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Exploring The Antioxidant And Antimicrobial Potential Of Acmella Oleracea: Synthesis And Evaluation Of Iron Nanoparticles

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Abstract

Plants have antioxidant, antibacterial, anti-inflammatory, and anticancer properties. Due to their phytochemical content, plants have several properties. These exercises may aid in the treatment and prevention of certain ailments. Ascorbic acid and *Acmella oleracea* extracts extracted in hexane, chloroform, and ethanol were evaluated for antioxidant activities. The ethanol extract showed the highest antioxidant activity, with inhibitory percentages of 51.53% at 100 µg/ml, 23.467% at 200 µg/ml, and 4.63% at 300 µg/ml. All percentages resulted in an IC50 value of 99.9 µg/ml. Hexane extract has moderate antioxidant activity, with inhibition rates of 43.766%, 30.014%, and 16.221% at the same dosages. Additionally, the IC50 value was 54.7 µg/ml. The chloroform extract was least active. With an IC50 value of 26.6 µg/ml, ascorbic acid remains the strongest antioxidant. These extracts produced iron nanoparticles that were examined using XRD. The study found significant peaks at 24.393°, 32.275°, and 35.772°, indicating hematite (α-Fe2O3) and magnetite (Fe3O4). The Debye-Scherrer equation predicted a 50-nanometer nanoparticle size. SEM showed the form was largely spherical with minimal aggregation. However, energy dispersive x-ray spectroscopy (EDAX) detected iron, oxygen, carbon, sodium, aluminum, and chlorine. These observations suggest that the hexane extract has antioxidant action and that the iron nanoparticles produce benefits for potential applications. *Acmella oleracea*-derived iron nanoparticles displayed diverse levels of antimicrobial efficacy as evidenced by a clearly defined zone of inhibition. The results indicate that iron nanoparticles have potential for investigation in the formulation of antimicrobial coatings, medical devices, or therapy applications that require specific antibacterial or antifungal characteristics.

1. Introduction

Since prehistoric times, medicinal herbs, have been identified and utilized in the practice of traditional medicine throughout history. Plants are capable of producing hundreds of different chemical compounds for a variety of purposes, including defense and protection against herbivorous mammals, fungi, insects, and illnesses. There is abundant evidence from a variety of sources that demonstrates the connection between man and his hunt for pharmaceuticals in nature dating back to the distant past. This evidence includes written documents, preserved monuments, and even original plant remedies. In the latter half of the 19th century and the early 20th century, there was a significant risk that medicinal plants would be no longer used in treatment. There are numerous writers who have written that the pharmaceuticals that were obtained from them had many drawbacks because of the destructive action of enzymes. These enzymes induce fundamental changes throughout the process of drying medicinal plants, which means that the healing action of medicinal plants is dependent on the mode of drying utilised. Plants are utilized not just for therapeutic purposes but also for commercial interests as well. The enhancement of the value of medicinal plants is of utmost significance, not only for the commercial application of raw medicinal goods but also for the therapeutic benefit they provide (Rao et al., 2022).
In order to combat the oxidative stress that is brought on by the sun and oxygen, plants manufacture a significant amount of antioxidants. As a result, plants have the potential to serve as a source of novel molecules that possess antioxidant activity (Scartezzini and Speroni, 2000). Natural antioxidants have the ability to boost the plasma's antioxidant capacity, which in turn lowers the chance of developing certain diseases, including cancer, cardiovascular disease, and stroke (Prior and Cao, 2000). Several studies have demonstrated that secondary metabolites derived from plants, such as flavonoids and phenolics, has the ability to effectively neutralize free radicals. Every portion of a plant, including the leaves, fruits, seeds, roots, and bark, contains instances of these organisms (Mathew and Abraham, 2006).
Throughout the course of human history, plants have served as a source of inspiration for the creation of novel pharmaceuticals and drugs derived from plants, both of which have made significant contributions to the health and well-being of humans (Vashisht and Jindal, 2012). Plants are used for the synthesis of nanoparticles from dates back. Since nanoparticles are so simple to create and control, the vast bulk of research that has been conducted on nanomaterials has been on nanoparticles. For the synthesis of nanoparticles, physical and chemical methods are typically utilized. However, because to the numerous limitations of existing approaches, the focus of research has recently switched towards the development of synthesis protocols that are both clean and environmentally benign. Due to the fact that they possess a

