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Original Research Article

The Antibacterial Activity of Ginkgo Biloba Extract and Its Green Synthesis of Iron Nanoparticles

Sundus Hameed Ahmed[1*]

[1]Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

***Corresponding Author:** Sundus Hameed Ahmed
Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

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Abstract: ***Background*:** The ancient plant species ginkgo biloba is thought to provide numerous health benefits to all living creatures. This plant is very bioactive and has a wide range of chemical constituents. There are several proven pharmacological and therapeutic benefits of *G. biloba*. ***Objective***: The objectives of the present study were to produce iron nanoparticles (FeNPs), isolate a natural compound from Ginkgo biloba leaves, and evaluate the antibacterial properties of the resultant nanoparticles (NPs). ***Materials and Methods***: Five extracts of Ginkgo biloba leaves were produced through various methods: cold water extraction, hot water extraction, cold alcoholic extraction, hot alcoholic extraction, and 70% alcoholic extraction. FeNPs biosynthesis occurred by reacting these Ginkgo biloba leaf extracts with iron sulfate Fe2 (SO4)3. Analysis techniques employed included GC/MS, SEM, XRD, and UV-Vis analysis. ***Results***: The iron nanoparticles (FeNPs) utilized in this using scanning electronic microscopy measurement and UV-VIS spectroscopy, which has a characteristic peak at 217nm, it was possible to detect irregular structures with dimensions of no more than 41 nm. AFM was also used in this study to examine the NPs-Fe's size and morphology. The FTIR measurement also revealed the principal iron oxide bands at 685 and 596cm-1, which are caused by Fe-O vibrational stretching. Bioactivity was also carried out against certain bacteria. ***Conclusions*:** Antimicrobial activity showed an inhibiting effect on a variety of harmful bacteria. *Klebsiella spp., Staphylococcus epidermidis and Candida albicans*.

Keywords: Spectroscopy, Aqueous Extracts, Ginkgo *Biloba,* FeNPs.

INTRODUCTION

The well-known plant species *Ginkgo biloba* has a multitude of bioactive components that contribute to its chemical variety and are thought to provide several health benefits to living creatures. Numerous pharmacological and therapeutic benefits of *G. biloba* have been shown. Ginkgo has been used in Chinese traditional medicine since at least the eleventh century C.E [1].

Ginkgo seeds, leaves, and nuts have historically been used to cure a variety of ailments, such as bronchitis, asthma, kidney and bladder problems, and dementia. However, there isn't much8] [2, 3] Ginkgo leaf extracts contain phenolic acids, proanthocyanidins, flavonoid glycosides (myricetin, kaempferol, isorhamnetin, and quercetin), and the terpene trilactones ginkgolides and bilobalides [1, 2]. The leaves also contain unique ginkgo biflavones, polyprenols, and alkylphenols [4]. Ginkgo leaf-containing medications have been shown to be effective in treating moderate age-related dementia and mild peripheral vascular disease in individuals following major illnesses, according to the European Medicines Agency Committee on Herbal Medicinal have been told not to by a doctor [5]. Although ginkgo leaf extract is often taken as a dietary supplement, there is no proof from science that it improves human health or effectively treats any illness [6, 7].

Applications for iron oxide nanoparticles are numerous and include sensors, solar energy conversion, environmental protection, drug delivery, and tissue repair [6]. The scientific community is more concerned about the production of iron nanoparticles due to their wide range of applications. These iron nanoparticles are also successfully

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applied in the therapy and diagnostic fields [7, 8]. Iron oxide nanoparticles are used as an adsorbent in wastewater treatment due to their exceptional capacity to remove a wide range of water pollutants.

The current study's goals were to manufacture iron nanoparticles (FeNPs), extract a natural substance from Ginkgo biloba leaves, and assess the newly formed nanoparticles' (NPs') antibacterial properties.

MATERIAL AND METHOD

Botanical Extracts

The following five Ginkgo biloba leaf extracts are made:

Cold Water Extraction

The preparation of cold water extract is done using [9], method. Add 500mL of distilled water to it. After the beaker was placed in the vibrating incubator for a full day at 37°C, the water extract was first filtered via a Buchner funnel fitted with a piece of gauze. After being centrifuged for 15 minutes at 2500rpm, the resulting filtrate was incubated for 48–72 hours at 37°C. Until used, the dried filtrate is kept in a refrigerator at 4°C.

