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Optimizing organically nano-fabricated Ni metal complexes for enhanced antioxidant and anticancer activity using response surface methodology

Swathi Aleti^{1*}, Savita Belwal¹ and Mukunda Vani Medala²

Abstract

Background Researchers, prompted by the toxicity and side effects associated with cisplatin, are exploring alternative approaches for developing transition metal-based anticancer agents. Employing a green biochemical approach, we transformed Nickel pyridine dicarboxylic acid compounds into the nanoscale using the aqueous extract of *Macrotyloma uniflorum* (horse gram).

Results Characterization of the biosynthesized nanoparticles involved electronic and IR spectroscopy. A scanning electron microscope revealed a predominant spherical shape for most Nickel nanoparticles (Ni-NPs), with XRD patterns indicating particle sizes ranging from approximately 30–150 nm. The nanoparticles were evaluated for their free radical scavenging efficiency and in vitro anti-malignant properties against HeLa and A549 cancer cell lines. Numerical optimization of the DPPH and MTT assays was conducted using response surface methodology (RSM), focusing on the effects of 3,4-pyridine dicarboxylic acid (ML₁), 2,4-pyridine dicarboxylic acid (ML₂), nickel nanoparticles concentration, and temperature. In this investigation, the incorporation of Horse Gram seed extract (*Macrotyloma uniflorum*) has unveiled its abundance in phenolic and flavonoid compounds, widely acknowledged for their robust antioxidant activity in the existing literature.

Conclusion The present study highlights the potential for refining the bio-toxicity and biochemical attributes of Ni-NPs to pave the way for a new generation of versatile anticancer agents with clinically established efficacy. Notably, the anticipated data closely corresponds with experimental outcomes, reinforcing the trustworthiness and validity of the RSM model for examining anticancer and antioxidant properties in this context. ML₂ exhibited heightened antioxidant and anticancer activities in comparison to ML₁ nanoparticles.

Keywords Biosynthesized Ni-NPs, *Macrotyloma uniflorum*, SEM, XRD, Antioxidant, Anti-cancer activity, Response surface methodology

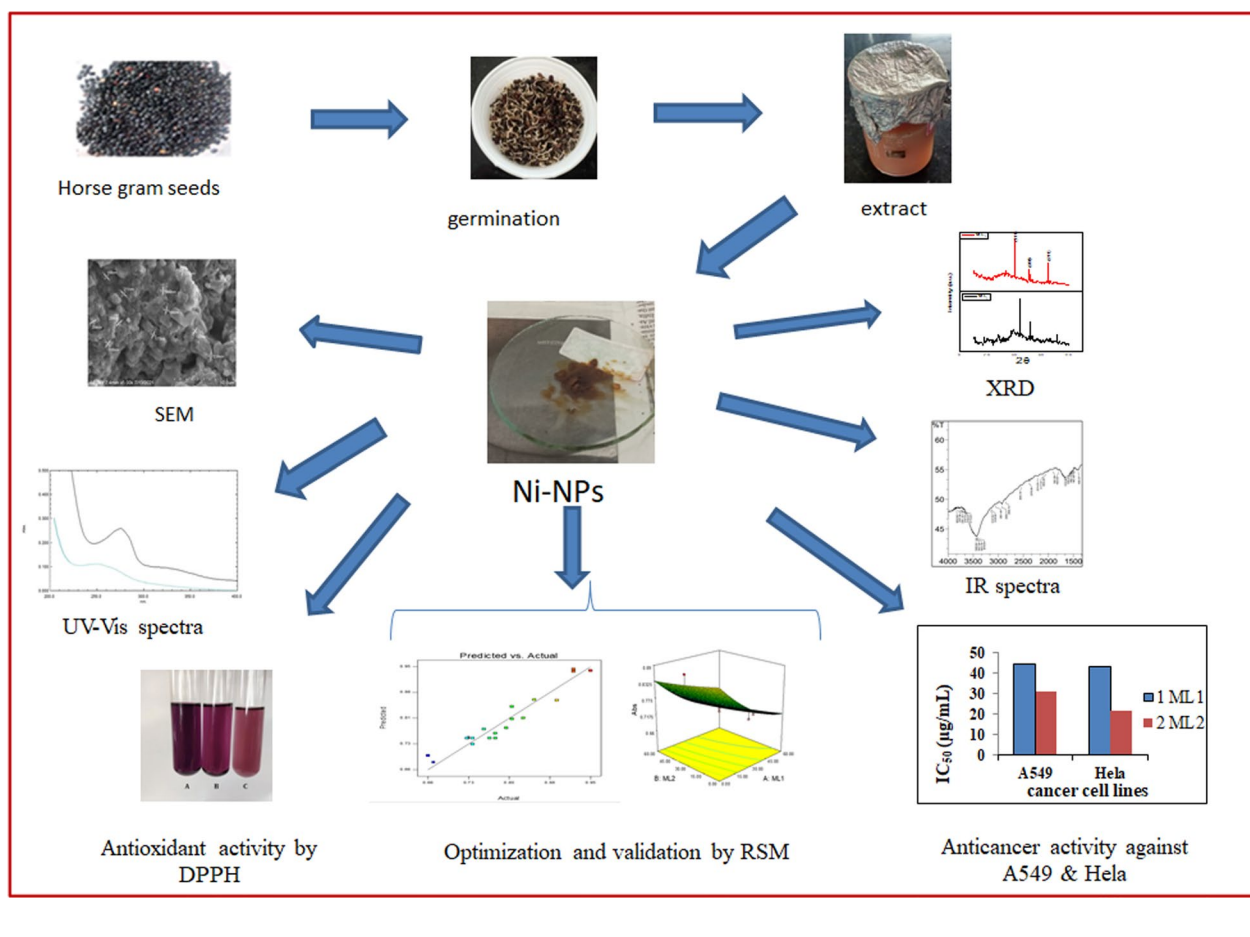
*Correspondence:

Swathi Aleti

swathihs@anurag.edu.in

Full list of author information is available at the end of the article

Graphical abstract



Background

Nanoparticles derived from metals exhibit a range of advantageous properties, including excellent conductivity, a significant surface-to-volume ratio, and notable nano-plasmonic characteristics [1]. The extensive study of metal nanoparticles is driven by their potential applications in sensor devices, bio-devices, data storage, catalytic processes, and spectrophotometric techniques [2]. Biogenic nano-sized particles obtained from plant-based materials offer a straightforward and time-efficient synthesis method, with plant extracts proving more conducive to size reduction compared to microbiological cultures [3]. Literature supports the efficacy of botanical extracts in treating skin diseases, outperforming results obtained from various microbes [4].

In this research, the focus is on exploring different ligands for the creation of Ni-nanoparticles. Two isomers of pyridine dicarboxylic acid, namely pyridine-2, 4-dicarboxylic acid (Lutidinic acid) and pyridine-3,4-dicarboxylic acid (Cinchomeric acid), have been selected as ligands for the study in conjunction with Nickel metal. Lutidinic Acid, recognized as a corrosion inhibitor [5], is also noted for its cytotoxic nature [6]. The objective is to examine the outcomes of these ligands in the synthesis of Ni nanoparticles.

The rationale behind selecting the organic moiety pyridine-2, 4-dicarboxylic acid and its derivatives for the study is clear. These compounds have demonstrated significant effects on physiological activity, acting as immuno-suppressants [7] and fibrous-repressive drugs crucial for the initiation and growth of certain plant

families [8]. Additionally, they play a protective role by preserving specific enzymes in the cells of *Bacillus subtilis* species under temperature reduction conditions [9]. Notably, the pyridine compound with 2,4-dicarboxylic acid features structure identical to 2-oxoglutarate, a well-known inhibitor of 2-oxoglutarate-dependent dioxygenases. This inhibition, as observed in the growth of tomato seedlings exposed to various concentrations of pyridine dicarboxylic acid, resulted in diminished root size and smaller epicotyl [10].

