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Biosynthesized MgONPs using *Syzygium cumini* seed extract: Characterization, *In vitro* anti-oxidant and anti-microbial activity

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ABSTRACT

The present study investigates *S. cumini* seed extracts which are considered as a promising and valuable source of bioactive compounds were prepared using different solvents such as methanol, ethanol, petroleum ether, acetone, chloroform, and diethyl ether. Among these solvents, methanol exhibited the highest extraction with a yield of 42 %. HPLC analysis revealed the highest concentration of quercetin flavonoids (49.62 mg/gm) in the methanolic *S. cumini* seed extract. Thus, the current work deals with the MgONPs synthesis through a biological approach using different *S. cumini* seed extracts. *In vitro* anti-oxidant properties were evaluated, which showed an IC_{50} value of 22.46 µg/mL for MgONPs synthesized from methanolic extract, surpassing the anti-oxidant potency of ascorbic acid by threefold. By leveraging the rich repository of bioactive compounds found within *S. cumini* seed extract, this study presents a novel approach to MgONPs synthesis. Exploring the symbiotic relationship between *S. cumini* seed extract and MgONPs, this research elucidates the pivotal role of bioactive compounds in guiding the formation and properties of nanostructures. Further anti-microbial studies on MgONPs from methanolic *S. cumini* seed extract were conducted against four different bacterial strains (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus,* and *S. typhimurium*), revealing potent anti-microbial activity with 5.3 mm of inhibition for 100 µl against *S. typhimurium*. These findings suggest that *S. cumini* is a source of bioactive compounds responsible for the successful synthesis of MgONPs. Characterization studies of MgONPs were also carried out using UV–vis spectroscopy, FTIR, SEM, XRD, DSC and HPLC.

1. Introduction

Nanoparticles have been widely used in the field of anti-microbial treatment for their properties [1,2,]. The spatial properties have allowed them to exert physiological effects on living organisms [3]. Magnesium Oxide (MgO) stands out as a fundamental metal oxide with appealing traits, catching significant interest due to its stability across different processing conditions and its reputation as a safe substance for both humans and animals [4]. Among the different metal oxides, MgO is a functional semiconductor with numerous uses in optoelectronics, cell signaling, drug administration, and imaging, particularly as an effective, potent anti-bacterial and anti-oxidant agent combating the most dangerous antibiotic-resistant diseases [5]. Magnesium oxide nanoparticles (MgONPs) synthesized from *Syzygium cumini (S. cumini)* showed potential microbial inhibition against *Escherichia coli, Streptococcus pneumonia, Staphylococcus aureus,* and *Salmonella typhimurium* [6]. In recent years, there has been a growing interest in exploring the synthesis of nanoparticles using natural sources as reducing and stabilizing agents due to their eco-friendly nature and biocompatibility [7,8]. MgONPs have emerged as a versatile nanomaterial with diverse applications in biomedicine, catalysis, and environmental remediation [9]. The synthesis of MgONPs from plant extracts offers several advantages, including cost-effectiveness, scalability, and the potential to harness the inherent bioactivity of plant-derived compounds [10].

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Despite the increasing prevalence of studies investigating the synthesis of nanoparticles using plant extracts, there remains a paucity of research focusing on the utilization of *S. cumini* seed extract for this purpose. Given the rich phytochemical composition of *S. cumini* seeds, there is a compelling rationale to explore their potential as a precursor for nanoparticle synthesis. Moreover, the integration of *S. cumini* seed extract-derived bioactive compounds into MgONPs could impart enhanced functionality and therapeutic efficacy to the resulting nanomaterials. Thus, in the present study, the focus has been on the antimicrobial action of MgONPs against pathogenic microorganisms influencing diabetes development and supplementation probiotics to increase essential gut microbiota to suppress insulin resistance, intestinal permeability, and inflammation.

The traditional medicinal system, centered on the use of herbal treatments, continues to play a vital role in the human healthcare system. Medicinal herbs have gained popularity in recent decades due to the idea that they are natural products with fewer side effects and higher efficacy than synthetic competitors [11]. Currently, traditional medicines serve as a crucial healthcare resource for nearly 80 % of the global population. Within the Myrtaceae family, there are approximately 121 genera and 3800–5800 species of shrubs, and trees predominantly found in tropical and subtropical regions worldwide [12]. Among these, the genus Syzygium stands out, boasting 1100 species, *S. cumini* (L.) Skeels (syn.: Eugenia jambolana, Syzygium jambolanum) being the most notable and renowned for its therapeutic applications, especially in the treatment of diabetes [13]. *S. cumini* is valued for the bioactive compounds present in different parts of the plant. Its leaves are utilized for curing dermopathies, leucorrhea, constipation, gastropathies, and diabetes; the fruits are employed to treat pharyngitis and splenic disorders; and the bark is known for its astringent, anthelmintic, and carminative properties [14]. Additionally, the seeds are also used as astringents, diuretics, and in diabetes treatment [15,16,]. The anti-diabetic qualities of *S. cumini* were first studied in the mid-nineteenth century, leading to its integration into Western medicine [16]. Native to the Indian subcontinent *S. cumini* is a giant tree widely farmed in various Asian, African, and South American nations [17]. In India, it is known as Jamun.

Pharmacological research has revealed that *S. cumini* exhibits various bioactive properties such as anti-bacterial, anti-hyperglycemic, anti-inflammatory, anti-oxidant, and cardioprotective [18–22,. Over the past decade, numerous outstanding and well-executed literature reviews have focused on the pharmacological activities, phytochemical contents, and nutritional significance of *S. cumini* [13,15,16]. The major bioactive component, quercetin present in *S. cumini* fractions has shown promising anti-oxidant and anti-glycation effects, along with the capacity to block digestive enzymes. This study aims to bridge this gap by elucidating the synergistic relationship between *S. cumini* seed extract and MgONPs and exploring their potential applications in biomedicine and beyond. Through comprehensive characterization techniques such as UV–vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and differential scanning calorimetry (DSC), the structural and functional properties of the synthesized MgONPs thoroughly investigated. This study describes unique bioactivities for *S. cumini* that have not previously been characterized, opening up new avenues for future research into the anti-diabetic activity from the fractions of *S. cumini*, particularly ethyl acetate and butanolic extracts [23]. Hence, in view of the above, we determined the anti-oxidant and anti-microbial activities of synthesized MgONPs using *S. cumini* seed methanolic extract.

2. Materials and methods

2.1. Plant material

Fresh fruits of *S. cumini* were obtained in the last week of May 2022 from the local region of Tirupati, Andhra Pradesh, India. To ensure cleanliness, the fruits were washed under running tap water to eliminate any dust particles. The pulp was separated, and the seeds were properly cleaned. The seed was then air-dried at room temperature for 1–2 weeks before being crushed into a fine powder using an electrical grinder.

2.2. Preparation of seed extract

For the preparation of the seed extract, a comparative study was conducted to assess the impact of various solvents on the extraction yield and content of alkaloids, flavonoids, phenolics, and terpenoids. The powdered seed sample was subjected to percolation by using a magnetic stirrer (48–72 h) with various organic solvents, including methanol/carbinol, ethanol, petroleum ether, acetone, chloroform, and diethyl ether (70% w/v) respectively. The resulting extracts were filtered twice using Whatman No.1 filter paper and stored at 4 °C for subsequent analyses.

2.3. Screening for phytochemical compounds

Phytochemical components such as alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, steroids, amino acids, and anthraquinone glycosides were detected in *S. cumini* seed extracts using standard procedures [24,25].