Hot Water Extraction

Take, 500mL of sterile distilled water is added to 100 grams of powdered Ginkgo biloba leaves to create the hot water extract. After being in a Soxhlet apparatus for a full day, the extract filtrate is dried at 40°C in an incubator and kept in storage at 4°C until required.

Cold Alcoholic Extraction

To prepare cold alcoholic extract, weigh out 100 grams of powdered Ginkgo biloba leaves, transfer them into a beaker, and then add 600mL of ethanol alcohol. After the beaker was placed in the vibrating incubator for a full day at 37 °C, the alcoholic extracts were filtered twice: once using Whatman No. 1 filter paper and once using a Buchner funnel with a piece of gauze. After centrifuging the filtrate for 15 minutes at 2500rpm, it was incubated for 48–72 hours at 40–45°C to produce a dry extract. It was then stored at 4 °C in the refrigerator until it was needed [10].

Hot Alcoholic Extract

One hundred grams of powdered Ginkgo biloba leaves were weighed, put inside a piece of gauze, and the soxhlet device was filled with 700 milliliters of 99% ethanol alcohol for a full day. After going through the procedure multiple times to get an adequate amount of the active ingredient, it was dried and put in an incubator between 40 and 45°C until it was suitable for use [11].

70% Alcohol Extraction

400 milliliters of the extract were produced when 100 grams of powdered *Ginkgo biloba* leaves were weighed and combined with 70% alcohol, or 420 milliliters of ethanol alcohol and 180 milliliters of distilled water [12].

Phytochemical Detection

Fresh, green *Ginkgo biloba* leaves were raised for this experiment in the College of Science's greenhouse. 10 grams of healthy leaves were cleaned, chopped, and in 0.1L double-distilled water for 30 minutes. Chemicals are used to create nanoparticles. Using the following two methods,

"The broth was filtered, brought to room temperature, and then maintained" at 4°C. Plants help decrease carbon dioxide agents [5]. Plant powder is dissolved in an aqueous solution that is distinct and all-inclusive. The test tube was shaken ferociously after the solution was added. A dense, persistent froth that forms over time may indicate the presence of soap1 [10]. When 5mL of the plant extract were combined with B-(1-3) milliliters of 1% mercuric chloride solution ($HgCl_2$), a white precipitate was generated, indicating that the detection was successful [11].

4% HCl is Hydrochloric Acid. Turbidity's Appearance Suggested the Presence of Resins

Ten milliliters of each extract were combined with twenty milliliters of distilled water that had been acidified with hydrochloric acid (4% HCl). Turbidity's there are two ways to find something: First, add a few drops of Marquis Reagent to one milliliter of the extract. The presence of alkaloids was indicated by the color changing to a gritty gray. Second, the presence of alkaloids was demonstrated by the white precipitate or cream that formed after one milliliter of the extract was treated with Mayer's reagent [12].

Proteins

The test solution was treated with a 4% NaOH solution. According to [13], the presence of proteins is shown by the emergence of a violet color after adding 1% $CuSo_4$ solution.

Biosynthesis of FeNPs

An aqueous extract of ginko leaves at concentration 0.2mg/mL was continually heated to 80°C, 0.01g of ferrous sulfate $Fe_2(SO4)_3$ was added, and the liquid instantly became black. After the solution cooled, the pH was measured again, and it came out to be 8.74. The colloidal solution of nanoparticles was kept at 4°C.

Assessment of the Prepared Nanoparticles' Characteristics

Nanoparticles were examined based on the color shift of the reaction mixture. The absorbance of the reaction mixture was measured between 200 and 700nm at 30-minute intervals. Zeta potential analysis established the typical size of the nanoparticles. We employed to quantify the generated FeNPs' hydrodynamic size and zeta potential. A red laser with a 633nm wavelength and a 173 degree scattering angle. Averaging of the measured particle sizes was done [14, 15].