number of distinct characteristics, iron nanoparticles are an important class of inorganic nanoparticles that can be utilized in a variety of different fields (Harlekar et al., 2014).
Infectious diseases are a significant contributor to morbidity and death rates among the general population, particularly in nations that are still in the process of building their economies. In light of this, pharmaceutical companies have been pushed to create novel antimicrobial medications in recent years, particularly as a result of the persistent appearance of microbes that are resistant to existing antimicrobials (Silva and Fernandes, 2010). The incidence of hazardous bacteria that are resistant to antibiotics has been seen to increase over the course of the past several decades, despite the fact that antibiotics have played a significant role in the treatment of infectious diseases caused by bacteria and fungus for the previous sixty years. As a result, there are several more plants that have yet to be discovered that contain novel phytochemicals that can infect bacteria.

2. Methodology

Acmella oleracea plants were collected, rinsed, and dried in the air before being pulverised into powder for extract production. Three extracts were made using hexane, chloroform, and ethanol, and then filtered and concentrated using a rotary evaporator. The antioxidant potential was assessed using DPPH (Williams et al., 1995) and FRAP (Stephanie et al., 2009) tests. The DPPH assay quantified the extract's capacity to scavenge free radicals by observation of absorbance variations at 517 nm, whereas the FRAP assay evaluated its ability to reduce radicals by measuring absorbance at 593 nm. The extract's capacity to mitigate oxidative stress was verified by the computation of IC50 values in both laboratory tests. In the production of iron nanoparticles (FeNPs), the extract of *Acmella oleracea* was employed as a natural reducing agent. An iron(III) chloride solution was introduced to the extract, and the mixture was repeatedly agitated at ambient temperature until a change in color was seen, indicating the presence of iron nanoparticles (FeNPs). Utilizing UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM), the produced nanoparticles were studied. UV-Vis spectroscopy verified the production of nanoparticles, XRD elucidated the crystalline structure and particle size, FTIR highlighted the functional groups implicated in nanoparticle synthesis, and SEM unveiled the size and morphology of the nanoparticles.
An agar well diffusion method was used to evaluate the antibacterial activity of plant mediated iron nanoparticles with bacterial strains including E. coli, S. aureus, and P. aeruginosa, as well as the antifungal activity with the pathogens Candida albicans. Zones of inhibition were measured and the minimum inhibitory concentration (MIC) was calculated by method of broth dilution (Kohner et al., 1994 and Mathabe et al., 2006).

3. Results and discussion

An assessment of antioxidant activity will provide a more comprehensive knowledge of the therapeutic capabilities of phytochemicals, enabling their optimization in different applications and contributing to the advancement of health-promoting goods. Each of the three extracts of *Acmella oleracea*—hexane, chloroform, and ethanol were tested for their ability to combat free radicals**.**

3.1 Antioxidant activity
3.1.1 DPPH assay

Table: DPPH assay of hexane, chloroform and ethanol extract of *Acmella oleracea*

Samples	Concentration			IC 50 Value
	100 µg/ ml	200 µg/ ml	300 µg/ ml	
Hexane	43.766 ± 0.008	30.014 ± 0.006	16.221 ± 0.03	**54.785**
Chloroform	56.452 ± 0.02	24.216 ± 0.027	13.581 ± 0.146	**111.628**
Ethanol	51.53 ± 0.04	23.467 ± 0.002	4.673 ± 0.098	**99.936**
Standard (Ascorbic acid)	44.526 ± 0.057	32.274 ± 0.095	26.692 ± 0.036	**26.6294**