Bio-nanotechnology involves the synthesis of nanoparticles through the utilization of organic biomolecules, encompassing living organisms such as fungi, bacteria, herb, yeast, as well as various naturally occurring moieties like proteins, peptides, sugar and vitamins [11, 12].

The integration of physical and chemical methodologies with fundamental standards, such as redox reactions, in the presence of biological adjuvants or natural phytonutrients, results in the production of nanoparticles with specific functions [13]. The biological synthesis of nanoparticles offers an environmentally friendly, uncomplicated, and cost-effective approach for researchers. This method also possesses the advantage of stabilizing nanoparticles through the utilization of plant secondary metabolites, serve equally reducing and capping molecules. Notably, nanoparticles produced using green technology exhibit minimal toxicity compared to chemically prepared counterparts, making them efficient carriers for drug delivery systems in *in vivo* applications [14].

Nickel nanoparticles, for instance, show promising applications in various fields including magnetism [15], microelectronics, power skills [16], and biomedical applications [17]. Due to their rapid reactivity, ease of operation, and environmentally friendly properties, nanoparticles play a pivotal role in accelerating various organic reactions. These include oxidative coupling of thiols with multiple reaction pathways [18], reduction of aldehydes and ketones [19], hydrogenation of olefins [20], preparation of stilbenes through alcohol by Wittig-olefination [21], and α -alkylation of ketones [22]. Furthermore, they serve as catalysts for the decomposition of ammonia and its products [23]. A cutting-edge application of nanoparticles involves the fabrication of nanotubes, particularly carbon nanotubes (CNTs) [24].

The literature extensively covers the biological synthesis, characteristics, and applications of both Ni and Ni-oxide nanoparticles, with numerous articles and reviews focusing on environmentally friendly approaches for their preparation [25]. Nasseri et al. presented a method for synthesizing NiO nanoparticles using an aqueous

extract of *Tamarix serotina*, showcasing their catalytic properties and confirming the nanoparticles' size to be in the range of 10–15 nm with a cuboid shape [26].

Presently, various plants segments, including leaves, flowers, seeds, fruits, barks, and peels, are employed for distillation to produce nanoparticles [27]. The plant distillate, rich in phytonutrients, antioxidants, and essential organic compounds, holds potential therapeutic significance [28, 29]. Over the past decade, the preparation and fabrication of nickel oxide nanoparticles have been continuously advancing, exploring their applications in various natural biological systems [30].

A strong statistical technique for experiment design and parameter optimisation is response surface methodology (RSM). Using process parameters, this can be utilized to create an accurate model for the response function in the optimal area [31]. Minimum quantity of research has been documented using RSM to optimize different ligands as parameters.

Our summary encompasses the accomplishments in the eco-friendly biotechnical preparation of Nickel nanoparticles and explores the influence of organic moieties to the physical characteristics of newly synthesized Ni-nanoparticles. Additionally, we present findings on the relative influence of various ligands using RSM, examining their structural effects and the biological properties of organically synthesized Nickel nanoparticles.

Methods

Chemical procurements

Chemicals are sourced from Sigma-Aldrich and employed in chemical reactions, with all solvents subjected to drying and additional purification through standard methods.

Synthesis of Ni complex

The compounds used in this study were obtained from the Department of Chemistry at the University of Rajasthan. The template condensation process was applied to produce Nickel (II) macrocyclic complexes, specifically utilizing benzyl dihydrazone with 3,4-pyridine dicarboxylic acid (ML₁) and 2,4-pyridine dicarboxylic acid (ML₂) in the presence of metal chloride. The synthesis followed established protocols as described in the literature. Subsequently, these complexes were further converted into nano-sized structures within our laboratory for their several applications. The illustrated macrocyclic structures are depicted in Fig. 1.

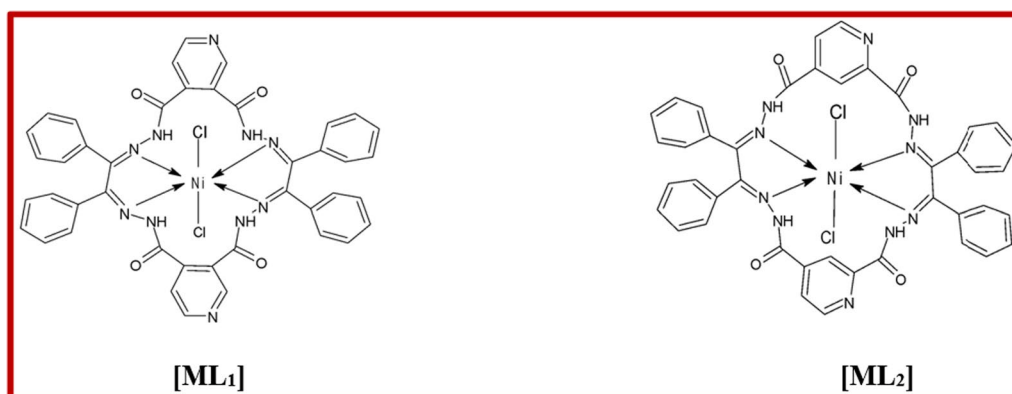


Fig. 1 Structures of the macro cyclic complexes

Preparation of plant extract

Macrotyloma uniflorum is acquired from the supermarket, cleaned with deionized H₂O, and drenched in distilled H₂O at 40 °C for 12 h. Subsequently, it undergoes a germination process for 48 h at 30 °C, with regular moistening using deionized H₂O every half day. The sprouts are then crushed to form a dense paste by incorporating

phosphate buffer solutions (pH 8). The resultant blend is strained through a twin layer of whatman No.1 filter paper, and the filtrate was centrifuged at 12,000 rotations per minute for 8 min below 40 °C. The weightless floating liquid is collected, and the extract solution is employed for the reduction of compounds into nanoform (Fig. 2).