Analysis of Nanoparticles

The following tools were utilized by Baghdad University of Technology's Nanoscale Research Center to study iron nanoparticles: Please list some of the features that a UV-Vis spectrometer exposes. UV light was used to examine the optical (meterteehsp 8001) from 300 to 800 nm [16].

Scanning Electron Microscope (SEM)

The type of scanning electron microscope (TESCAN-VEGA / USA) that Using 30 kv electric voltage and 3 nm beams.

X-ray (Shimadza Maxima-A)

The crystal phases and sizes were determined using the X-Ray Diffract (XRD700) with an electric voltage of 40KV, a current of 30MA, and a scanning range of (100.000-20.000) degrees". Making use of copper (Cu) Using tubes with a copper wavelength of 1.54A, the XRD patterns were acquired within 0.12 seconds of the scan speed [17].

Scanning Electron Microscope (SEM)

A 30 kilovolt electrical voltage and a 3 nanometer wavelength were used when using a scanning electron microscope (TESCAN-VEGA) [15].

GC/MS for Analysis

Two fused silica capillaries (film thicknesses of 0.3, 0.2, and 0.2 mm) and one polar stationary phase StabilaxR (film thicknesses of 0.6, 0.2, and 0.2 mm) were utilized for the GC/MS analysis. The following was the column's temperature profile: 50 °C for three minutes, then an ascent of 2 °C each minute to 250 °C, and a hold of 15 °C per minute at that temperature. At 250 degrees Celsius, split mode injection was performed.

The carrier gas (He) flowed at a rate of 1.2 mL/min. The Mi70eV was used. The sample was then injected into the microlitre once it was decided where to look for the components. By comparing the relative retention indices obtained from a series of C5-C28 n-alkanes injected into the GC and GC/MS under identical conditions as the oils with those in the data bank (Wiley library) and the literature, we were able to ascertain the ionization mechanism. The same criteria that applied to the oils were also used to compare these indices [16].

The Bacterial That Use in Study

The carrier gas (He) flowed at a rate of 1.2mL/min. The Mi70eV was used. The sample was then injected into the microlitre once it was decided where to look for the components. By comparing the relative retention indices obtained from a series of C5-C28 n-alkanes injected into the GC and GC/MS under identical conditions as the oils with those in the data bank (Wiley library) and the literature, we were able to ascertain the ionization mechanism.

Fill each of the five milliliter bottles with the medium, and autoclave the bottles to get rid of any remaining debris. Then, before continuing, let the medium come to room temperature. Pure bacterial colonies should then be added to the medium. The bacteria that are growing on the conveyor belt (Loop) and impeding progress should be the source of these pristine colonies. -The optimal temperature for bottles is 20 degrees Celsius.

Using a clean culture diffuser (Spreader), apply 0.1 milliliter of the bacterial suspension that has already been created in the spine onto the surface of the culture medium. The culture was allowed to absorb and the medium was allowed to dry after the plates were exposed to the room's air for twenty to thirty minutes. Subsequently, six extract tablets (200µg/ml) and six FeNPs pills (200µg/ml) were meticulously placed, with the use of sterile forceps and gentle to moderate pressure, atop the culture material. The dishes were left out in the open for the entire day and night at a height of 37 meters, as per the specified parameters. The procedure of measuring led to the discovery of the findings [19]. States that the regions of inhibition brought on by the extract and FeNPs identified the resistance (Resistant) or sensitivity (Sensitive) of the bacteria.

RESULTS AND DISCUSSION

Phytochemical Component Detection in Extracts of Ginkgo Biloba

Table 1 lists the phytochemical substances found in Ginko biloba extracts. The results demonstrate that the following chemicals are present in cold, hot, and mixed alcohol extracts as well as in 70% alcoholic water extract, flavonoids, glycosides, phenols, protein, and coumarins.

All extracts are free of terpens, steroids, alkaloids, and carbohydrates; however they do include resin, both hot and cold alcoholic. All extracts, with the exception of hot alcoholic extract, include phenols [10].