The percentage of inhibition is 43.766% at a concentration of 100 µg/ml, 30.014% at 200 µg/ml and 16.221 % at 300 µg/ml for hexane extract of *Acmella oleracea*. Similarly, it is 56.452%, 24.216% and 13.581% for chloroform extract and 51.53%, 23.467% and 4.63% for ethanol extract. An inhibition of hexane comparable to that of the standard was observed which is 44.526%, 32.274% and 26.692% with an increase in concentration. The IC50 values of the samples were in the order of Standard (26.6) < hexane (54.7) < ethanol (99.9) < chloroform (111.6). Antioxidant activity of hexane is moderate and comparable to hexane extract whereas the ethanol and chloroform extract has minimum antioxidant activity. An earlier study was conducted to investigate the antioxidant activity of various sections of Acmella olearcea, and the results were compared subsequently (Weintraub et al., 2020). In order to regulate the therapeutic benefits of a plant, the most important

criteria that are responsible for this regulation are bioactive chemicals (such as phenolics and flavonoids) and antioxidant activity (Chandur et al., 2011). In a different study, it was demonstrated that the secondary metabolites of *Acmella oleracea*e played a significant part in the anti-oxidant action (Abeysinghe et al., 2014).

3.1.2 Ferric Reducing Antioxidant Potential (FRAP) Assay

Table: FRAP assay of hexane, chloroform and ethanol extract of *Acmella oleracea*

Sample	Concentration			IC 50 Value
	100 µg/ ml	200 µg/ ml	300 µg/ ml	
Hexane	79.068 ± 0	24.726 ± 0.006	10.359 ± 0	**164.251**
Chloroform	71.837 ± 0.012	37.771 ± 0.006	23.054 ± 0.006	**176.306**
Ethanol	77.188 ± 0.014	54.026 ± 0.006	38.551 ± 0.006	**244.104**
Standard (Ascorbic acid)	47.432 ± 0.005	27.302 ± 0.008	18.443 ± 0.011	**69.3228**

At concentrations of 100 µg/ml, 200 µg/ml, and 300 µg/ml, the hexane extract exhibited inhibition percentages of 79.068%, 24.726%, and 10.359% respectively, with an IC50 value of 164.251 µg/ml. These findings suggest that the hexane extract shown the greatest antioxidant activity compared to the other extracts that were examined. The chloroform extract exhibited inhibition at concentrations of 100 µg/ml (71.837%), 200 µg/ml (37.771%), and 300 µg/ml (23.054%), with an IC50 value of 176.306 µg/ml. With inhibition percentages of 77.188% at 100 µg/ml, 54.026% at 200 µg/ml, and 38.551% at 300 µg/ml, the ethanol extract had a higher IC50 value of 244.104 µg/ml, indicating more modest antioxidant activity.
At an IC50 value of 69.3228 µg/ml, the conventional antioxidant ascorbic acid had the highest activity, inhibiting 47.432% at 100 µg/ml, 27.302% at 200 µg/ml, and 18.443% at 300 µg/ml. Overall, the Hexane extract exhibited the greatest antioxidant capacity among the examined extracts, although Ascorbic acid maintained its position as the most potent antioxidant. In previous investigations, the antioxidant activity of *Acmella oleracea* was determined using the FRAP assay, revealing the presence of numerous antioxidants (Abeysiri et al., 2013). The findings of the FRAP test revealed that the leaves of *Acmella oleracea*e possess the greatest antioxidant potential, likely attributed to constituents such as phenolics and flavonoids (Nascimento et al., 2020).

3.2 Characterisation of iron nanoparticles

The noticeable visual indication of iron nanoparticle creation was the transition in colour from yellow to a deep black shade. Validation of the formation required scientific evidence, such as characterization studies.