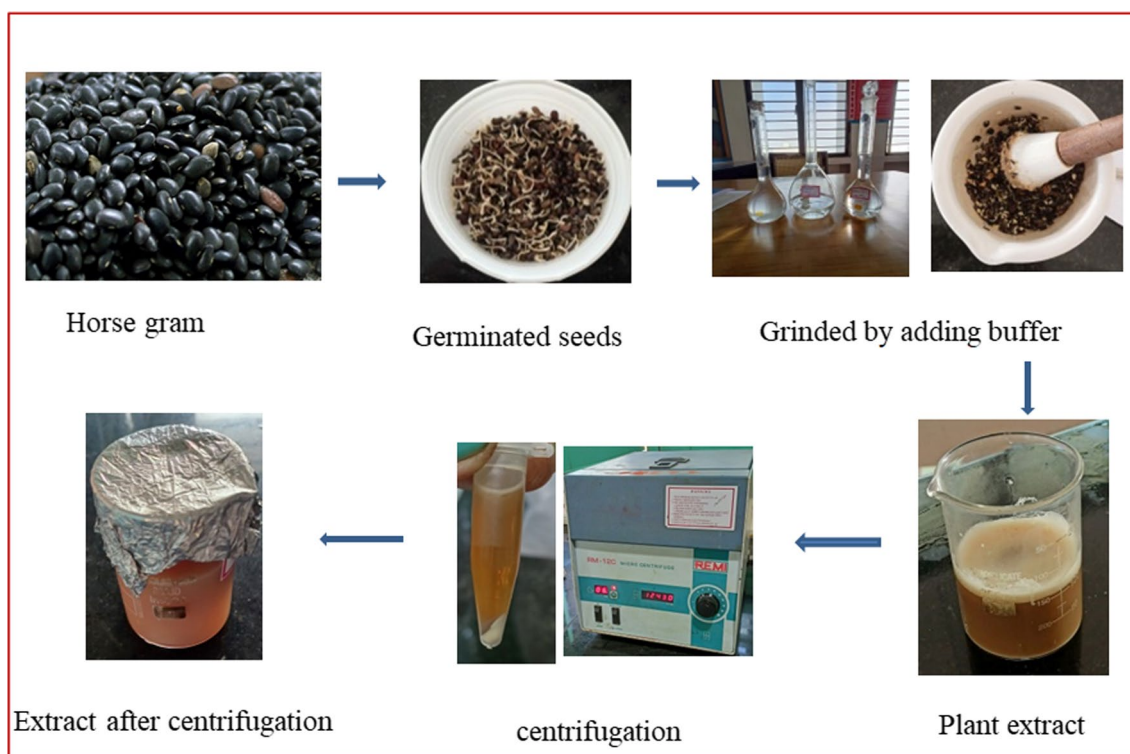


Fig. 2 Synthesis of extract of *Macrotyloma uniflorum*

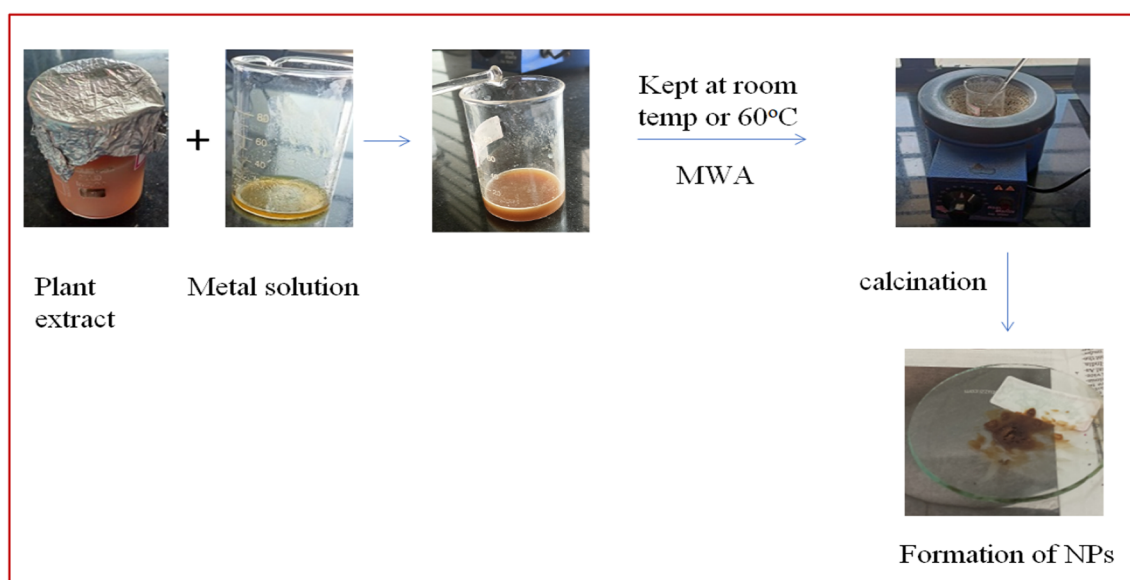


Fig. 3 Synthesis of Ni nanoparticles

Synthesis of Ni nanoparticles

The synthesis of Ni nanoparticles through a green route involves mixing the seed extract with the specific quantity of aqueous solution of nickel compounds, followed by allowing mixture to remain undisturbed until a noticeable colour change indicates nanoparticle formation. This process occurs either at room temperature or at 60–70 °C, depending on specific requirements.

For the microwave-assisted synthesis, a reduced amount of extraction solvent and a shorter reaction time yield efficient results. The reaction mixture is heated in a microwave at a power of 100–150 W for about 1–2 min. Later the extract undergoes centrifugation to room temperature, and the resulting precipitate is dried to obtain the desired metal nanoparticles. Notably, the percentage yield of nanoparticles from both synthesis methods was found to be nearly identical.

Utilizing an aqueous solution comprising plant material and Ni metal complexes in a 10:1 ratio, nanoparticles were synthesized following literature guidelines [32]. The mixture underwent incubation over a water bath, with careful pH control using 1 Normal H_3PO_4 . Within 2–3 h, a distinct colour transformation from yellow to dark brown signified the conversion of the mixture into Nickel nanoparticles (Fig. 3).

The synthesized nanoparticles were subsequently subjected to electronic spectroscopy analysis. The size and crystallinity of Ni-nanoparticles were determined through XRD. The surface morphology of Ni-NPs was then examined using a scanning electron microscope

(SEM) to obtain micro/nano-images of the complexes (ML_1 and ML_2). SEM analysis revealed that the Ni-nanoparticles exhibited a round shape within the nano size range. Furthermore, the nanoparticles have been evaluated for their anti-malignant action.

Statistical analysis (factorial design and optimization)

The experimental strategy, data scrutiny, and statistical optimization were carried out using Design-Expert software from Stat-Ease, USA. The objectives were to minimize the absorbance level in the sample, employing three variables: ML_1 with concentrations ranging from 0 to 60 $\mu\text{g/l(ppb)}$, ML_2 the concentrations range from 0 to 60 $\mu\text{g/l}$, and temperature varying from 20 to 50 °C. These variables were explored at three levels, and the absorbance value was observed as the response.

Design-Expert software generated a 2^3 factorial design consisting of 20 experiments to systematically explore the parameter space. Table 1 presents the range and variables—both in actual and coded forms—that were

Table 1 Depiction of independent variable levels in both actual and coded formats

Variables (independent)	Signs	Levels of code		
		– 1	0	+ 1
ML_1 concentration ($\mu\text{g/l}$)	X_1	0	30	60
ML_2 concentration ($\mu\text{g/l}$)	X_2	0	30	60
Temperature (°C)	X_3	20	35	50

investigated. Notably, the chosen mid-level values for ML_1 concentration, ML_2 concentration, and temperature were 30 $\mu\text{g/l}$, 30 $\mu\text{g/l}$, and 35 $^{\circ}\text{C}$, respectively.

The outcomes of the experiments were displayed using a second-order polynomial equation.

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i \neq j=1}^3 \sum_{j=1}^3 \beta_{ij} X_i X_j \quad (1)$$

In the context of the regression equation, where Y denotes the anticipated value of the response variable and β denotes the coefficient, the outcomes were analysed using Design Expert. The influence of independent terms on dependent variables was evaluated, and through resolving the regression equation and scrutinizing the graphs, optimal conditions were determined. These optimal conditions were then employed to assess antioxidant and anticancer activities. The experimentally acquired responses were juxtaposed with the numerically predicted counterparts for comparison. To statistically assess the variance between mean values at a 95% confidence interval, analysis of variance (ANOVA) was employed. As per the quadratic model of ANOVA, the significance of model terms is signified by “Prob > F ” values below 0.0500.