2.3.1. Test for alkaloids

To perform the alkaloids test, a solution was prepared by dissolving 1.36 gm of mercuric chloride in 60 ml of distilled water, and 5 gm of potassium iodide in 10 ml of pure water. These two solvents were then combined and diluted to a total volume of 100 ml with distilled water. A few drops of this reagent were added to 1 ml of acidic aqueous solution of samples. The presence of alkaloids was indicated by the formation of white or pale precipitates.

2.3.2. Test for tannins

2.3.2.1. Ferric chloride test. 5 ml of seed extract (5 mg/ml) was dissolved in 2 ml of distilled water, and a few drops of neutral 5 % ferric chloride solution was added. The appearance of a dark green or black precipitate indicates the presence of tannins.

2.3.2.2. Lead acetate test. In another test, 5 ml of the sample was placed in a test tube, and a few drops of 1 % solution of lead acetate were added. The formation of a bulky white precipitate confirmed the presence of tannins.

2.3.3. Test for saponins

For the saponins test, about 5 ml of the seed extract (5 mg/ml) was placed in the test tube, and a few drops of sodium bicarbonate were added. The mixture was shaken vigorously and left to stand for two minutes. The formation of a honeycomb-like froth indicated the presence of saponins.

2.3.4. Test for flavonoids

In a test tube containing about 0.5 ml of alcoholic extract from the sample, 7–10 drops of diluted hydrochloric acid (HCl) and a small amount of magnesium (Mg) were added. The solution was boiled for a few minutes, and the appearance of a reddish pink or dirty brown color confirmed the presence of flavonoids.

2.3.5. Test for phenols

For the phenols test, 1 ml of the alcoholic solution of the seed extract was placed in the test tube, and 2 ml of distilled water followed by a few drops of an aqueous solution of ferric chloride (10 %) were added. The formation of a blue or deep green color confirmed the presence of phenols.

2.3.6. Test for terpenoids

In a test tube containing 5 mg of plant extract, 2 ml of chloroform ($CHCl_3$), and 5–10 drops of concentrated sulfuric acid (H_2SO_4) were added. The observation of a reddish-brown color confirmed the presence of terpenoids.

2.3.7. Test for steroids

About 100 mg of dried extract from the sample was dissolved in 2 ml of chloroform ($CHCl_3$). A few drops of sulfuric acid (H_2SO_4) were carefully added to form a lower layer. The presence of a reddish-brown color at the interface indicated the presence of a steroidal ring.

2.3.8. Test for amino acids

About 2 ml of the extracted sample was placed in the test tube, and treated with 1–2 drops of ninhydrin reagent. The appearance of violet or purple color indicated the presence of amino acids.

2.3.9. Test for anthraquinone glycosides

About 5 ml of seed extract was placed in the test tube, then added 2 ml of dilute sulfuric acid (H_2SO_4), and boiled for 5 min, then filtered. To the filtrate, equal volumes of chloroform ($CHCl_3$) were added and mixed well. The organic layer was separated, and 10 % ammonia solution was added. The appearance of brick-pink color in the ammonia layer indicated the presence of anthraquinone glycosides.

2.4. Preparation of MgONPs

The *S. cumini* seed extracts of different solvents (methanol/carbinol, ethanol, petroleum ether, acetone, chloroform, and diethyl ether) were taken for the preparation of MgONPs [5]. About 20 ml of 1 M aqueous solution of magnesium chloride was prepared and placed in an Erlenmeyer flask. Then, 25 ml of *S. cumini* seeds extract was added drop by drop to the 10 ml of 1 M magnesium chloride solution at room temperature and stirred for a few minutes. A change in the solution's color from pale yellow to brown and then to dark brown indicated the formation of MgONPs. The resultant solution was used for further characterizations. A portion of the extract was dried in a hot air oven for preservation and future use. This synthesis method for MgONPs with *S. cumini* seed extracts in organic solvents is considered a pure green and eco-friendly process, as it does not involve harmful chemicals.

2.5. Determination of extraction yield

The extraction yield percentage was calculated by using the following formula [26].

$$\%\ Extraction\ yield = \frac{(weight\ of\ extract\ after\ evaporating\ solvent\ and\ freeze\ drying)}{(Dry\ weight\ of\ the\ sample)} \times 100$$

2.6. Total flavonoids content (TFC)

The total flavonoids were determined by using the modified colorimetric method of aluminum chloride [27]. A mixture of 2 ml extract, 0.5 ml of 5 % aluminum chloride ($AlCl_3$) solution, and 0.5 ml of 1 M potassium acetate solution was incubated for 15 min at room temperature. As a control, absolute ethanol was used. Using a V-730 Uv-vis spectrophotometer, the absorbance of all samples was measured at 415 nm. Quercetin was used as a reference standard to calculate flavonoid content and expressed as milligrams of quercetin equivalent (QE) per gram of extract (dry weight). The calibration curve equation was, $y = 0.0015x + 0.3173$, where $R^2 = 0.993$.

2.7. Total phenolics content (TPC)

Total phenolic levels were determined using a modified Folin-Ciocalteu assay, as described by McDonald et al. [28]. One milliliter of each extract was mixed with 5 ml of 10 % Folin-Ciocalteu reagent, and 4 ml of 2 % Sodium carbonate (Na_2CO_3) was added to the mixture. As a control, a reagent without extract was used. After 60 min of incubation at room temperature, the absorbance of all samples was measured at 765 nm using a V-730 UV–vis spectrophotometer. A gallic acid calibration curve (0–100 g/mL) was used to calculate phenolic content, expressed in milligrams of gallic acid equivalent (GAE) per gram of extract. The calibration curve equation was $y = 0.0012x + 0.2509$, where $R^2 = 0.964$.

2.8. Total alkaloids content (TAC)

Total alkaloids were determined using a colorimetric method following the procedure by Ajanal et al. [29]. One milliliter of plant extract was washed three times with 10 ml of chloroform before adjusting the pH to neutral. After that, 5 ml of phosphate buffer and 5 ml of bromocresol green solution were added to the extract. After vigorously shaking the mixture with chloroform, it was collected in a 10 ml volumetric flask. The absorbance of the solution at 470 nm was measured using a UV–Vis spectrophotometer. Atropine was used as the reference standard to calculate the alkaloid content, expressed as milligrams of atropine equivalent (AE) per gram of extract (dry weight). The calibration curve equation was $y = 0.0011x + 0.0277$, where $R^2 = 0.984$.

2.9. Determination of anti-oxidant activity

The anti-oxidant activity of MgONPs derived from methanolic *S. cumini* seed extracts was evaluated by using a slightly modified DPPH-free radical scavenging assay based on the method described by Mahdi-Pour et al. [30]. Different dilutions of the extract (50, 100, 150, 200, 250, and 300 µg/ ml) were mixed with 1 ml of DPPH solution (0.004 % in ethanol) and incubated at 37 °C for 30 min. The absorbance of the mixture was measured at 517 nm using a UV–vis spectrophotometer. Ascorbic acid was used as a positive control and absolute ethanol served as a negative control. The IC_{50} value, representing the concentration required for a 50 % inhibition of DPPH (IC_{50}) was determined from the plot of the percentage of residual DPPH against the sample concentration using the calibration curve equation was $y = 0.0023x - 0.0569$, where $R^2 = 0.9796$.

The following formula was used to calculate the DPPH scavenging activity:

% RSA= ((Absorbance of control (A0)-Absorbance of sample(A)) / (Absorbance of control) X 100)

where A is the absorbance of the sample containing extract and A_0 is the absorbance of the negative control (0.004 % DPPH solution).

