium. The absorption of visible radiations due to the excitation of SPR imparts various colours to the nanomaterials. As the nanomaterial size changes, the colour of the solution also changes (Breck, 2016). Hence, the UV-VIS absorption spectrum is quite sensitive to the formation of nanomaterials.

Table 1. Absorption spectra of Iron oxide nanomaterials in UV-VIS spectrophotometer

S.No.	Varieties of dates	Absorption spectra in UV-VIS Spectrophotometer	
		Lowest absorption peak (nm)	Highest absorption peak (nm)
1.	Ambera	275	398
2.	Sukhri	273	395
3.	Khudri	270	390

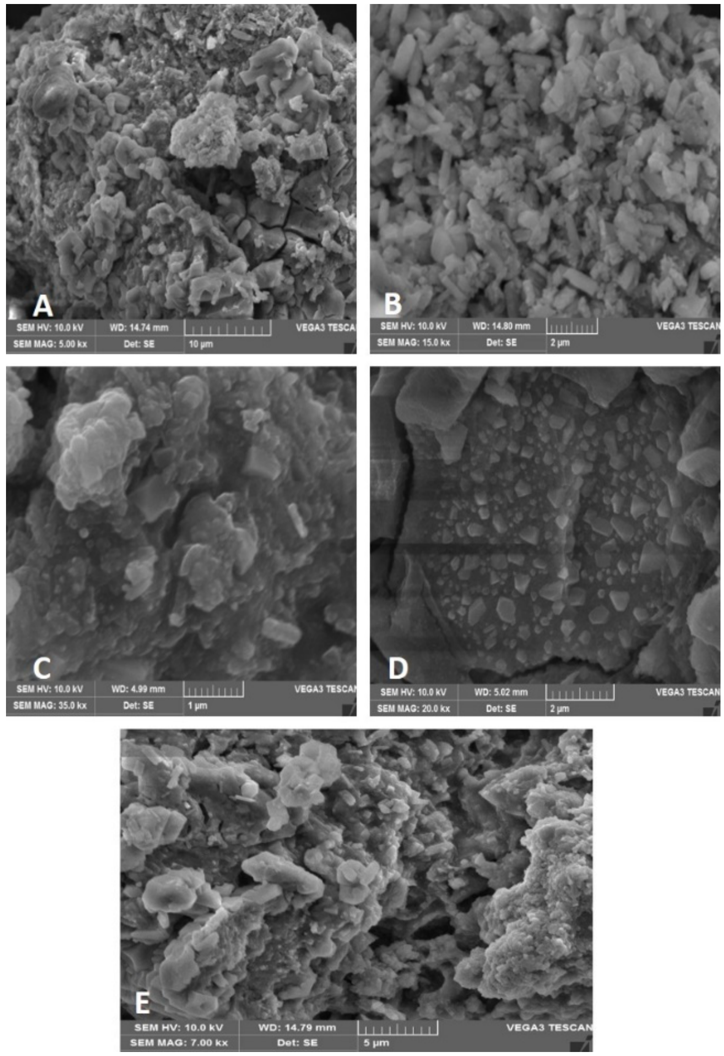
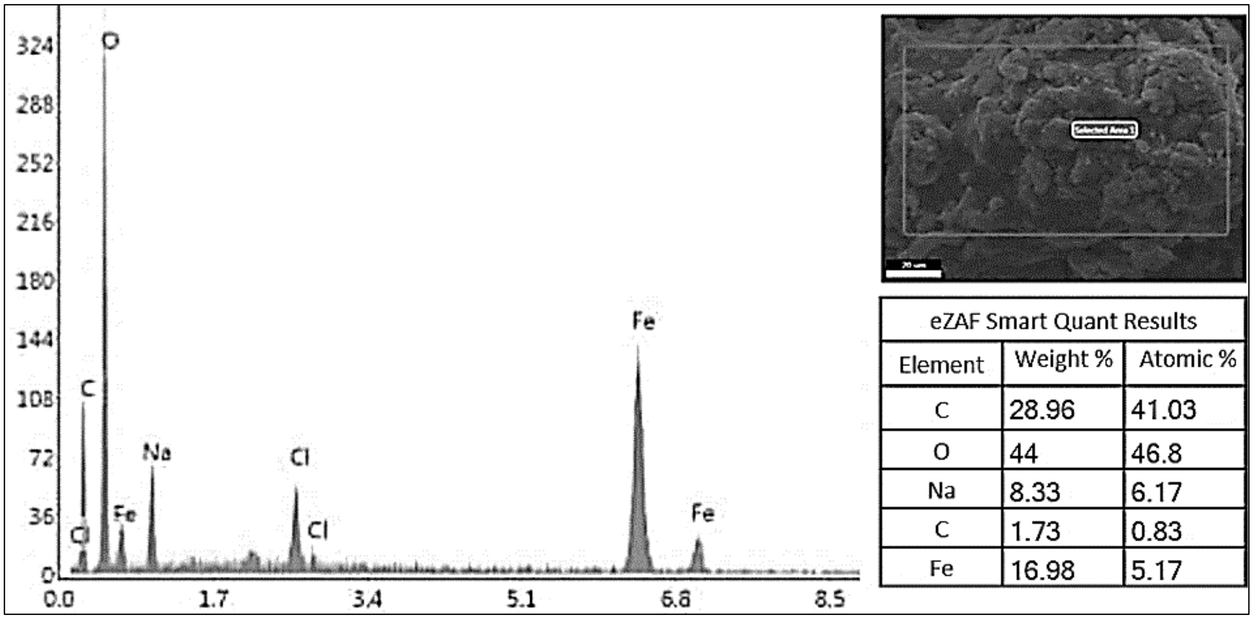