3.2.1 UV visible spectrometry

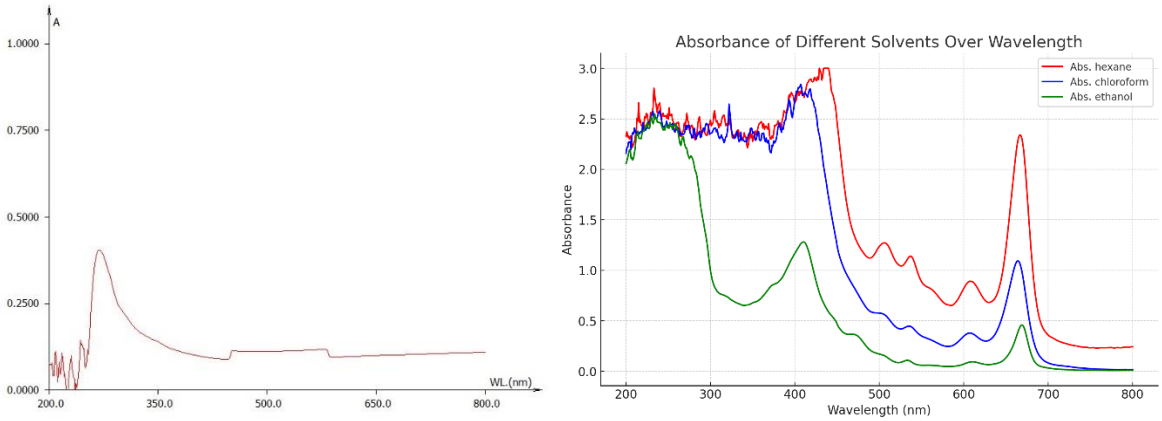


Fig: UV visible spectroscopy of iron nanoparticles and *Acmella oleracea*

The UV spectrum shows a significant absorption peak at 270nm. This provides further evidence that the generation of iron nanoparticles by the use of *Acmella oleracea* extract was effective. The broadness of the peak suggests the polydispersed nature of the nanoparticles. It was absorbed that the iron nanoparticles that were synthesised from fungus showed peaks at 226 and 276 nm. (Mazumdar and Haloi, 2011). In distilled water, the most prominent peaks that are observed for iron nanoparticles are at 216 nm and 268 nm. Changes in the peaks of nanoparticles could be the result of changes in the composition of the media or the size of the particles (Blanco et al., 1994 and Guo et al., 2001).

3.2.2 FTIR of iron nanoparticles

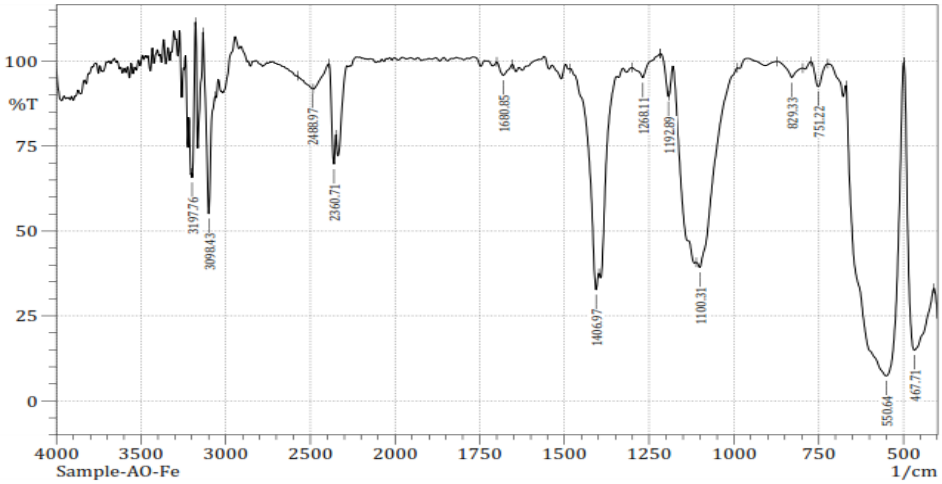


Fig: FTIR of plant mediated iron nanoparticles

Table: Functional gropus and structure displayed in the FTIR spectrum of plant mediated iron nanoparticles

Wave length	Functional groups	Structure
467.71	Alkyl halides	R-Br
550.64	Alkyl halides	R-Br
751.22	Aromatics	meta-disub.
829.33	Aromatics	Para-disub.
1100.31	Misc.	P=O phosphine oxide
1192.89	Misc.	P=O phosphate
1268.11	Misc.	P=O phosphoramide
1406.97	Aromatics	C-C in ring
1680.85	Alkenes	cis RCH=CHR
2360.71	Misc.	Si-H silane
2488.97	Misc.	P-H phosphine
3098.43	Alkenes	R2C=CH2
3197.76	Carboxylic acids	RCO-OH