2.10. *Characterization of MgONPs*

The MgONPs were prepared by reducing the magnesium ion solution with *S. cumini* seed extracts and characterized using various techniques including, UV-visible spectroscopy (UV–vis), Scanning Electron

Microscope (SEM), Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), and Differential Scanning Calorimetry (DSC).

2.10.1. UV-visible spectrophotometer

The MgONPs synthesized using *S. cumini* seed extracts of different solvents (methanol/carbinol, ethanol, petroleum ether, acetone, chloroform, and diethyl ether) were subjected to using UV-visible spectroscopy (Shimadzu Model No. UV – 1800, ENG 240 V). Spectra of the suspension were collected and analyzed within the wavelength range of 200–500 nm [31].

2.10.2. Fourier transform infrared spectroscopy (FTIR)

The FTIR analysis was performed using a 1600 Perk-inElmerÒ spectrophotometer (PerkinElmer do Brasil Ltda, Sa˜o Paulo, SP, Brazil) in the range of 4000–400 cm^{-1}. The curves were plotted using the OPUS software, and the spectrum of the synthesized MgONPs of different solvent extractions was taken, with the help of a micropipette, a drop of synthesized nanoparticles was placed over the FTIR instrument, and the spectrum was recorded [32].

2.10.3. Scanning electron microscope (SEM)

The size and morphology of the MgONPs of methanolic *S. cumini* seed extract were investigated using a field emission scanning electron microscope (FE-SEM, Hitachi S-4200, Japan) [33]. Thin films of the sample were prepared on a carbon-coated copper grid, and the samples were allowed to dry before examination.

2.10.4. X-ray diffraction (XRD)

The XRD analysis for MgONPs of methanolic *S. cumini* seed extract was performed using AERIS PAN analytical Instrument to identify the crystalline nature of the sample [34].

2.10.5. Differential scanning calorimetry (DSC)

The thermal properties of synthesized MgONPs from seeds of *S. cumini* methanol extract were investigated using a TA 2920 modulated DSC thermal analyzer (TA Instruments, New Castle, DE) [32]. The analysis was conducted for a calcinated sample containing approximately 10 mg weighed into the aluminum pan and hermetically sealed. Then it was heated from 35 to 140 °C at a rate of 10 °C/min. The onset temperature (Tm), enthalpy of denaturation (ΔH), denaturation temperature (Td), and cooperativity, represented by the width at half-peak height (ΔT1/2), were computed from the thermograms by the Universal Analysis Program, Version 1.9 D (TA Instruments).

2.10.6. High-performance liquid chromatography (HPLC)

2.10.6.1. Analysis for quercetin flavonoid determination. Quercetin flavonoids were determined using an HPLC (HPLC-2160, SPD 20A) instrument equipped with a UV detector (Agilent Series 1100) and an Eclipse Plus C18 column (4.6 × 250 mm). Methanol and Acetic acid (a ratio of 98:2 v/v) were used as mobile phase at the flow rate of 1 ml/min, and the temperature of the column was set at 30 °C. The sample (20 µl) was injected into the column, and the absorbance was measured at 257 nm. A calibration curve using quercetin at 1 mg/ml concentration was used for comparison with the methanolic extract of *S. cumini* [33].

2.11. Anti-microbial activity

The anti-microbial activity of the MgONPs synthesized from methanolic *S. cumini* seed extract was evaluated using the agar well plate method [6]. The anti-microbial activity of the MgONPs of *S. cumini* seed methanol extract was investigated against four bacterial strains namely, *E. coli, Bacillus subtilis, Staphylococcus aureus,* and *S. typhimurium*. The anti-microbial activity studies were conducted for both normal MgONPs and methanolic *S. cumini* seed extract based MgONPs. Different concentrations (25, 50, 75, and 100 µg/ml) of the samples were tested, and the extent of the anti-bacterial effect was quantitatively assessed by measuring the zone of inhibition.

3. Results

3.1. Screening of phytochemical compounds

Phytochemical screening revealed the presence of secondary metabolites such as alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, steroids, amino acids, and anthraquinone glycosides was observed. Among all the extracts tested, methanol and ethanol showed almost all the secondary metabolites except glycosides. Alkaloids, flavonoids, and phenolics were expressed in all the solvents and the results of other metabolites were depicted in Table 1.

3.2. Effects of different solvents on extraction yield

The effect of various organic solvents, including methanol, ethanol, acetone, petroleum ether, chloroform, and diethyl ether, on the extraction yield of *S. cumini* was investigated. The results demonstrated significant in the extraction yield using different solvents. Methanol showed the highest extraction yield (42 %), followed by ethanol (29.5 %), acetone (24.5 %), petroleum ether (22.1 %), chloroform (17.2 %), and diethyl ether (2.4 %). The findings indicated that highly polar solvents favored better extraction efficiency (Fig. 1).

3.3. Effects of different solvents on phenolic, flavonoid and alkaloid content

The impact of different solvents on the phenolic, flavonoid, and alkaloid content of *S. cumini* seed extracts was also studied (Table 2). Significant variations in the content of these bioactive components were observed. Methanol was found to be the most effective solvent for extracting these compounds, resulting in the highest content of flavonoids (12.76 mg QE/g DW), phenolics (1.72 mg GAE/g DW), and alkaloids (1.32 mg AE/g DW). Ethanol also showed high efficiency in extracting flavonoids (5.95 mg QE/g DW). However, its phenolics and alkaloids content was relatively lower. Acetone exhibited less efficiency compared to methanol and ethanolic extracts, which showed the extraction of flavonoids (3.97 mg QE/g DW), phenolics (1.02 mg GAE/g DW), alkaloids (0.67 mg AE/g DW). Petroleum ether resulted in lower flavonoid content (1.93 mg QE/g DW) and a little higher phenolic content (0.87 mg AE/g DW) and alkaloid content (1.21 mg AE/g DW) compared to ethanolic extract. Chloroform and diethyl ether resulted in the lowest flavonoid content (1.38 mg QE/g DW and 0.63 mg QE/g DW) respectively. Similarly, phenolic and alkaloid content is also lowest in these two solvent extracts.

3.4. Anti-oxidant activity of MgONPs

The anti-oxidant activities of MgONPs prepared from the *S. cumini* seed extracts of different solvents were evaluated by the DPPH radical scavenging assay. As illustrated in Table 3, MgONPs of extracts from different solvents possessed varying free-radical scavenging activity ($p < 0.05$). Lower IC_{50} values indicate greater DPPH scavengers activity of MgONPs, while higher IC_{50} values indicate lower scavenging activity. The effect of MgONPs on the anti-oxidant activity of DPPH radicals is depicted in Fig. 2. The MgONPs of methanolic extract demonstrated the most potent anti-oxidant activity with an IC_{50} value of 22.46 µg/ml. MgONPs of Ethanolic and acetone extracts exhibited significant radical scavenging activity with IC_{50} values of 31.47 µg/ml and 32.49 µg/ml, respectively. These MgONPs (of ethanolic and acetone) showed significantly higher radical scavenging activity than MgONPs of ascorbic acid (IC_{50} value 50.94 µg/ml). MgONPs of Petroleum ether and chloroform extracts exhibited a lower radical scavenging activity, compared to

Table 1
Phytochemical screening analysis of *S. cumini* seed extracts of different solvents.