Table 1: Phytochemical compound in M. oleifera extracts

No.	Detection type	Reagent used	Indication of reagent	cold Alcoholic Extract	Cold Water Extract	Hot water	Hot Alcoholic Extract	70% Alcoholic Extract
1	Tannins Test	ferric chloride test	Greenish black colour	+	+	+	+	+
2	Glycosides Test	Benedict detector	A red precipitate appears	+	+	+	+	+
3	Phenols Test	ferric chloride	Development of violet or dark blue colour	+	+	+	_	+
4	Resins Test	acidified water HCL	turbidity appears	+	_	_	+	_
5	Flavonoids Test	alcoholic potassium hydroxide 50%	Yellow colour in bottom layer	+	+	+	+	+
6	Saponin Test	mercuric chloride solution	Thick foam that stays on for long	+	+	+	+	+
White Precipitate		+	+	+	+	+	+	+
7	Protein Test	-	-	+	+	+	+	+

The reason for using UV-vis spectroscopy is that nanoparticles interact with wavelengths, this technique that was applied to verify the particle creation. The first indication that Ginkgo biloba plant extract was the cause of the iron ion-based nanoparticle production in the presence of rosemary plant extract was a shift in color from yellow to brown. The 400 nm surface plasmon resonance (SPR) signal served as confirmation for this which attests to the extract's nanoparticle content (Figure 1). The unbound electrons in the nanoparticles have a resonant frequency at specific wavelengths, which causes SPR. A relationship between absorption and particle size can be established, and the SPR peak can be lowered, by reducing the particle size [13, 14]. There was a difference in the compounds produced by the nanoparticles manufactured from rosemary extract.

Gc-Mass

The GC-Mass spectroscopy fragments show the following compounds with its percentages:

5-Methyl-2-trimethylsilyloxy-benzoic acid trimethylsilyl ester, region 3-deutrophrnyl-4-phenylpyrazole trimethylsilyl ester, 47.12%; 2-octene (E), 27.03%; and 4-amino-2(1H)-pyridine, 20.202-1-(2,5-dimethoxy-4-methylphenyl)-15.17%,(+)-(1R,4S)-1,7,7-trimethyl-3-methylenebicyclo(2,2,1)-1 propanone-11.24 percent heptane-2-one 5-trimethylsilyl ester's trimethylsilyl ester Bis(2-ethylhexyl) phthalate, 34.71%, (+)-"(1R,4S), 5-methyl-2-Trimethyl-2-trimethylsilyloxy-benzoic acid area 39.053%2,2,1)-trimethyl-3-methylenebicyclo-1,7,7-trimethyl"-heptan-2-one, 15.139%, dodecamethyl-, 14.345%, and cyclohexasiloxane, 14.345%."

Ginkgo biloba extract Phenol, 4-(2-aminoethyl)-, 36.609%, Heneicosane, 28.453%, and Nonadecane, 25.2825, according to the Gc - MASS result Cycling [4.4.0]12-Oxatricyclo[4.4.3.0(1,6)]; dec-1-ene, 2-isopropyl-5-methyl-9-methylene-, 20.836%3,11- 19.762% dione, 1H-Cycloprop[e][1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)] azulen-7-ol, decahedron-1,1,7-trimethyl-4-methylene--, 19.648%.

The extract revealed sixteen chemicals through investigation, however the nanoparticles revealed only six, and the results of the tests on both the extract and the nanoparticles demonstrated that the compounds are entirely distinct from one another. As a result of the reaction that occurred, new chemicals Tables 2 and 3.

Table 2: GC-MS results of iron particles for "(*Ginkgo biloba*) extract

No	RT (min)	Area%	Name	Quality	CAS Number
1	4.237	15.17	2-Propanone, 1-(2,5-dimethoxy-4-methylphenyl)-	46	029907-70-8
2	12.959	47.12	3-DEUTEROPHENYL-4-PHENYLPYRAZOLE	59	024768-85-2
3	14.345	10.66	Cyclohexasiloxane, dodecamethyl-	58	000540-97-6
4	15.139	11.24	(+)-(1R,4S)-1,7,7-trimethyl-3-methylenebicyclo(2,2,1)-heptane-2-one	52	016161-84-5
5	34.71	8.54	Bis(2-ethylhexyl) phthalate	38	000117-81-7
6	39.053	7.28	TRIMETHYLSILYL ESTER OF 5-METHYL-2-TRIMETHYLSILYLOXY-BENZOIC ACID	30	000000-00-0