Fig 3. SEM micrographs of iron oxide nanomaterials synthesised from date palms.

The nanostructure of iron oxide nanomaterials was further investigated using SEM. The SEM micrographs obtained from the ready sample exhibited that iron oxide nanomaterials are sphere shaped and rod-shaped, clustered together and bear an overall rough morphology with aggregation in solution (Figure 3).

EDAX spectrum (Figure 4) shows the presence of compounds such as carbon, oxygen, sodium, potassium and chloride in the reaction mixture or the date palm extract other than iron oxide nanomaterials. The date palm extract recorded 17% iron and 44% oxygen in the total weight of Fe_2O_3NPs. This is based on the bremsstrahlung X-ray intensity as a function of energy. Potassium modifies the general form of the enzyme iota, revealing the suitable chemically active sites for reaction. It also defuses various organic anions and other compounds within the date fruits, serving to ease the optimum pH between 7.0 and 8.0, suitable for most enzyme reactions



eZAF Smart Quant Results		
Element	Weight %	Atomic %
C	28.96	41.03
O	44	46.8
Na	8.33	6.17
C	1.73	0.83
Fe	16.98	5.17

Fig.4. EDAX spectrum of iron oxide nanomaterials in the selected area and their characteristics.

The features of the molecular structure of iron oxide nanomaterials were explored in XRD data (Figure 5), the XRD pattern is $2\theta = 31.2^{o}$, 44.3^{o} and 45.1^{o}andthe XRD geometry data of the iron oxide nanomaterials corresponded well to rhombohedra geometry. The typical size of the synthesised nanomaterials was calculated in nanometers from the XRD peaks using the Scherrer equation. The range of the peak received by X-ray diffraction is based on the apparatus and crystallite size (Jahja *et al.,* 2018). The small particle below 100 nm in size broadening from the apparatus is negligible. Scherrer's method permits the calculation of the crystal size: DRX= 0.9 lambda/Δ cosθ, where 0.9 is a dimension constant of the equipment, lambda is the wavelength of the radiation corresponding to the peaks, and θ is the Bragg's angle. Hence, the grain of the particle is 80-90nm.

The infra-red spectra (IR) of the synthesised iron oxide nanomaterials were acquired by an FTIR spectrometer (Figure 4); they were used to find the possible biomolecules accountable for the reduction and stabilisation of the nanomaterials, and the IR spectra were collected between 400 and 4000cm^{-1}. The IR spectrum of iron oxide nanomaterials displayed prominent absorption bands at 705, 845, 1115, 1295, 1480, 1650, 1960 and 3650 cm^{-1}and would have resulted from (O-H) stretching vibration. The IR portion of the electromagnetic spectrum has been separated into 3 regions, viz. near, middle and far infrared, named for the visible spectrum (1980 – 2450cm^{-1}). The C = O stretching has also been observed in the IR spectrum.

3.3 Phytochemical analysis of three date palm varieties

Among the phytochemicals, terpenoids and phenols were produced by all three varieties of date palms, whereas cardioglycans were found only in one variety i. e. Ambera. None of the varieties produced flavonoids, saponins, tannins and alkaloids (Table 2).