Phytoconstituents	Methanol	Ethanol	Petroleum ether	Acetone	Chloroform	Diethyl ether
Alkaloids	✓	✓	✓	✓	✓	✓
Tannins	✓	✓	X	✓	X	✓
Saponins	✓	✓	✓	✓	✓	X
Flavonoids	✓	✓	✓	✓	✓	✓
Phenols	✓	✓	✓	X	✓	✓
Terpenoids	✓	✓	✓	✓	X	X
Steroids	X	✓	X	✓	✓	✓
Amino acids	✓	X	✓	✓	✓	X
Anthraquinone glycosides	X	X	X	X	X	X

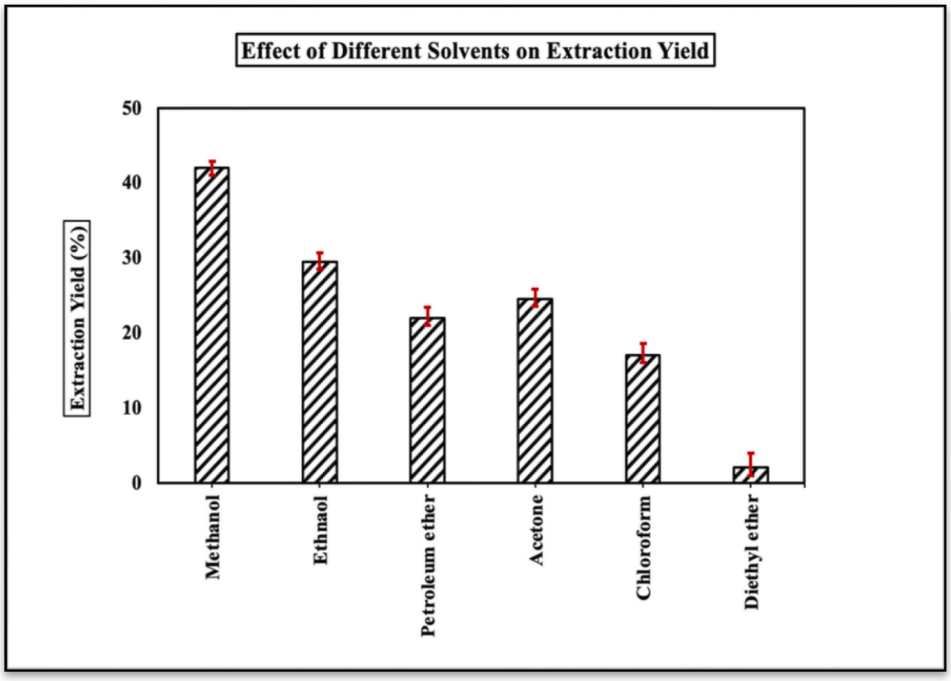

Fig. 1. Comparison of extraction yields using different solvents: The effect of various solvents (Methanol, Ethanol, Petroleum ether, Acetone, Chloroform, and Diethyl ether) on the extraction yield from *S. cumini* seeds. The vertical bars represent the standard deviation calculated from three independent experiments ($n = 3$).

Table 2
Effect of different solvents on phenolic, flavonoid, and alkaloid content of *S. cumini* seeds.

Extraction solvent	Flavonoids (mg QE/g DW)	Phenolics (mg GAE/g DW)	Alkaloids (mg AE/g DW)
Methanol	12.76 ± 0.34	1.72 ± 0.08	1.32 ± 0.03
Ethanol	5.95 ± 0.10	0.53 ± 0.04	0.36 ± 0.02
Petroleum ether	1.93 ± 0.03	0.87 ± 0.02	1.21 ± 0.03
Acetone	3.97 ± 0.04	1.02 ± 0.02	0.67 ± 0.02
Chloroform	1.38 ± 0.06	0.71 ± 0.03	0.19 ± 0.004
Diethyl ether	0.63 ± 0.01	0.43 ± 0.02	0.42 ± 0.03

Table 3
The 50 % inhibitory concentration (IC_{50}) values of DPPH scavenging activity of synthesized MgONPs using different *S. cumini* extracts.

Samples	IC_{50} Values (µg/ml)
Methanolic Extract	22.46 ± 0.8
Ethanolic Extract	31.47 ± 5.70
Petroleum Ether Extract	44.29 ± 8.55
Acetone Extract	32.49 ± 4.56
Chloroform Extract	41.21 ± 10.84
Diethyl ether Extract	48.64 ± 13.60
Ascorbic Acid (Control)	45.23 ± 8.03

All values are mean ± SD ($n = 3$) with in a column significantly differ by Tukey's test at $P < 0.05$. Statistical analysis was carried out by ANOVA.

MgONPs of methanolic extract with IC_{50} values of 44.29 µg/ml and 41.21 µg/ml, respectively. Comparatively diethyl ether extract exhibited the lowest radical scavenging activity with an IC_{50} value of 48.64 µg/ml compared to ascorbic acid (50.94 µg/ml). Finally, it can be interpreted that the radical scavenging activity for the MgONPs prepared from methanol ($IC_{50} = 22.46$ µg/ml) > ethanol ($IC_{50} = 31.47$ µg/ml) > acetone ($IC_{50} = 32.49$ µg/ml) > chloroform ($IC_{50} = 41.21$ µg/ml) > Petroleum ether ($IC_{50} = 44.29$ µg/ml) > diethyl ether ($IC_{50} = 48.64$ µg/ml) > ascorbic acid ($IC_{50} = 50.94$ µg/ml).

3.5. Characterization of MgONPs

The synthesized MgONPs were characterized using various techniques.

3.5.1. UV - visible spectrophotometer

The UV-visible spectrophotometer revealed absorption surface plasmon resonance peaks at 290 nm wavelength for the various solvent extracts, indicating the presence of MgONPs (Fig. 3). There was a higher intensity of absorption peak for methanolic extract followed by ethanolic extract, petroleum ether extract, acetone extract, and, chloroform extract.

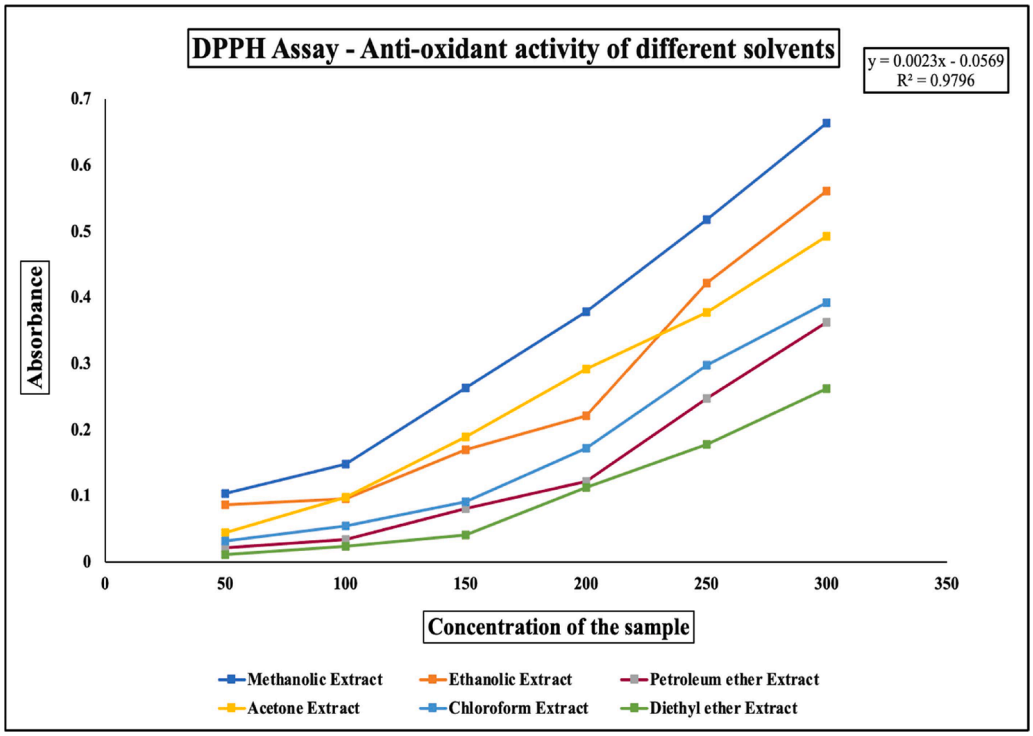

Fig. 2. DPPH Scavenging Activity of *S. cumini* Seed Extracts: The 50 % inhibitory concentration (IC_{50}) values depicting the anti-oxidant activity measured by DPPH scavenging assay for different solvents of *S. cumini* seed extracts.