There is evidence of the existence of carboxyl groups (RCO-OH) in the presence of the absorption peak at 3197.76 cm^{-1}, which corresponds to the stretching vibrations of the O-H bond in carboxylic acids.An association has been made between the stretching vibrations of terminal alkenes (R2C=CH2) and the peak that occurs at 3098.43 cm^{-1}. The absorption band that is located at 1680.85 cm^{-1} is associated with the cis-configuration of alkenes, which is represented by the equation cis RCH=CHR. This particular arrangement of double bonds indicates the presence of double bonds.The presence of phosphine functional groups is indicated by the presence of P-H bonds, which are suggested by the absorption at 2488.97 cm^{-1}. The presence of Si-H bonds, which are characteristic of silanes, is indicated by the peak at 2360.71 cm^{-1}.On the other hand, the peak at 1192.89 cm^{-1} corresponds to the P=O link in phosphate, while the absorption band at 1100.31 cm^{-1} is related with the P=O bond in phosphine oxide. The existence of a P=O bond in phosphoramide is indicated by the peak at 1268.11 cm^{-1}, which also reveals the presence of a number of other groups that include phosphorus.In addition, the peak that is located at 1406.97 cm^{-1} is indicative of the stretching vibrations that occur between carbon atoms within the aromatic ring, which provides additional evidence that aromatic compounds are present. Iron nanoparticles synthesized from tea extract were found to exhibit FTIR spectra that exhibited similar stretching patterns (Gottimukkala et al., 2017).