Antioxidant activity

Antioxidant estimation employs the use of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), a compound that aids in assessing the radical capacities of antioxidants [33]. The assessment relies on the capacity of antioxidants to diminish DPPH, measured through the DPPH radical assay principle. This involves determining the reduction in optical density (Abs) at 517 nm using a spectrophotometer after the interaction with nanoparticles (NPs). The reduction in DPPH colour during the reaction is tracked, and the antioxidant assay is computed through spectrometric analysis. The percentage of DPPH radical scavenging is calculated using the equation provided below [34].

The DPPH scavenging percentage is determined using the following formula:

$$\text{DPPH scavenging(\%)} = 100 \times \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}$$

Here Absorbance of control represents the absorbance before adding test sample to DPPH and Absorbance of sample is the absorbance after reaction has taken place between DPPH and test sample.

The formula quantifies the reduction in absorbance due to the scavenging activity of the tested sample against the DPPH radical.

Anticancer activity

Upon procurement of cell lines from NCCS, Pune, the cells were maintained in DMEM augmented with 10% Fetal Bovine Serum (FBS) with antibiotic drugs (0.5 mL^{-1} penicillin/streptomycin) at 38 $^{\circ}\text{C}$ in a 4–5% CO_2 /97% air atmosphere. To examine the MTT assay, cells were planted in a 96-well plate at a density of 5.0×10^3 cells/well in 100 μL of culture and allowed to incubate for 8–12 h at 35–40 $^{\circ}\text{C}$, as previously documented [35]. To assess cell viability, three independent triplicate experiments were conducted three times using six concentrations of test chemicals ranging from 5 to 100 $\mu\text{g/mL}$. After 24 h of incubation, each treatment was removed, and fresh media with varying concentrations of test compounds were added. Subsequently, the test solution was replaced with fresh media containing MTT solution (0.5 mg/mL), and the plates were further incubated at 35–37 $^{\circ}\text{C}$ for three hours. Following the reduction of MTT salt to chromophore formazan crystals, a precipitate was formed at the end of the incubation period. This conversion was facilitated by cells with metabolically active mitochondria. The absorbance of solubilized crystals in DMSO was then measured at 560 nm using a microplate reader.

$$\% \text{Inhibition} = \frac{100 \times (\text{Control} - \text{Treatment})}{\text{Control}}$$

Characterization of nanoparticles

The prepared samples underwent characterization using various analytical techniques. UV–visible spectroscopy was carried out using a UV1800S instrument from Shimadzu, Japan. SEM analysis was performed using a JEM-IT 800 SHL version, Japan. Fourier-transform infrared (FTIR) analysis was conducted using an Alpha-T instrument from Bruker. X-ray diffraction (XRD) analysis was accomplished using the X'Pert PRO XRD PW 3040 system.

Results

IR-spectroscopy

IR spectroscopy offers high reliability in characterizing the bulk of nanoparticles. By estimating vibrations of

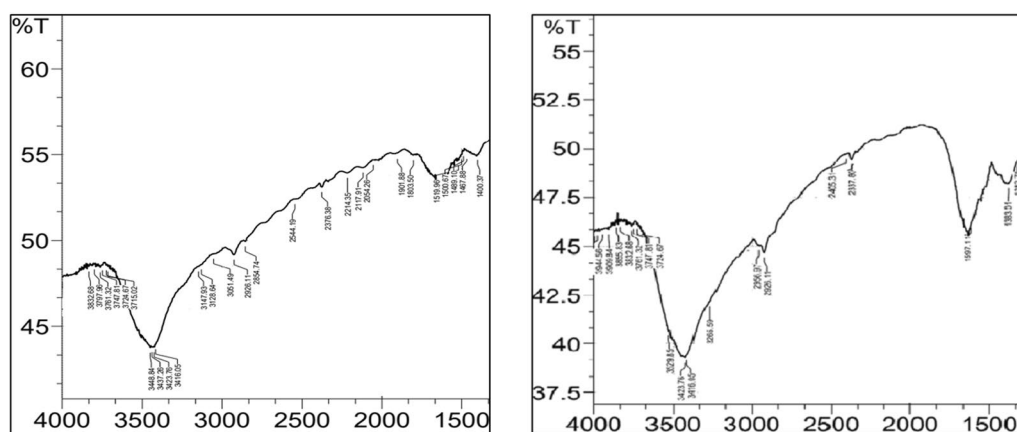


Fig. 4 IR-spectra of Ni-NPs of ML₁ and ML₂

functional groups on their surface, FTIR, in particular, is incredibly versatile for surface analysis of nanoparticles in specific conditions, enabling determination of their surface chemical composition with precision.

The IR spectra analysis of both metal nanoparticle complexes was conducted to elucidate their structural features and bonding interactions. Remarkably, the spectra of the complexes show absence of bands corresponding to $\nu_{as}(-NH_2)$ at 3360 cm^{-1} , $\nu_{as}(-NH_2)$ at 3280 cm^{-1} , and $\nu(C=O)$ with the range of $1680\text{--}1690\text{ cm}^{-1}$. This absence in the spectra indicates the condensation process and the construction of a macrocyclic structure. Peaks observed in the vicinity of $1648\text{--}1580\text{ cm}^{-1}$ were accredited to $\nu(C=N)$. The band position for $\nu(C=N)$ was found to be lower than the typical values associated with azomethine groups, supporting the inference of coordination of the group with the metal atom and the creation of macrocyclic complexes. Furthermore, the absorption bands corresponding to the phenyl ring were identified in the regions of $1465\text{--}1495\text{ cm}^{-1}$ and $1355\text{--}1390\text{ cm}^{-1}$, assigned to $\nu_{asym}C_6H_5$ and $\nu_{sym}C_6H_5$, respectively (Fig. 4).

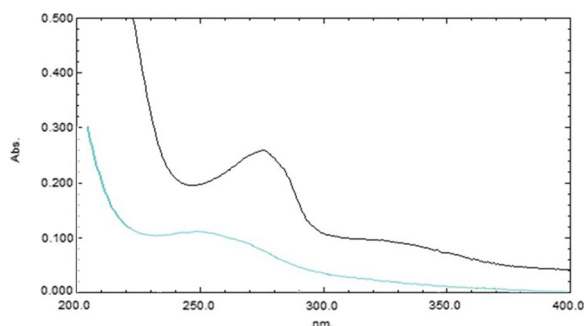


Fig. 5 UV-Vis spectra of Ni-NPs of ML₁ and ML₂

Electronic spectra of Ni-NPs

UV-Visible spectroscopy is a powerful method for studying the growth of metal nanoparticles within a polymer network or the formation of a polymer network around a metal nanoparticle core. UV-Visible spectroscopy is commonly employed to investigate the plasmonic resonance of nanoparticles. By analysing absorbance data in the range of $200\text{--}700\text{ nm}$, we have confirmed the formation of Ni nanoparticles and their plasmonic resonance. This enables us to characterize and confirm the presence of reduced Ni metal in the form of nanoparticles.

The observation of electron oscillation points to the occurrence of surface plasmon resonance (SPR), where absorption bands are evident. The phenomenon arises from the absorption of UV light by Ni-NPs, leading to consistent oscillation of conduction electrons. This resonance is achieved when the frequency of surface electrons of metal NPs aligns with that of incident photons. The UV-Visible spectrophotometer is employed to analyse the optical density of the absorbed light [36] (Fig. 5).

XRD analysis

X-ray diffraction (XRD) is a technique utilized to analyse the crystalline properties of materials, providing insights into their structural characteristics, phase nature, lattice parameters, and grain size. The lattice parameter is determined through the application of the Scherrer equation, which involves assessing the broadening of the most prominent peak observed in an XRD pattern for a given sample.