Table 3: GC_MS results of *Ginkgo biloba* extract

No	RT (min)	Area%	Name	Quality	CAS No.
1	4.237	5.66	[1,2'-"Binaphthalene"]-5,5',8,8'-tetrone, 1',4-dihydroxy-2,3'-dimethyl-, (-)-	91	020175-84-2
2	5.659	5.39	1,3,5,5-Tetramethyl-1,3-cyclohexadiene	94	004724-89-4
3	6.816	27.03	2-Octene, €-	25	013389-42-9
4	9.052	5.89	Phenol, 2-methoxy-	55	000090-05-1
5	12.524	20.20	4-Amino-2(1H)-pyridine	49	000000-00-0
6	12.959	8.78	4-(trimethylsilyl)dibenzofuran	64	017943-24-7
7	14.345	2.97	Cyclohexasiloxane, dodecamethyl-	52	000540-97-6
8	15.139	3.31	3-Acetylnoradamantane	43	058275-58-4
9	16.42	2.13	TRANS(.BETA.)-CARYOPHYLLENE	99	000000-00-0
10	17.977	3.25	"Silanamine, N-[2,6-dimethyl-4-[(trimethylsilyl)oxy]phenyl]-1,1,1-trimethyl-"	25	072088-09-6
11	19.648	1.68	"1H-Cycloprop[e]azulen-7-ol, decahedron-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-"	95	006750-60-3
12	19.762	1.38	12-Oxatricyclo[4.4.3.0(1,6)]tridecane-3,11-dione	47	034813-12-2
13	20.836	1.98	"Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-"	91	000000-00-0
14	25.282	4.81	Nonadecane	98	000629-92-5
15	28.453	2.77	Heneicosane	98	000629-94-7
16	36.609	2.77	Phenol, 4-(2-aminoethyl)-	30	000051-67-2

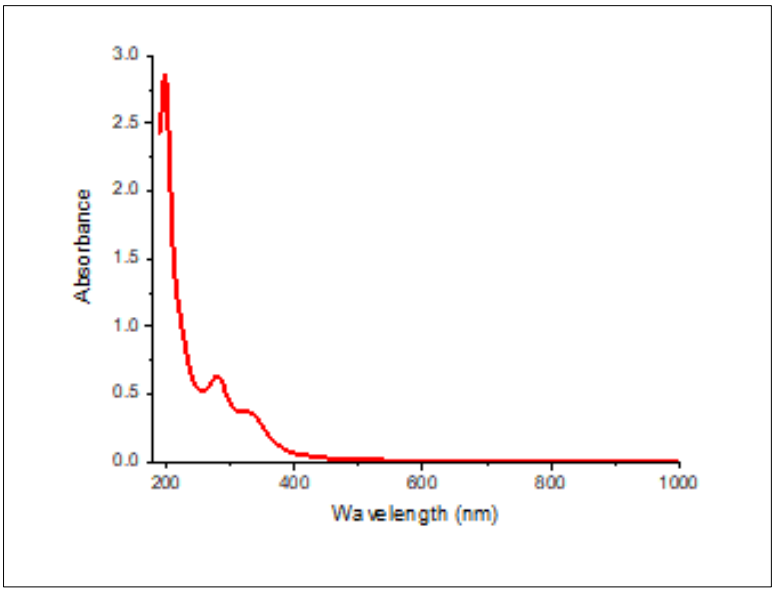

Figure 1: UV-Vis of IronNPs

1. UV-Vis

The majority of metallic nanomaterials and their oxides exhibit distinctive SPR peaks in the UV-Vis spectrum. FeNPs displayed a distinctive peak at 217 nm that is consistent with data from earlier research [15]. According to Fig. (1), "the spectrum also showed some low-intensity peaks which may be attributed to the electronic transitions for the active groups in the active" compounds in the extract.

2. SEM

These attributes were ascertained using the SEM technique, which is employed to ascertain the size and form of FeNPs. The measurement revealed the existence of asymmetrical structures that were no larger than 41 nm. The measurement might be clearer in the TEM measurement since the surface is devoid of visible particles, which is proof of the manufactured particles' modest size Figure (2).