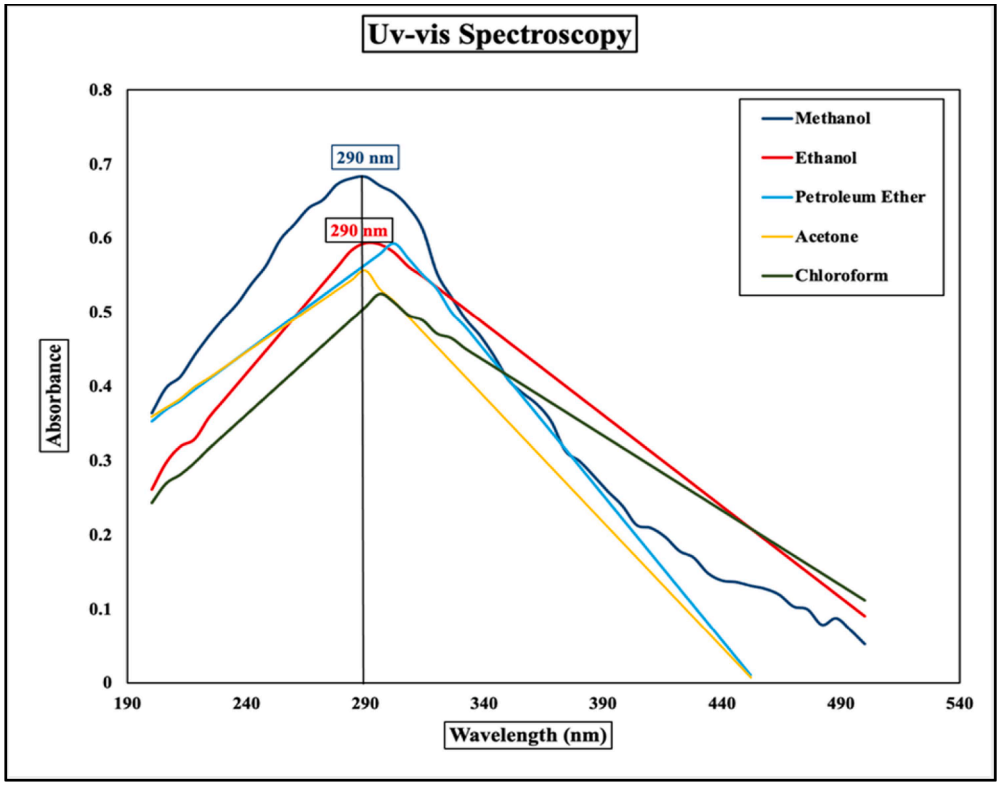

Fig. 3. UV–vis Absorption Spectrum of MgONPs: UV–visible absorption spectra illustrating the optical properties of MgONPs synthesized using various solvents of *S. cumini* seed extracts.

3.5.2. Fourier transform infrared resonance (FTIR)

The FTIR spectra peak found at 3337.46 cm^{-1}, 3333.20 cm^{-1}, 3325.91 cm^{-1}, and 3382.3 cm^{-1} shows O—H stretch and H-bonded, which represents the alcohols and phenolic band, which may proclaim the presence of alcohols and phenols. Another peak found at 1652.17 cm^{-1}, 1641.23 cm^{-1}, 1625.67 cm^{-1}, and 1621.19 cm^{-1} represents the

-C═C- stretch which may proclaim the presence of alkenes. The peak found at 1017.16 cm^{-1} represents the presence of C—O stretch that may further proclaim the presence of alcohols, carboxylic acids, ethers, and esters (Table 4 and Fig. 4).

3.5.3. Scanning electron microscope (SEM)

The particle size and morphology of the synthesized MgONPs of *S. cumini* seed methanolic extract could be affected by several factors, such as temperature, solution concentration, and pH used. In the current study, all these parameters were kept constant for morphological analysis. The SEM image displayed here was used to study the formation of the MgONPs and their morphological size. The SEM image shows the particle size and shape of the synthesized MgONPs after annealing and it displayed spherical nanoparticles below 100 nm in size (Fig. 5). Most of these synthesized MgONPs were aggregated, and a few individual nanoparticles were also displayed.

3.5.4. X-ray diffraction (XRD)

The XRD analysis revealed variations in the crystallite size and composition of MgONPs. Fig. 6, shows the XRD pattern of the MgONPs synthesized with *S. cumini* seed methanolic extract. The diffractogram confirmed the presence of MgO in crystalline phase. As evidenced by the peak position with 2θ values of 15.7°, 20.3°, 22.7°, 30.2°, 32.5°, 41.1°, and 50°, corresponding to the crystalline planes of (463), (484), (734), (729), (1127), (798), and (330), respectively. These peaks confirmed the formation of the monoclinic crystal structure for MgO.

3.5.5. Differential scanning calorimetry (DSC)

The DSC experiments determined the melting temperature of different compounds synthesized during the process. At a scan rate of 10 °C/min in N2, four melting points were identified at 117.6 °C, 172.3 °C, 212.6 °C, and 276.8 °C. The initial peak around 100 °C was attributed to the loss of moisture indicating an endothermic progression. The degradation of peaks observed between 170 °C – 300 °C also demonstrated the endothermic behavior of the compounds. The comparison analysis with standard flavonoid (Quercetin) also showed a similar melting point compared to the synthesized nanoparticles. These results indicate the thermal decomposition reactions for the main component of the biomasses (Fig. 7).

3.5.6. High-performance liquid chromatography (HPLC)

Flavonoid compounds of the 70 % methanolic fraction were analyzed for identification purposes. The fraction showed the peak with the recorded standard which showed the increased peak height at almost the same retention time. This indicates the presence of compared standard (quercetin) in the sample. The other minute peaks showed the presence of other phenolic or alkaloid compounds but they were showed in trace amounts. The amount of quercetin present in the methanolic seed extracts of *S. cumini* was found to be 49.62 mg/gm and the peak height and the area were depicted in Table 5 and Fig. 8.

Table 4
FTIR absorption spectra of synthesized MgONPs of different solvents and their possible peak assignments.