The XRD spectra of Nickel-nanoparticles, synthesized through the reduction of Ni metal complexes

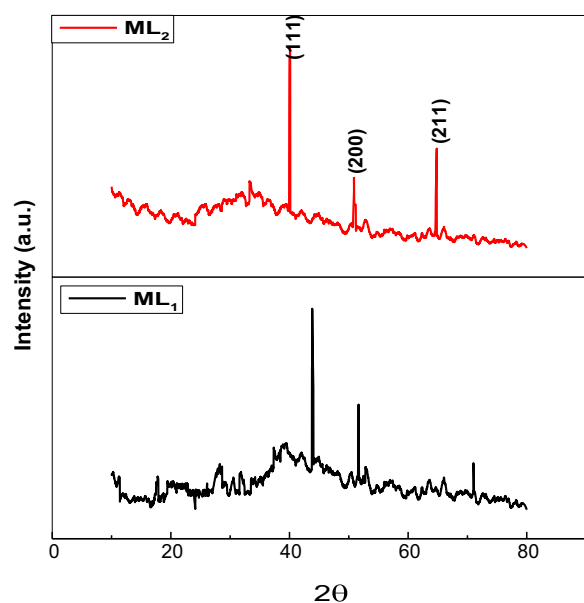


Fig. 6 XRD spectra of Ni-NPs of ML_1 and ML_2

(ML_1 and ML_2) using the seed extract of *Macrotyloma uniflorum*, were examined. For the nano-particles of ML_1 compound, five unique diffraction peaks were identified with corresponding 2θ values of 18.85, 20.56, 22.55, 17.65, and 16.17. Particularly, the peak at 18.85° exhibited the maximum intensity. Similarly, the XRD spectrum of the ML_2 compound displayed six characteristic diffraction peaks at 2θ values of 31.13, 22.38, 19.82, 25.1, 27.3, and 16.5, with the peak at 31.13° demonstrating the highest intensity (Fig. 6).

SEM (scanning electron microscope) of nickel-NPs

SEM is a powerful technique used for analysing particle characteristics, such as size, shape, and texture with high precision. Operating with only small amounts of material, typically in the milligram range, SEM employs a focused electron beam that scans the prepared sample in a series of parallel tracks. Figures 7 and 8 depict SEM images of two distinct metal nanoparticles.