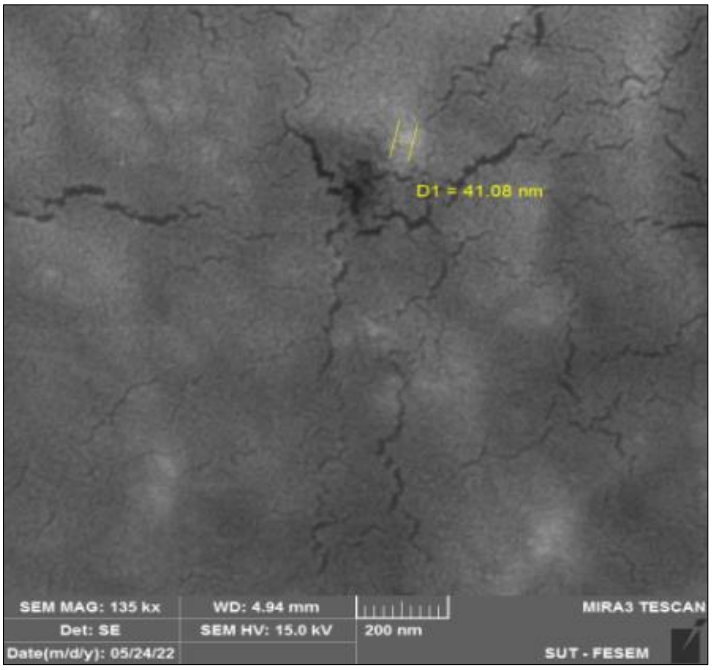

Figure 2: SEM of IronNPs

3. XRD

The iron oxide was shown to be amorphous by the XRD analysis, which revealed a broad peak at 22.64 degrees. A low-intensity peak centered at 36.39 degrees and another peak located at 46 and 0.84 degrees demonstrate the formation of FeNPs e with few crystalline characteristics. Figure 3.

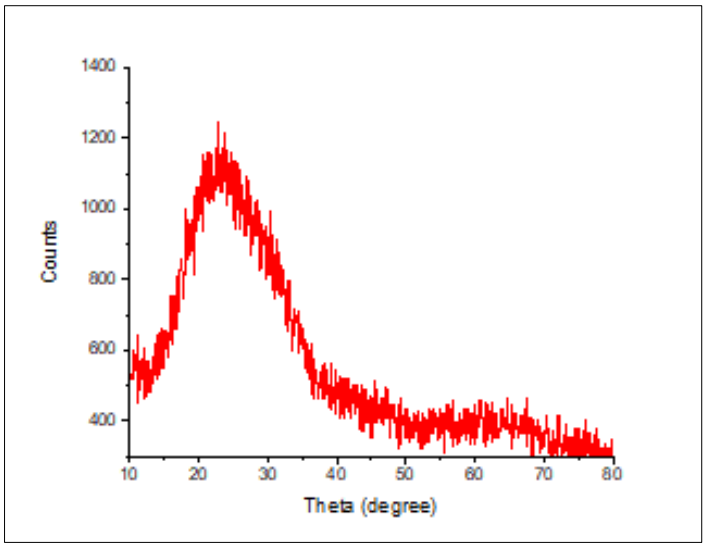

Figure 3: XRD of FeOAG

Characterization of IronNPs Nanoparticles

The stretching vibrational and bending of OH in water are responsible for the bands observed in the FTIR spectra of iron oxide nanoparticles at 3315 and 1633 cm-1, respectively. Since these materials may absorb water on their surfaces due to their high surface area, the existence of these bands is indicative of the production of nanoparticles.

Due to Fe-O vibrational stretching, the measurement also revealed the main bands of FeNPs at 685 and 596cm^{-1} [4].