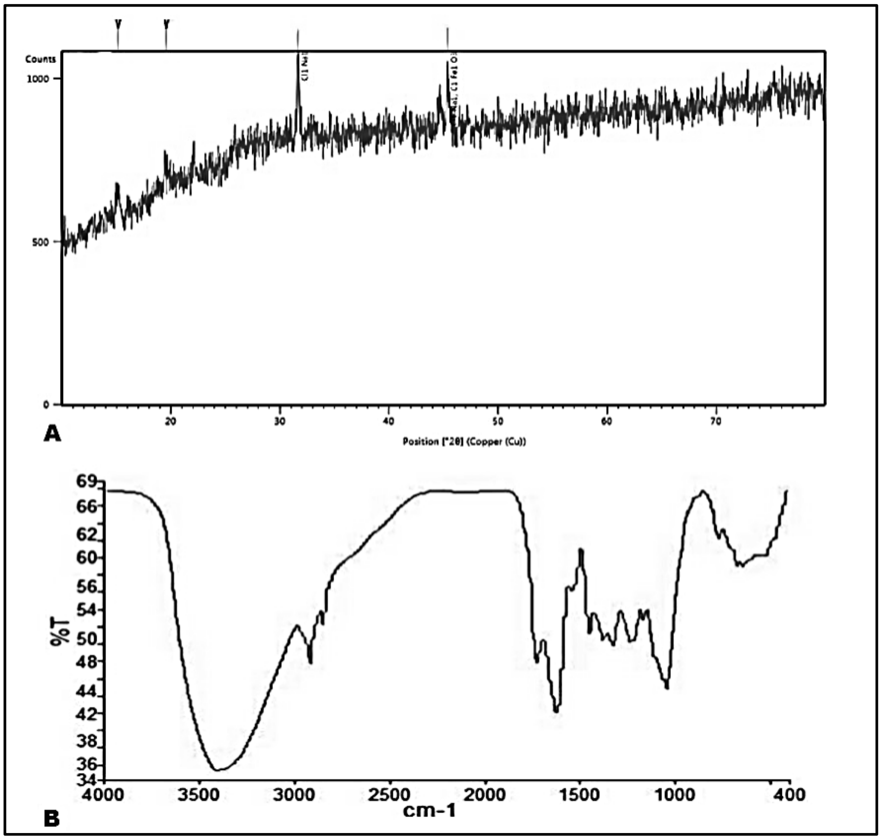


Fig. 5. A- XRD pattern of the iron oxide nanomaterials and B. FTIR spectrum of the iron oxide nanomaterials

Table 2 Phytochemical constituents of three varieties of Date palms

Dates varieties	Terpenoids	Flavonoids	Phenols	Saponins	Cardioglycans	Tannins	Alkaloids
Ambera	++	-	+++	-	+++	-	-
Sukhri	+++	-	++	-	-	-	-
Khudri	+++	-	++	-	-	-	-

-not detected, ++moderate quantities, +++ high quantities

3.4 Determination of the antimicrobial activity of synthesised iron oxide nanomaterials

The antimicrobial property of the synthesised iron oxide nanomaterials revealed that they have high antimicrobial activity against *Staphylococcus* when compared with the standard antibiotic, ampicillin (Table 3). In contrast, moderate antimicrobial activity was observed against *Bacillus.* Synthesised iron oxide nanomaterials have equal antimicrobial activity compared to date palm fruit extract. Moreover, results reveal that the more susceptible bacteria were *Staphylococcus* and the more resistant were *Bacillus*. The iron oxide nanomaterials were naturally stabilised and has more surface area that could be used for various applications.

Table 3. Turbidity readings are taken at 600nm (*Staphylococcus* sp. and *Bacillus* sp.)

Extracts	Growth inhibition (absorbance at 600nm)					
	Staphylococcus sp.			*Bacillus* sp.		
	100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
Ambera	0.09	0.12	0.07	0.61	0.72	0.94
Sukhri	0.11	0.10	0.09	0.82	0.08	0.68
Khudri	0.15	0.16	0.13	0.84	0.69	0.90
Fe NP	0.20	0.26	0.30	0.88	0.62	0.19
+ve Control		0.18			0.93	
-ve Control		0.25			0.90	

± Values are the mean of triplicates

3.5 Determination of the anticancer activity of synthesised iron oxide nanomaterials

The iron oxide nanomaterials were assessed for anticancer activity in human cancer cell lines exclusively, adeno carcinomic alveolar basal epithelial cell line (A 549) and breast cancer cell line (MDA – MB – 231) by the MTT assay method (Senthilraja & Kathiresan, 2015).