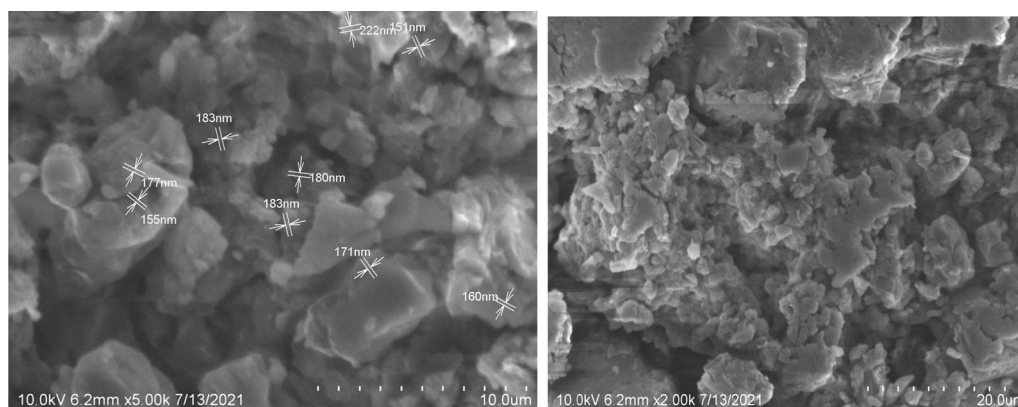


Fig. 7 SEM images of ML_1 NPs

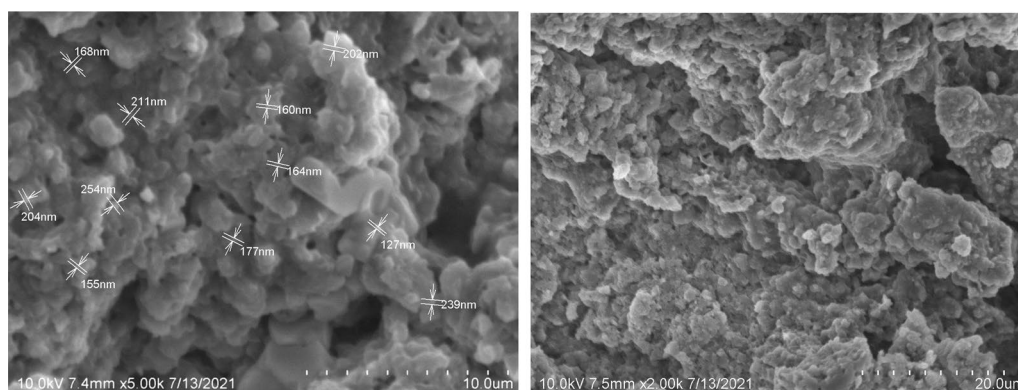


Fig. 8 SEM images of ML_2 NPs

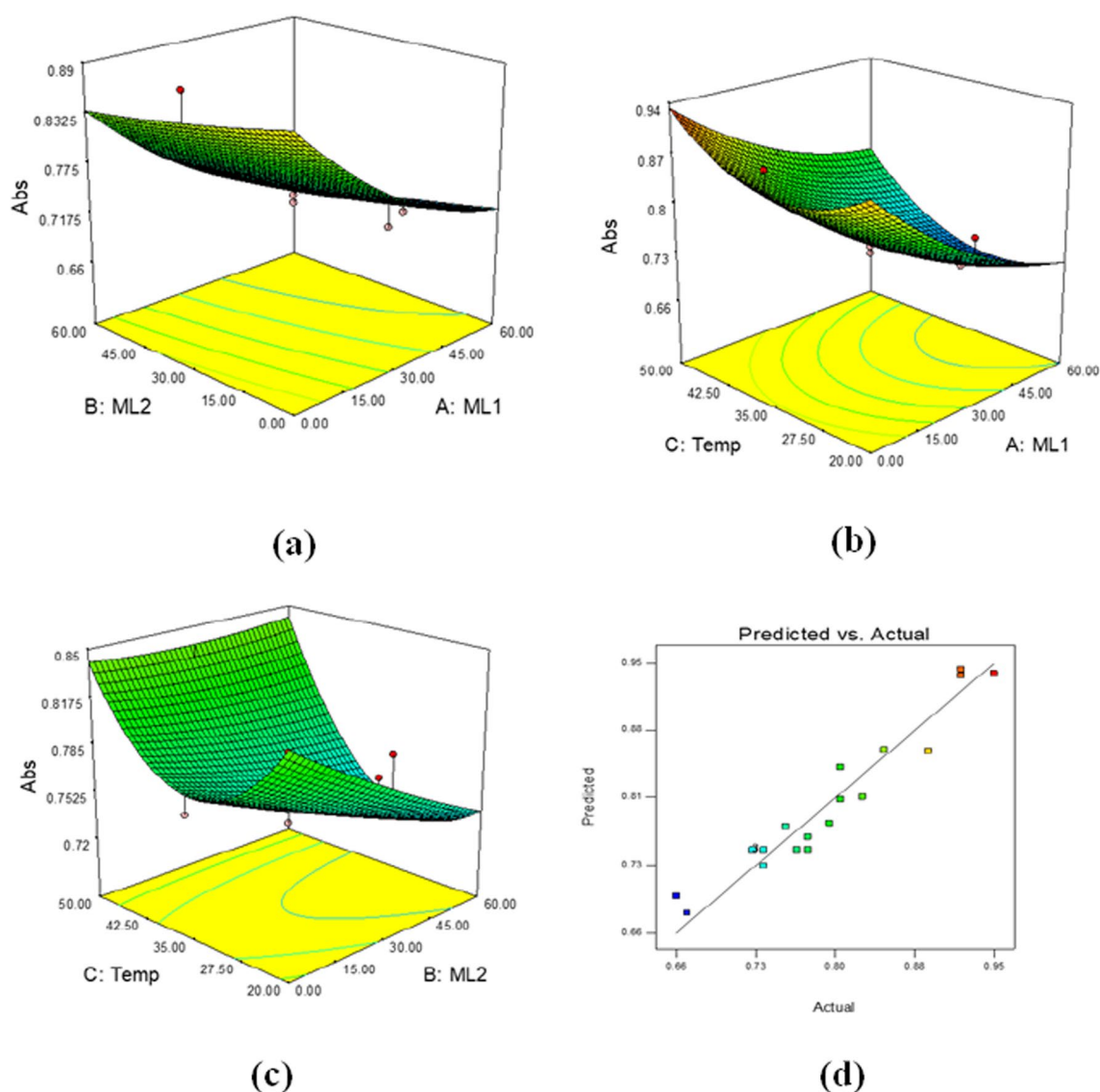


Fig. 9 **a** 3D plot for absorbance as a function of ML₁ and ML₂ concentration. **b** 3D plot for absorbance as a function of ML₁ concentration and temperature. **c** 3D plot for absorbance as a function of ML₂ concentration and temperature. **d** Predicted versus actual values of absorbance

Statistical analysis using response surface methodology

Response surface methodology (RSM) stands out as a highly efficient technique within the realm of design of experiments (DOE). Its primary goal is to streamline the optimization of crucial parameters to achieve an optimal response while ensuring a robust model fit. By employing RSM, we have effectively optimize key factors such as ML₁ concentration, ML₂ concentration, and temperature, all while conducting a limited number of experiments (20). This approach is particularly advantageous as it avoids the need for an extensive array of experiments (Figs. 9).

The design table was obtained (Table 2) and experiments were conducted following the conditions of the table. The absorbance was measured and a quadratic model with 20 runs was built. It was investigated how design process parameters affected absorbance.

In this context, key model terms include ML₁ and ML₂ concentrations, temperature, and the interaction term involving temperature. The model's importance is substantiated by an *F*-value of 15.05. Furthermore, the "Lack of Fit *F*-value" of 3.18 suggests that the lack of fit is not significantly different from pure error, underscoring the adequacy of the model. This signifies that the

Table 2 Central composite design of input variables with responses

S. no	Independent variables			Response	
	Factor 1 (ML ₁ , µg/l)	Factor 2 (ML ₂ , µg/l)	Factor 3 (Temperature, °C)	Experimental (Absorbance)	Predicted (Absorbance)
1	60.00	30.00	35.00	0.66	0.7
2	30.00	60.00	35.00	0.74	0.75
3	0.00	0.00	20.00	0.92	0.94
4	30.00	30.00	50.00	0.81	0.807
5	30.00	30.00	35.00	0.74	0.75
6	30.00	30.00	35.00	0.74	0.75
7	0.00	60.00	50.00	0.92	0.94
8	60.00	0.00	50.00	0.81	0.84
9	60.00	60.00	50.00	0.83	0.81
10	0.00	60.00	20.00	0.85	0.86
11	30.00	30.00	35.00	0.73	0.75
12	60.00	60.00	20.00	0.67	0.68
13	30.00	30.00	35.00	0.78	0.76
14	30.00	30.00	35.00	0.77	0.75
15	30.00	30.00	20.00	0.80	0.78
16	0.00	30.00	35.00	0.89	0.86
17	0.00	0.00	50.00	0.95	0.94
18	60.00	0.00	20.00	0.78	0.75
19	30.00	0.00	35.00	0.76	0.77
20	30.00	30.00	35.00	0.74	0.738

model fits the data well. This indicates a good fit of the model to the data. The coefficient of determination (R^2) for the response is 93%, affirming the appropriateness of the applied models. The adjusted coefficient of determination (Adj. R^2) for the response is determined to be 86%. As a result, it can be said that the experimental value meets Eq. (2) and the regression coefficients fit into a second-order polynomial equation.

$$Y = 1.209 - 0.00546x_1 - 0.00267x_2 - 0.01855x_3 + 1.388 \times 10^{-6}x_1x_2 + 2.5 \times 10^{-5}x_1x_3 + 4.722 \times 10^{-5}x_2x_3 + 3.232 \times 10^{-5}x_1^2 + 4.545 \times 10^{-6}x_2^2 + 2.626 \times 10^{-4}x_3^2 \tag{2}$$

In the given equation, where Y represents absorbance, x_1 stands for ML₁ concentration, x_2 for ML₂ concentration, and x_3 for temperature; it is noteworthy that concentration of ML₁ and ML₂ as well as temperature all exhibit a negative impact on absorbance. In order to explore the impact of interactions, 3D plots were created by graphing absorbance versus independent variables while maintaining optimal circumstances for the other variables. These graphs effectively interpreted the interactions between the two factors.

Variables optimization and experimental validation

To guarantee the precision and dependability of the formulated model, experiments were conducted to compare predicted and actual responses, as shown in Fig. 11. Once validated, the model was employed to optimize conditions for maximum antioxidant and anticancer activities. As depicted in Fig. 10a, an increase in ML₁ concentration up to approximately 40 µg/l resulted in a decrease in absorbance. This suggests that higher concentrations of ML₁ may induce cytotoxic effects on the cells, leading to a decline in cell viability, reflected by lower absorbance measurements and further increases in ML₁ concentration did not



Fig. 10 Preparation of DPPH solution and DPPH with Ni-NPs solution

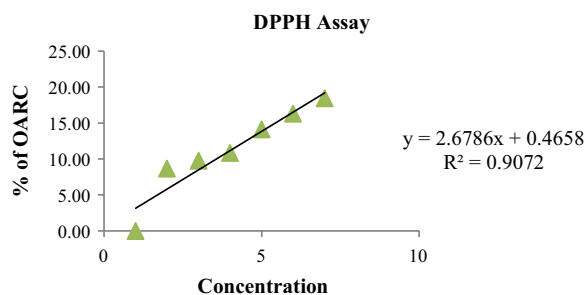
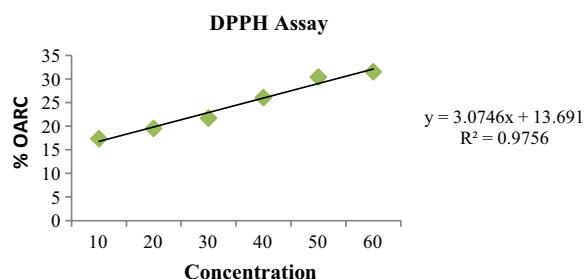
Table 3 Analysis of variance with the second order polynomial fit equation

Source	Sum of squares	DF	Mean square	F value	p Value
Model	0.11	9.0	0.013	15.05	0.0001
Residual	0.008355	10.0	0.0008355		
Lack of fit	0.006355	5.0	0.001271	3.18	0.1151
Pure error	0.002	5.0	0.0004		
Total	0.12	19.0			

significantly affect absorbance. Conversely, the increase in ML₂ concentration did not exhibit a notable change in absorbance, possibly indicating that ML₂ concentration had already reached its saturation point (Table 3).