3.2.3 XRD of iron nanoparticles

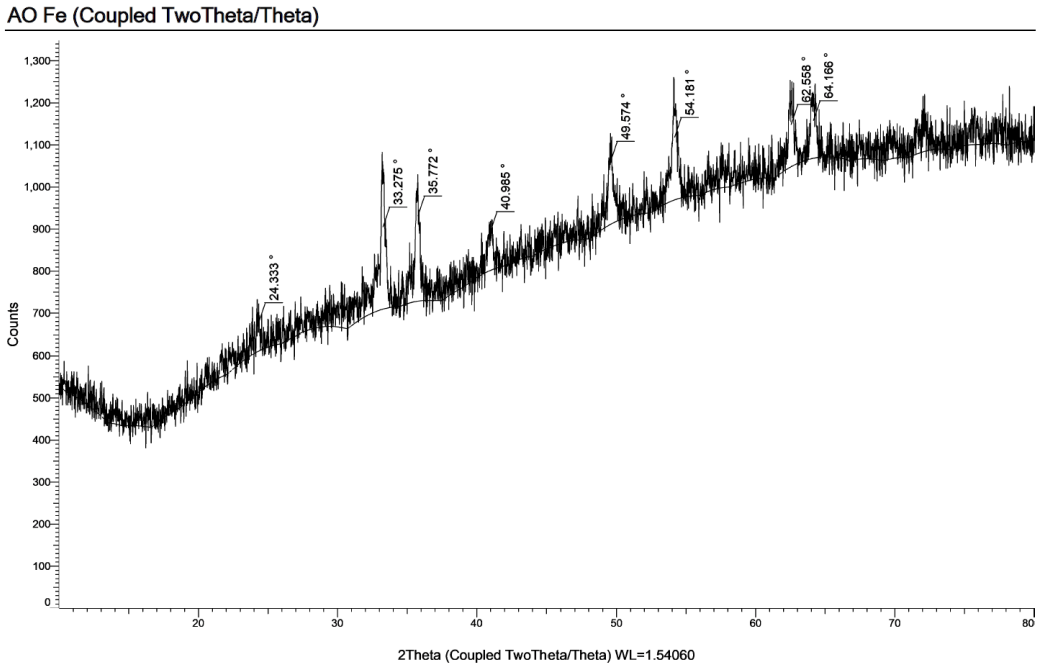


Fig: XRD of iron nanoparticles synthesised from *Acmella oleracea*

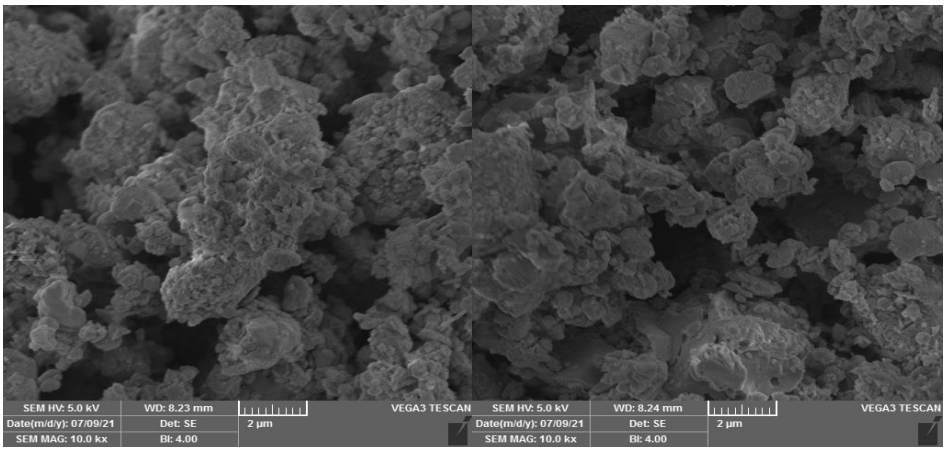
The 24.393° peak is possible to be associated with the (111) plane of an iron oxide phase such as hematite (α-Fe2O3) or a similar oxide structure.The 32.275° peak may be associated with the (220) electron plane of Fe3O4 (magnetite) or γ-Fe2O3 (maghemite). Iron oxide nanoparticles often have magnetite and maghemite as their characteristic phases. The significant peak observed at 35.772° is most likely associated with the (311) plane of magnetite (Fe3O4). The debye-scherrer equation was used to determine the size of the nanoparticles, and the average size was found to be 50 nm.

3.2.4 SEM and EDAX of iron nanoparticles

Combining the high surface-to-volume ratio with the size effects of nanoparticles results in the introduction of a wide variety of phenomena that are depending on the size of the particles, including chemical, electrical, magnetic, and mechanical properties (Akbari et al., 2011). The plant extract was shown to be effective as a reducing and stabilizing agent, as evidenced by the nanoparticles which has primarily spherical morphology and consistent size distribution within the nanoparticles.The surface of the nanoparticles looked to be smooth, which indicated that there was minimal aggregation and that the nanoparticles were effectively stabilised. Iron nanoparticles were determined to have a size of approximately 50 nm using image J software.The nanoparticles exhibited a spherical geometric form, with some being polydispersible due to reaction kinetics and surface chemistry.

The elemental composition of the nanoparticles was validated through the utilization of Energy Dispersive X-ray Spectroscopy (EDAX) analysis on the iron nanoparticles (FeNPs) that were manufactured through the utilization of *Acmella oleracea* extract technology. A peak at around 6.4 keV is prominently displayed in the spectra. This peak corresponds to the presence of iron (Fe), which validates the successful synthesis of FeNPs. In addition to iron, other elements such as oxygen, carbon, sodium, aluminum, and chlorine were found to be present. Several investigations have demonstrated the existence of iron and oxygen in EDAX, which was produced by chemical-based techniques (Prabhu et al., 2015 and Karami, 2010). Our instance involves Acmella, which possesses phytochemicals that promote the existence of additional elements such chlorine, aluminium, sodium, and carbon in the sample.

A possible source of oxygen is the oxidation of iron or the bioorganic molecules that are derived from the plant extract. On the other hand, carbon is most likely derived from organic compounds that function as capping agents. The presence of trace elements such as chlorine and salt in the extract or synthesis leftovers, as well as the presence of aluminum, may be indicative of contamination in the sample preparation process.