Experimental Frequencies (cm^{-1})	Literature Frequencies (cm^{-1})	Possible peak assignments
3337.46 3333.20 3325.91 3382.34	3500–3200	O—H stretch, H–bonded: alcohols, phenols
1652.17 1641.23 1625.67 1621.19	1680–1640	–C═C– stretch: Alkenes
1017.16	1320–1000	C—O stretch: alcohols, carboxylic acids, esters, ethers

3.6. Anti-microbial activity

The anti-microbial activity of MgONPs of methanolic *S. cumini* seed extract was determined by using the zone of inhibition method. The MgONPs of methanolic *S. cumini* seed extract exhibited an inhibitory effect on both gram-negative and gram-positive bacteria such as *E. coli, Bacillus subtilis, Staphylococcus aureus,* and *S. typhimurium.* Four different concentrations were tested for all four test organisms as described. As the concentration increased, the zone of inhibition also increased, which indicates MgONPs of methanolic *S. cumini* seed extract showed potential anti-microbial activity. In the test organism *E. coli* it showed 5.2 mm of inhibition for 100 µl, 4.6 mm of inhibition for 100 µl in bacillus subtilis, 4.7 mm of inhibition for 100 µl in *Staphylococcus aureus* and 5.3 mm of inhibition for 100 µl in *S. typhimurium.* MgONPs of methanolic *S. cumini* seed extract showed more potent anti-microbial activity against *S. typhimurium* followed by *E. coli, Staphylococcus aureus*, and *Bacillus subtilis* (Table 6 and Fig. 9).

4. Discussion

Plants provide a superior platform for synthesized nanoparticles as they naturally produce capping agents and are free of hazardous chemicals [34]. Utilizing nanoparticles derived from plant extracts and magnesium in anti-bacterial and medicinal research applications is safe. Exploring medicinal plants that can produce nanoparticles holds the potential to create a new market for natural medicines in nanoscience, particularly for biomedical applications. The study focused on utilizing bioactive substances from natural sources like *S. cumini* seed that acts as functional foods to improve human health and treat several ailments [35]. Through phytochemical screening, it is possible to identify secondary metabolites with a range of medically and commercially significant actions, such as alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, steroids, amino acids, and anthraquinone glycosides [36]. Numerous studies conducted on various *S. cumini* sections demonstrated the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, steroids, amino acids, and anthraquinone glycosides [37,38]. The sample needs to undergo several steps to obtain these biochemical compounds from plants, including drying, maceration, homogenization, and extraction, which play a crucial role in obtaining these bioactive compounds. The extraction method, extraction duration, temperature, phytochemical makeup, and solvent employed all significantly impact how effectively the extraction is carried out [36–39,].

In this study, the extraction was carried out using various organic solvents such as methanol, ethanol, petroleum ether, chloroform, acetone, and diethyl ether to find out the best solvent to extract the bioactive compounds from *S. cumini* seeds. The current findings demonstrated that different solvents had variable extraction yields and it is due to variations in the solvent polarity and their impact on the concentration of bioactive chemicals in the extract. Among the solvents tested, methanol proved to be the most effective in extracting phenolics, flavonoids, and alkaloids from *S. cumini* seeds. This preference for methanol might be attributed to the high concentration of polar chemicals soluble in the plant material, which are better soluble in highly polar solvents like water, methanol, and ethanol. Further research was conducted to quantify the concentration of bioactive chemicals in the extract, aiming to understand the solvents effect on extraction yield. Methanolic extracts exhibited the highest quantities of flavonoids, phenolics, and alkaloids, resulting in the maximum extraction yield. This enhanced yield can be attributed to the increased solubility of these compounds in methanol compared to the other solvents studied [40–42]. The data from this study strongly suggests that methanol is the most efficient solvent for extracting bioactive components from *S. cumini* seed extracts. The choice of extraction solvent significantly influences both the extraction yield and the content of bioactive components, thereby impacting the biological activity of the extract [41].

In this study, crude seeds of *S. cumini* were successfully used for the

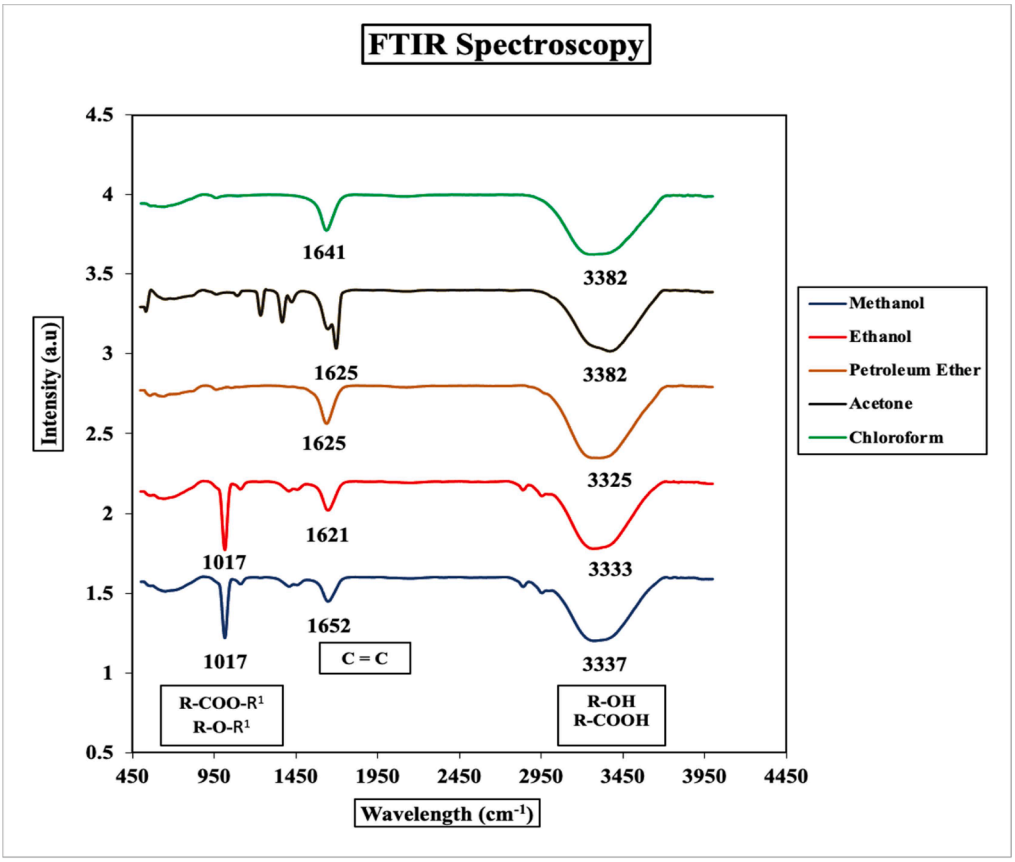

Fig. 4. FTIR Spectra of Synthesized MgONPs: Fourier-transform infrared (FTIR) absorption spectra showing the functional groups and chemical bonds present in the synthesized MgONPs derived from different solvents of *S. cumini* seed extracts.

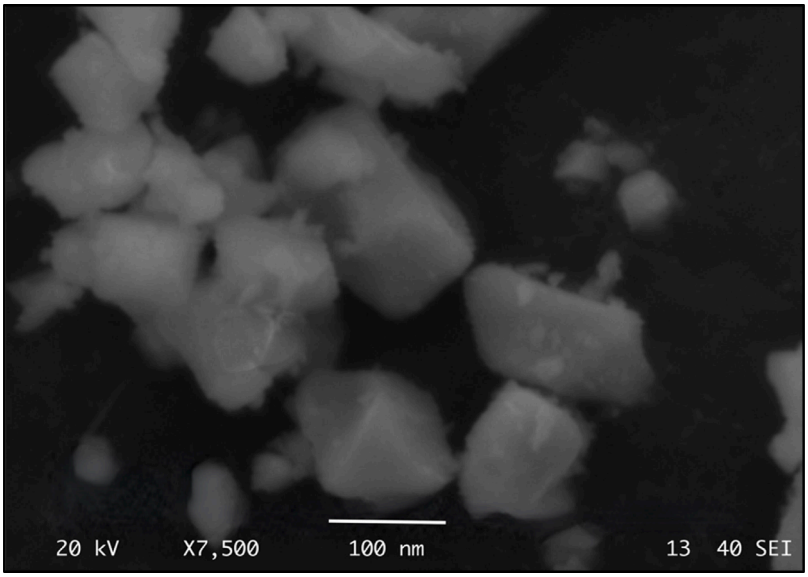

Fig. 5. SEM Image of MgONPs from Methanolic Extract: Scanning electron microscopy (SEM) image depicting the morphology of synthesized MgONPs from methanolic extract of *S. cumini* seeds, revealing both aggregates and individual particles with a size of approximately 100 nm.

bioreduction of magnesium chloride solutions to produce MgONPs. MgONPs were synthesized biologically using *S. cumini* seed extracts of different solvents and the conformation of MgONPs was determined using UV-visible spectroscopy by observing the SPR and functional groups via FTIR. Further characterization of MgONPs obtained from methanolic extract was done using SEM, XRD, and DSC investigations.