Antioxidant activity

To quantify the antioxidant potential of the Ni NPs, 2 ml volume of a methanol-dissolved 100 μ M DPPH solution was combined with 2 ml of diverse concentrations of Ni NPs solution. The resultant mixture was left undisturbed at room temperature for 30 min. Following this, the absorption at 520 nm for the samples was measured utilizing a spectrophotometer (Figs. 10, 11, 12). The antioxidant activity was subsequently calculated using the provided formula. The calculation of IC₅₀ value of ML₁ and ML₂ (NPs) in DPPH assay is given in Table 4 and 5.

**Fig. 11** Assessment of the scavenging activity of ML₁ (NPs) against DPPH radicals**Fig. 12** Assessment of the scavenging activity of ML₂ (NPs) against DPPH radicals**Table 4** IC₅₀ value of ML₁ (NPs) in DPPH assay

S. no	Concentration of the sample(μg/ml)	Absorbance	% of OARC	IC50
1	Blank	0.92	0.00	19.33
2	10	0.84	8.70	
3	20	0.83	9.78	
4	30	0.82	10.87	
5	40	0.79	14.13	
6	50	0.77	16.30	
7	60	0.75	18.48	

Table 5 IC₅₀ value of ML₂ (NPs) in DPPH assay

S. no	Concentration of the sample (μg/ml)	Absorbance	% of OARC	IC50
1	Blank	0.92	0	10.49
2	10	0.76	17.39	
3	20	0.74	19.56	
4	30	0.72	21.73	
5	40	0.68	26.08	
6	50	0.64	30.43	
7	60	0.63	31.52	

$$\% \text{ of DPPH free radical scavenging} = \frac{\text{Blank} - \text{Test sample}}{\text{Blank}} \times 100$$

Cytotoxic activity

Assessing the cytotoxicity of the synthesized compounds against HeLa (cervical) and A549 (lung) cancer cell lines involved evaluating the number of viable cells remaining after a specific incubation period with macrocyclic complexes. The in-vitro cytotoxic activity results were quantified as IC₅₀ values, representing the concentration of the compound in μ g/mL that inhibits cell proliferation by 50% compared to untreated control cells is also summarized. The macrocyclic complexes exhibited a dose-dependent impact on the viability of both types of cancer cell lines (Figs. 13, 14, 15, 16). The growth inhibition percentage of cancer cell lines demonstrated an increase with the rising concentration of the tested compounds (Table 6).

The statistical analysis of MTT cytotoxicity study data reveals noteworthy findings regarding the cytotoxic potential of test compounds ML₁ and ML₂ against A549 and HeLa cell lines. In the case of A549 cell lines, both ML₁ and ML₂ exhibit significant cytotoxicity, with IC₅₀ concentrations of 44 μ g/mL and 31.20 μ g/mL,

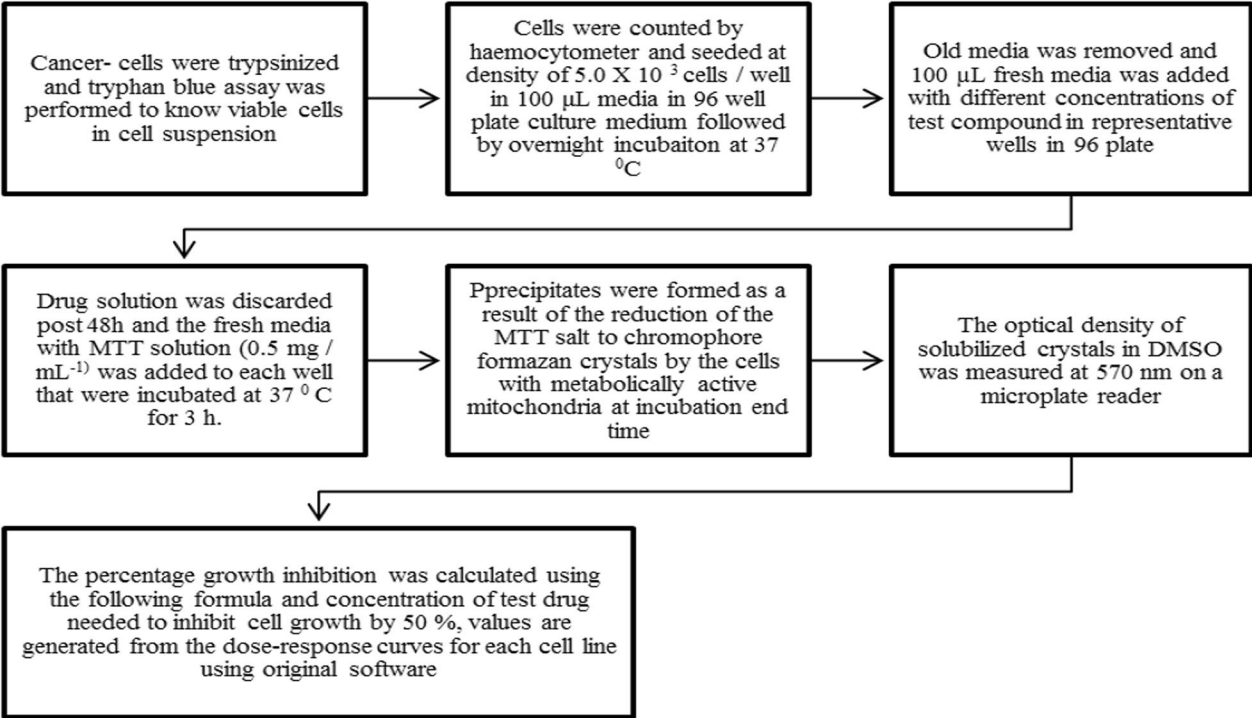


Fig. 13 Flow diagram showing the route of complete anticancer activity

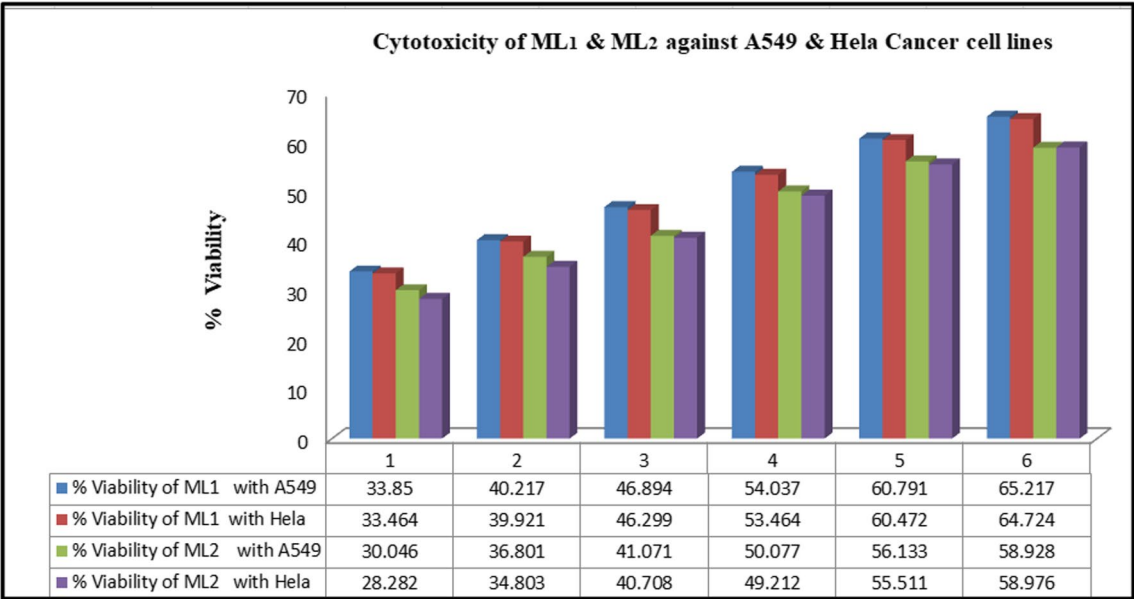


Fig. 14 Bar diagram showing the % Viability and Cytotoxicity of ML₁ and ML₂ against A549 and HeLa Cancer cell lines

respectively. Markedly, ML₂ stands out for its substantial cytotoxicity against HeLa cells, suggesting its potential as a potent anti-lung cancer agent, given its low IC₅₀. Similarly, against HeLa cell lines, ML₁ and ML₂ demonstrate substantial cytotoxic potential, displaying IC₅₀ concentrations of 43.37 μg/mL and 21.42 μg/mL, respectively.