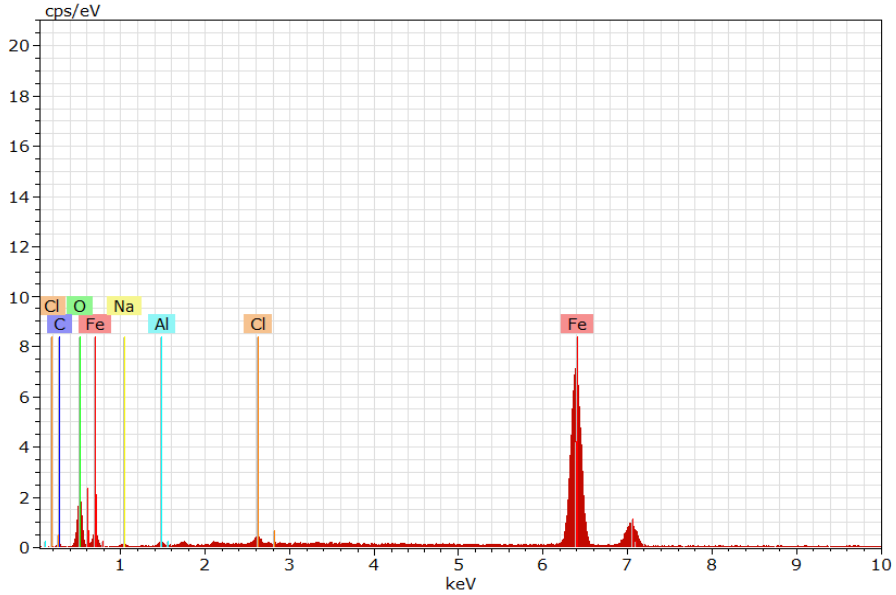


Fig: SEM and EDAX images of iron nanoparticles

Elements	Weight %	Atomic %
Fe	86.30%	64.42%
O	11.18%	29.14%
C	1.33%	4.61%
Na	0.65%	1.18%
Cl	0.50%	0.58%
Al	0.05%	0.07%

3.3 Antimicrobial activity of iron nanoparticles

Table: antibacterial activity of plant mediated iron nanoparticles against pathogens

Sample	***E. coli***	***B. subtilis***	***S. aureus***	***K. pneumoniae***	***E. faecalis***	***P. vulgaris***
Ao - FeNps	18 mm	15 mm	15 mm	12 mm	12 mm	NZ
+ ve	24 mm	21 mm	21 mm	15 mm	21 mm	16 mm
- ve	NZ	NZ	NZ	NZ	NZ	NZ