To assess anti-oxidant activity, DPPH scavenging activity tests were conducted on MgONPs prepared from the *S. cumini* seed extracts of different solvents. Among the tested extracts, the methanolic extract demonstrated the highest activity with the lowest I C_{50}values. This enhanced activity could be attributed to the extract's highest concentration of flavonoid, phenolic and alkaloid components [42–44], which

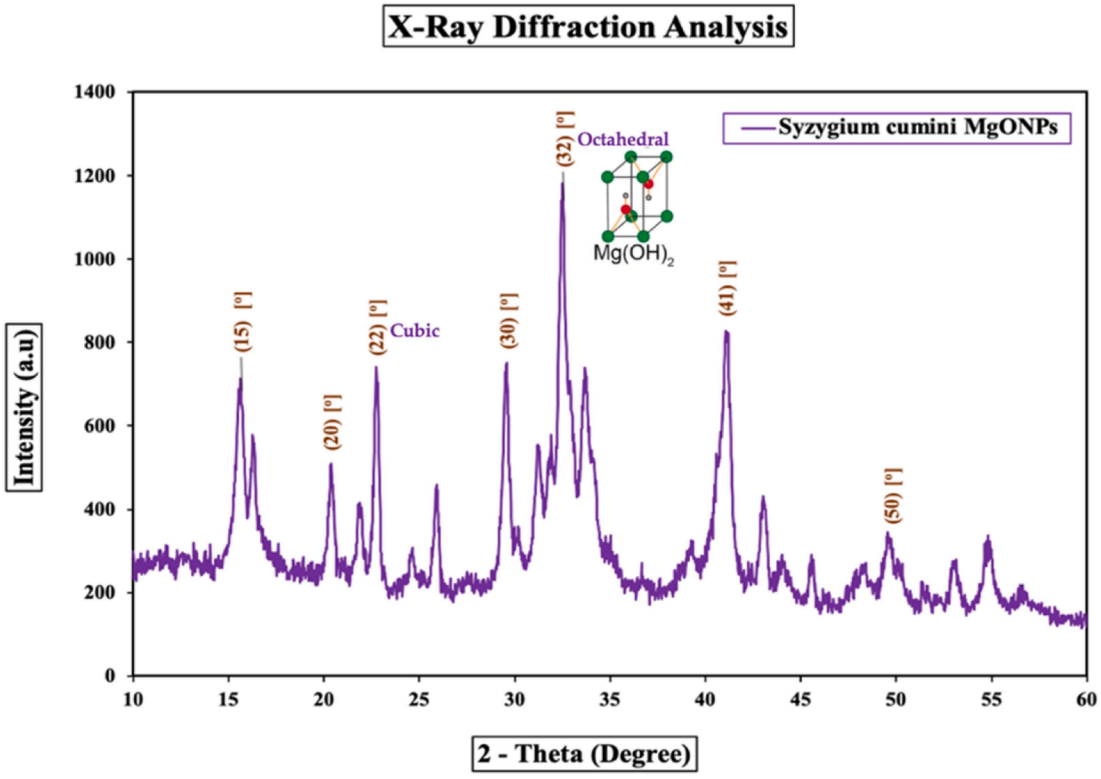

Fig. 6. XRD Pattern of MgONPs: X-ray diffraction (XRD) pattern demonstrating the crystalline structure and phase composition of MgONPs prepared using *S. cumini* seed methanolic extract.

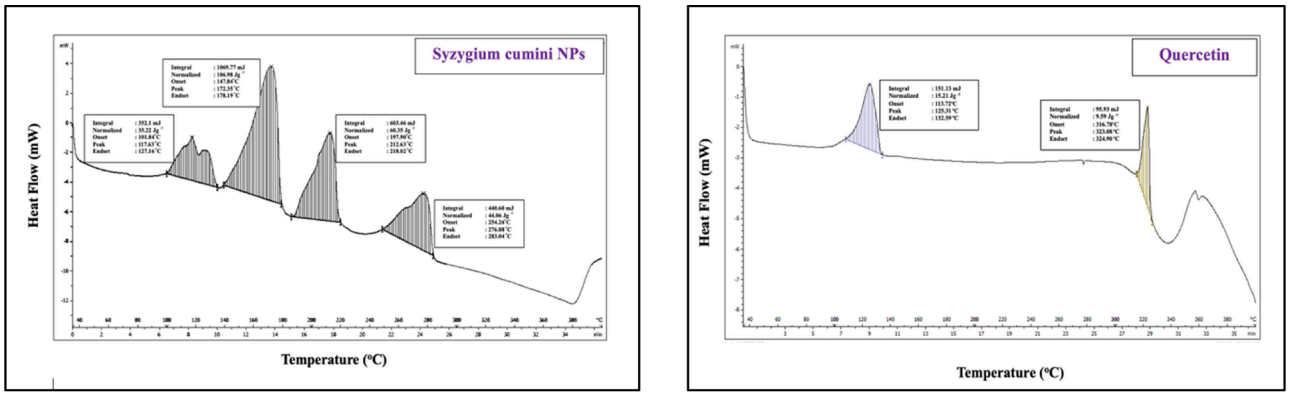

Fig. 7. DSC Analysis of MgONPs and Quercetin: Differential scanning calorimetry (DSC) analysis showing the thermal behavior and transitions of synthesized MgONPs derived from methanolic *S. cumini* seed extract (a), alongside quercetin as a reference standard (b).

Table 5
Amount of Flavonoid present in the methanolic seed extracts of *S. cumini*.

S. No	Peaks	Retention Time	Mean Peak Area	Concentration/Amount of Flavonoid (mg/gm)
1	Peak 1	1.52	265,752	5.821
2	Peak 6	17.3	3,105,349	49.62
3	Standard Quercetin	18.2	1,571,655	–

are known for their strong anti-oxidant properties. These compounds effectively protect the human body from oxidative damage by scavenging reactive oxygen species such as hydroxyl radicals, peroxyl radicals, hypochlorous acid, peroxynitrite, and superoxide anions [42]. Surprisingly, the methanolic seed extract of *S. cumini* exhibited twice the DPPH scavenging activity compared to ascorbic acid. These exciting findings suggest that the methanolic extract of *S. cumini* seeds holds potential as an anti-oxidant agent for future medication development.

Therefore, the anti-bacterial studies on MgONPs of methanolic *S. cumini* seed extracts were carried out against four different bacteria (E. coli, Bacillus subtilis, Staphylococcus aureus, and S. typhimurium). The anti-bacterial capabilities depend on their morphology and size. The findings disclosed that the MgONPs of *S. cumini* seed methanolic extract exhibited strong anti-bacterial efficacy against all four bacteria with the highest activity against Salmonella species, followed by E. coli, Bacillus subtilis, and Staphylococcus aureus.