MTT assay of the synthesised iron oxide nanomaterials effectively against the cancer cell lines (Figure 5), and the half-maximal inhibitory concentration IC_{50} can be a source to determine the cytotoxicity concentration for testing the behaviour of probable drug molecule under these experimental conditions. Therefore, the iron oxide nanomaterials obtained from Ambera, Sukhri and Khudri extracts were tested for repressive activities against the human adeno carcinomic alveolar basal epithelial cell line, A 549. The test sample concentration, 400 µg/ml of iron oxide nanomaterials, was used based on the value of IC50, 491.5 µg/ml. The assay exhibited that 44% of the lung cancer cells (A 549) had been obstructed by the iron oxide nanomaterials obtained from the Sukhri variety date palm extracts. The viability was thus reduced to 56%, making the iron oxide nanomaterials from date palms a green substitute for treating malignant cancer (Figure 5). Moreover, the iron oxide nanomaterials obtained from date palm extracts were also examined for inhibitory activity against the MDA-MB-231 cell line with the sample concentration as 400 µg/ml since the IC_{50} value was 290 µg/ml. The results showed that 80% of the breast cancer cells (MDA - MB – 231) were inhibited, as the viability of the iron oxide nanomaterials obtained from date palms was 20%. The results reflect that the iron oxide nanomaterials from date palms effectively against cancer cells (Figure 5). The synthesised nanomaterials, their morphology, uniform distribution without any segregation, the FTIR spectroscopy findings to demonstrate physicochemical interaction between nanomaterial and moieties, X-ray diffraction analysis for the formation of nanostructures and its crystallinity are vital for the characterisation of nanoparticles (Farah *et al.,* 2021).

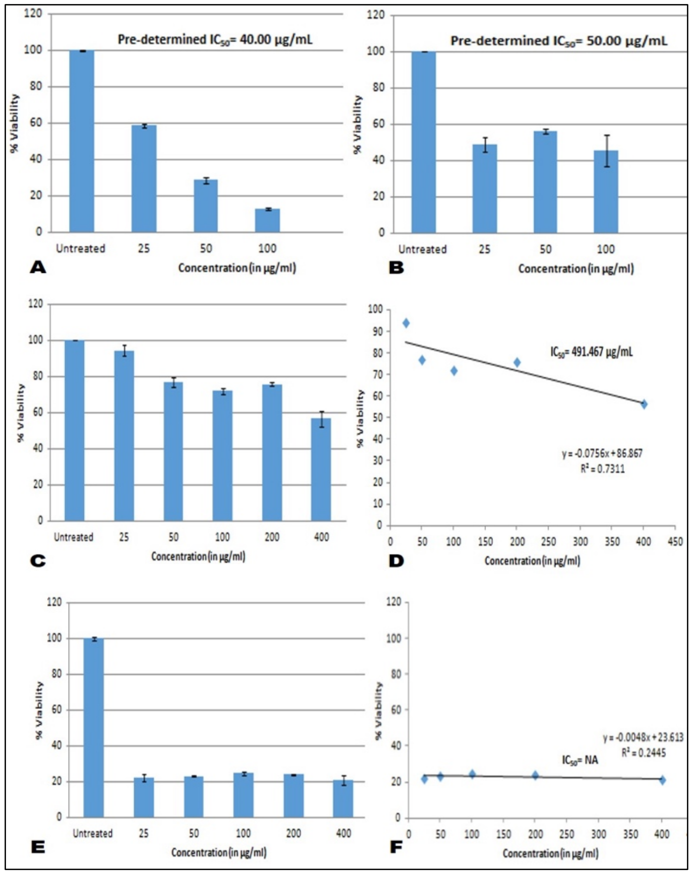


Fig. 6. MTT assay to test the anticancer effect of Fe NP and drugs on the breast (MDA MB 231) and lung cancer cell lines (A549). A. Effect of Cisplatin drug on the breast cancer cell line, B. Effect of Cisplatin drug on the lung cancer cell line, C. Effect of different concentration of Fe NP on lung cancer cell lines, D. IC_{50} Concentration of Fe NP on A549, E. Effect of different concentration of Fe NP on the breast cancer cell line and F. IC_{50} Concentration of Fe NP determined on the breast cancer cell line.

4. Conclusion

Accomplished the eco-friendly synthesis method of iron oxide nanomaterials from three varieties of date palms, characterised the iron oxide nanomaterials using state-of-the-art techniques, and determined the sizes. The phytochemicals present in the extract of date palm fruits have served as capping and reducing agents and have stabilised the iron oxide nanomaterials. The iron oxide nanomaterials from the Sukhri date palm variety have shown substantial antibacterial and anticancer activity against human pathogens, *Bacillus* sp. and lung cancer cell lines (A 549). The yield of iron oxide nanomaterials obtained by the green synthesis method would be the most reasonable approach due to its nontoxic and eco-friendly nature. The major outcomes of the present study would form a basis for future research in iron oxide nanomaterials synthesis from dates and its therapeutic application studies.

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