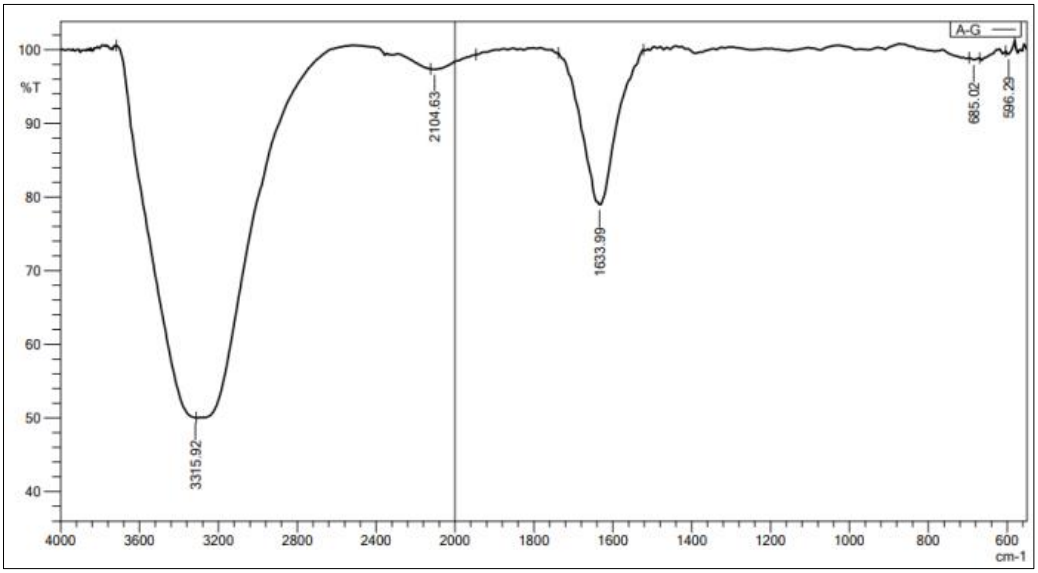

Figure 4: FTIR of IronNPs

The Antibacterial Activity

The antibacterial efficacy of a particle is determined by its small size and rounded shape. Our results are consistent with several previous studies showing strong antibacterial action. Table 3 shows biosynthesized aqueous leaf extract Antibacterial FeNPs exist. S. aureus was discovered when compared to aqueous extract results, the two bacteria that were most inhibited by iron nanoparticles were S. epidermidis [23mm] and [28mm] respectively. These findings align with [16].

Table 3: Antimicrobial activities of IronNPs

Strain	Wayer Extract (200µg/ml)	FeNPs(200µg/ml)
S.aureus	15mm	28mm
S.epidermidis	18mm	23mm
K.pneumoniae	13mm	19mm
Candida albicans	12mm	21mm

In Wang *et al.*, (2020) [17], research, they showcased the eco-friendly production of iron nanoparticles utilizing Ginkgo biloba leaf extract. These nanoparticles displayed strong antibacterial properties against various bacteria types, encompassing both Gram-positive and Gram-negative strains, such as methicillin-resistant S. aureus (MRSA) and multidrug-resistant E. coli strains. Furthermore, the study highlighted the biocompatibility of these nanoparticles, indicating promising prospects for their utilization in biomedical fields.

Iron nanoparticles offer potential in antibacterial uses owing to their natural antimicrobial attributes. Researchers have effectively synthesized iron nanoparticles with heightened antibacterial potency by utilizing Ginkgo biloba extract as a reducing agent. This eco-friendly synthesis method not only diminishes environmental repercussions but also maintains the bioactivity of Ginkgo biloba components, potentially bolstering the antibacterial effectiveness of the nanoparticles [18, 19].

CONCLUSIONS

- Ginkgo biloba extract was successfully synthesized using five different methods.
- Iron nanoparticle of Ginkgo biloba was successfully synthesized.
- Different physicochemical techniques were used to characterize the new iron NPs.
- Ginkgo biloba extract offers a sustainable approach for producing antimicrobial agents with enhanced efficacy and biocompatibility.
- The leaf extract shows lower bioactivity than the new FeNPs.

- ❖ It is recommended to conduct additional research on the special FeNPs as they show promise as anticancer materials.
- ❖ Future research endeavors should focus on elucidating the mechanisms underlying the antibacterial activity of Ginkgo biloba constituents and optimizing nanoparticle synthesis protocols for broader clinical applications.

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