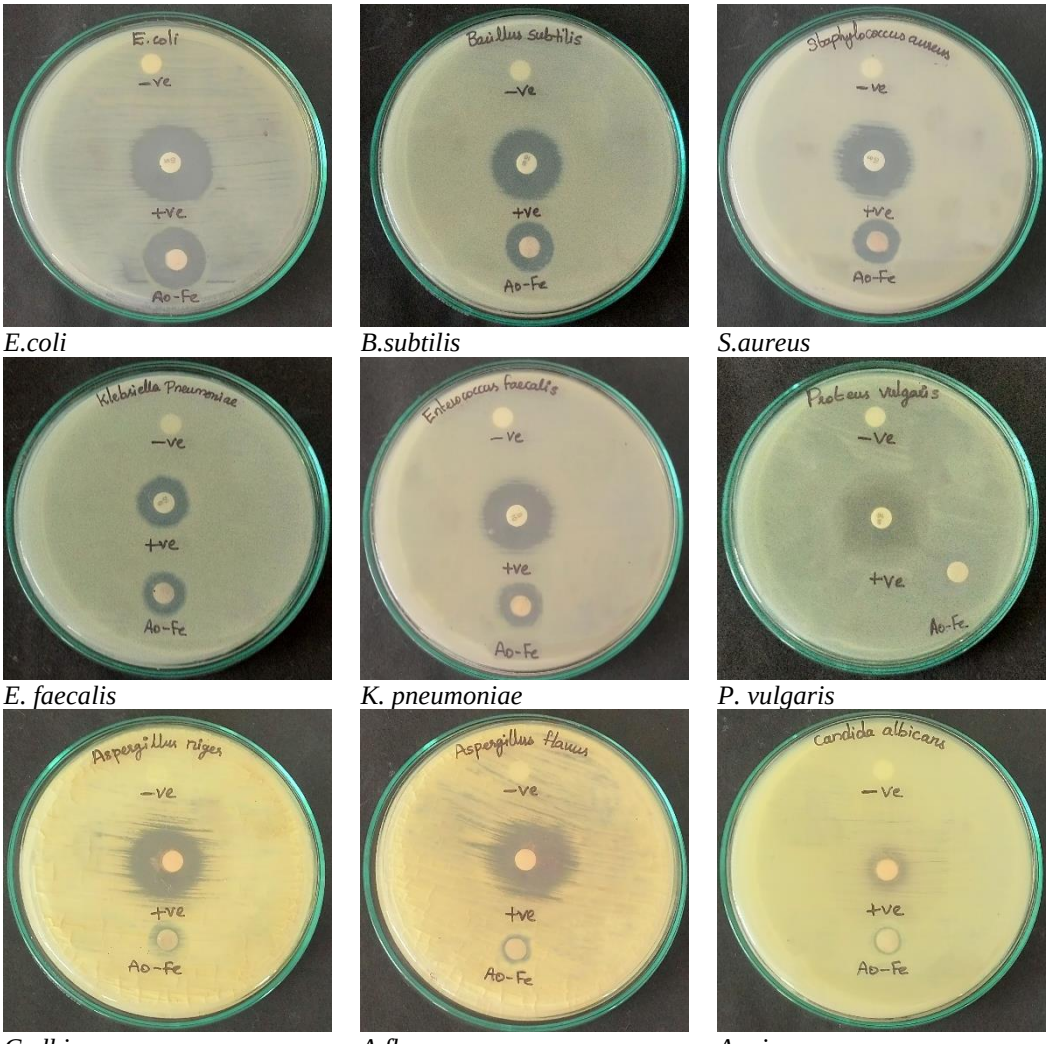
Iron nanoparticles synthesised form *Acmella oleracea* was tested for antibacterial activity against three gram positive (Bacillus subtilis, Staphylococcus aureus and Enterococcus faecalis) and three-gram negative bacteria (Escherichia coli, Klebsiella pneumoniae and Proteus vulgaris). The ZOI of FeNPs was 18 mm, which suggests that they have a strong antibacterial impact against Escherichia coli; however, their effectiveness was lower than that of the positive control, which was 24 mm. In comparison to the positive control, which had a zone of inhibition (ZOI) of 21 mm, FeNPs showed

a ZOI of 15 mm against Bacillus subtilis and Staphylococcus aureus, which indicates that they had moderate antibacterial activity. 12 mm zones were seen in the agar plates of Klebsiella pneumoniae and Enterococcus faecalis in which the positive controls showed 15 mm and 21 mm of zones respectively which suggest that they have lesser antibacterial activity than the positive controls. Proteus vulgaris had no impact by the nanoparticles whereas the control showed 16mm of inhibition zones.

Table: antifungal activity of plant mediated iron nanoparticles against pathogens

Sample	*A. niger*	*A. flavus*	*C. albicans*
Ao - FeNps	11 mm	11 mm	9 mm
+ ve	21 mm	21 mm	16 mm
- ve	NZ	NZ	NZ

Aspergillus niger and Aspergillus flavus exposed 11 mm of zones by the iron nanoparticles which is less than the positive control that had 21 mm of zones on both. 9 mm of zone of inhibition was noticed against Candida albicans that has a positive control with 16 mm of inhibition.



E.coli *B.subtilis* *S.aureus*

E. faecalis *K. pneumoniae* *P. vulgaris*

C.albicans *A.flavus* *A. niger*

Fig: antimicrobial activity of *Acmella oleracea* mediated Fe nanoparticles against different pathogens

4. Conclusion

Acmella oleracea exhibited substantial antioxidant activity, which can be ascribed to its diverse range of bioactive metabolites. The metabolites are probably accountable for the plant's physiological effects, serving a crucial function in suppressing different infections and counteracting free radicals. Furthermore, the phytochemicals found in *Acmella oleracea* played a crucial role in the environmentally friendly production of iron nanoparticles, acting as natural molecules

that reduce and stabilize iron. *Acmella oleracea*-derived iron nanoparticles (FeNPs) exhibit promise as antibacterial agents, although some improvement is needed to augment their efficacy. The potential of *Acmella oleracea* for medicinal and nanotechnology-based advancements is demonstrated by its biological activities.

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