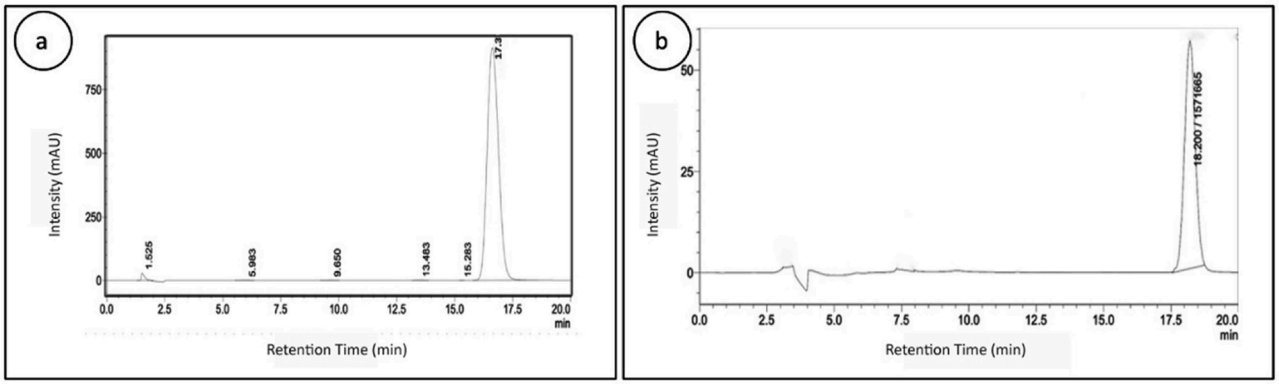

Fig. 8. HPLC Chromatogram of *S. cumini* seed extract and Quercetin: High-performance liquid chromatography (HPLC) chromatogram depicting the presence of quercetin flavonoids in the methanolic extract of *S. cumini* seeds (a), compared with a quercetin standard (b).

Table 6
Anti-microbial activity of MgONPs methanolic seed extracts of *S. cumini* in four different organisms and their zone of inhibition.

Test Organisms	Zone of Inhibition (mm)				
	25 µL	50 µL	75 µL	100 µL	Control
E. coli	4.06 ± 0.08	4.1 ± 0.09	4.4 ± 0.08	5.2 ± 0.12	3.8 ± 0.06
Bacillus subtilis	3.96 ± 0.04	4.06 ± 0.08	4.5 ± 0.44	4.6 ± 0.27	—
Staphylococcus aureus	3.5 ± 0.36	3.0 ± 0.04	3.96 ± 0.27	4.7 ± 0.05	3.6 ± 0.08
S. typhimurium	4.1 ± 0.06	4.5 ± 0.12	5.0 ± 0.1	5.3 ± 0.05	3.6 ± 0.1

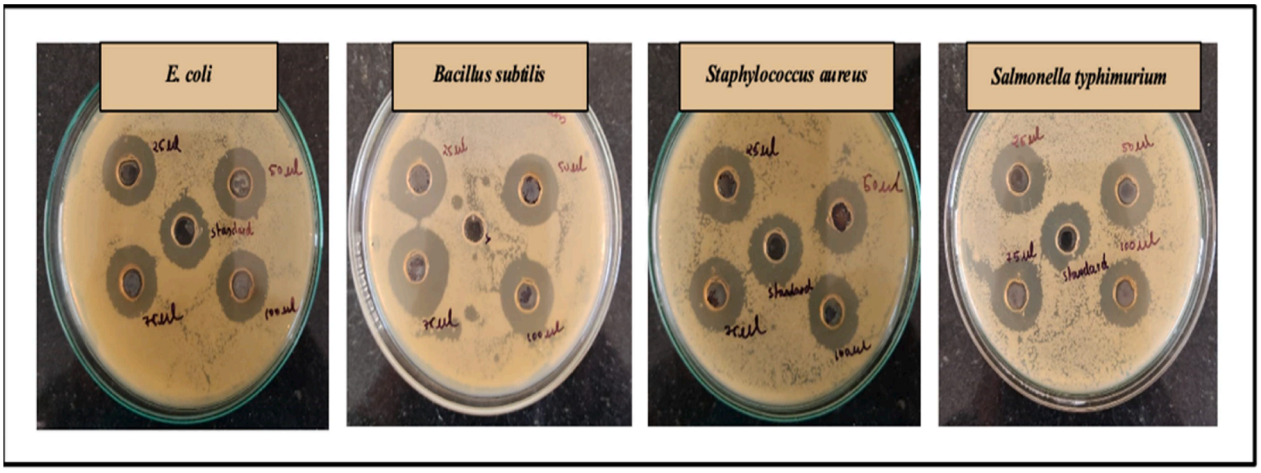

Fig. 9. Anti-microbial Activity of MgONPs from methanolic seed extracts: Assessment of anti-microbial efficacy against selected bacterial strains (*Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Salmonella typhimurium*) by MgONPs synthesized from methanolic *S. cumini* seed extract, showing inhibition zones.

The results of the current study highlight the synergistic anti-bacterial properties of the MgONPs produced from *S. cumini* seed extract, likely due to the presence of phytochemicals with medical applications.

5. Conclusion

The study demonstrates the *S. cumini* as a promising and valuable source of bioactive compounds with potential applications in pharmacology. The *S. cumini* seed extracts were prepared using different solvents like methanol, ethanol, petroleum ether, acetone, chloroform, and diethyl ether. Among these tested solvents, methanol proved to be the most effective for extracting bioactive compounds, exhibiting the highest extraction with a yield of 42 %, along with the highest amounts of flavonoids (12.76 mg QE/g DW), phenolics (1.72 mg GAE/g DW), and alkaloids (1.32 mg AE/g DW). The qualitative and quantitative analysis of *S. cumini* seed extract through HPLC showed a high flavonoid (quercetin) concentration of 49.62 mg/gm. Additionally, the study synthesized MgONPs using *S. cumini* seed extract. The formation of MgONPs was confirmed by observing the SPR peak at 290 nm in UV–vis spectroscopy. The FTIR studies identified the presence of alcohols, carboxylic acids, ethers, and esters. SEM showed spherical and aggregated morphology with a size <100 nm. XRD exhibited a monoclinic structure, and DSC was used to study the thermal properties. These findings suggest that *S. cumini* is a promising source of bioactive compounds, particularly flavonoids like Quercitin, which are responsible for the successful synthesis of MgONPs, exploring its potential applications in pharmacognosy. The *In vitro* anti-oxidant and anti-microbial studies directed MgONPs as effective anti-oxidants as well as antibacterial agents. In conclusion, the high concentration of flavonoid (Quercitin) in *S. cumini* seed extract played a key role in the synthesis of MgONPs.

CRediT authorship contribution statement

Sai Manogna Kotakadi: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Manpreet Jivin Bangarupeta:** Writing – original draft, Methodology, Investigation. **Kusuma Kandati:** . **Deva Prasad Raju Borelli:** Writing – review & editing, Validation, Supervision, Resources. **Jaheera Anwar Sayyed:** Methodology, Investigation. **Mannur Ismail Shaik:** Writing – review & editing, Validation, Resources, Funding acquisition, Data curation, Conceptualization. **John Sushma Nannepaga:** Writing – review & editing, Validation, Supervision, Software, Resources, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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