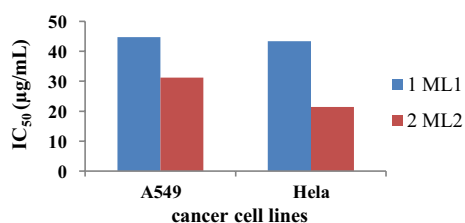


Fig. 15 IC₅₀ values of Ni-NP against A549 and HeLa cell lines

Discussions

All the characterization techniques we have utilized have effectively provided valuable insights into the newly synthesized nanoparticles. The infrared (IR) spectral data offered valuable information about presence of function groups, the structural modifications and bonding configurations within the synthesized macrocyclic complexes. In UV–visible studies, the size and dispersion of nanoparticles (NPs) significantly influence the characteristics, breadth, and position of the surface plasmon resonance (SPR) peak observed. The UV–Visible spectrophotometer data within the 250–300 nm range for the synthesized Ni-NPs reveals their plasmonic nature, signifying electron oscillation. This emphasizes the pivotal role of NP size and distribution in shaping the optical properties and behaviour

of synthesized Ni-NPs. X-ray diffraction (XRD) analysis findings offer valuable insights into the structure and size distribution of the synthesized Ni-NPs, thereby informing their potential applications across various domains. Utilizing the Debye–Scherrer formula, particle sizes for both ML₁ and ML₂ compounds were determined to fall within the nano-range. Scanning electron microscopy (SEM) enables a meticulous examination of nanoparticle boundaries, enhancing precision in assessing nanoparticle size and distribution. The images obtained by the Scanning Electron Microscope for Ni-NPs of metal complexes (ML₁ and ML₂) approve that the nanoparticles are almost spherical in form with the size of maximum 170 nm. Response surface methodology (RSM) studies reveal a notable trend wherein as temperature rises to approximately 30 °C, absorbance initially decreases before rising again. This temperature sensitivity underscores its crucial role in optimizing results. The most favourable outcomes were achieved when experimentally validated data were fitted into the equation, yielding a minimum absorbance of 0.73 at ML₁ concentration of 35 µg/l, ML₂ concentration of 15 µg/l, and a temperature of 30 °C, closely aligning with the predicted response.

The antioxidant radical-scavenging potential of Ni NPs was evaluated using a methanolic solution

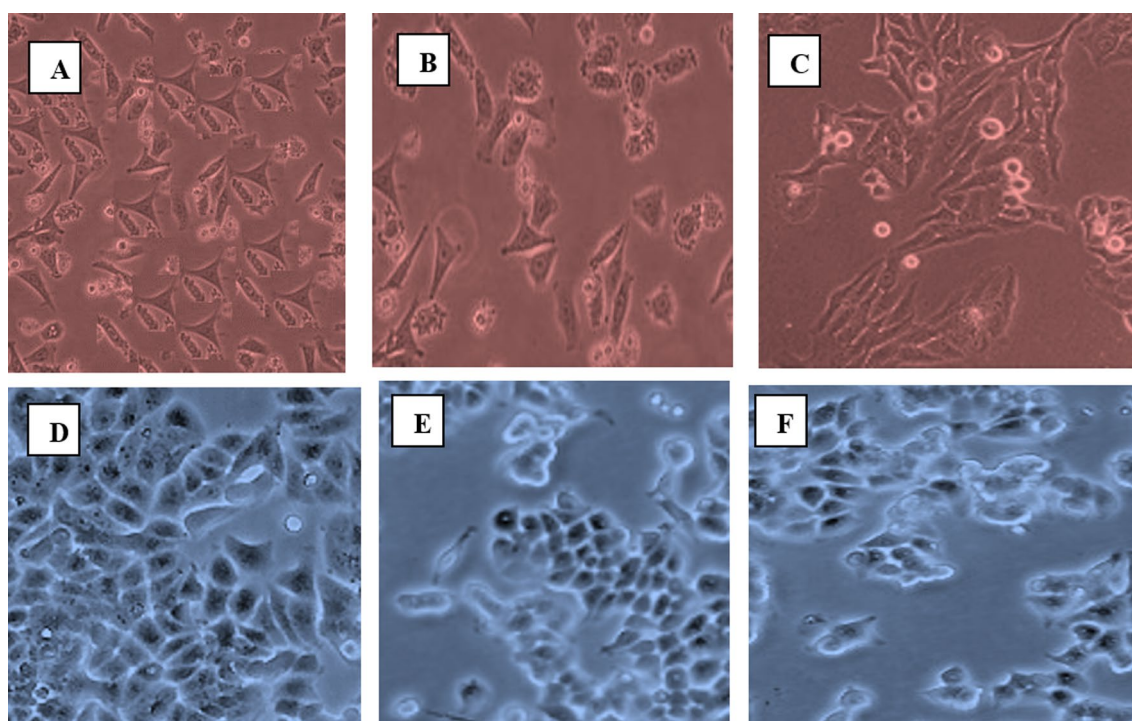


Fig. 16 **A** HeLa cell line-control. **B, C** Variations in the morphology of HeLa cell line after exposure to NPs of ML₁ and ML₂. **D** A549 cell line-control. **E, F** Variations in the morphology of A549 cell line following exposure to NPs of ML₁ and ML₂

Table 6 IC₅₀ Comparison of ML₁, ML₂ Compounds against A549 and HeLa cell Lines after 24-Hour Incubation: MTT Study

S. no	Compound	IC ₅₀ (ug/L)	
		A549	HeLa
1	ML ₁	44.76	43.37
2	ML ₂	31.2	21.42

containing 2,2-diphenyl-1-picrylhydrazyl (DPPH), a well-known radical and scavenger for other radicals. Initially, the methanolic DPPH solution exhibits a deep violet colour, which transitions to colourless or fades to a pale yellow upon neutralization with antioxidants, indicating a reduction reaction and confirming the radical nature of the test sample. The calculations of IC₅₀ values for both nanoparticles in the DPPH assay indicate that ML₂ exhibits greater antioxidant potential compared to ML₁. The anticancer activity results indicated that all the complexes exhibited moderate to good anticancer activity against both HeLa and A549 cancer cell lines. In summary, both ML₁ and ML₂ demonstrate effective cytotoxicity against human lung and cervix cancer cells, positioning them as promising candidates for further exploration in cancer therapeutics. ML₂, in particular, emerges as a potent anti-cervix cancer agent, characterized by its low IC₅₀ value.

Conclusions

Researchers, prompted by the toxicity and side effects associated with cisplatin, are exploring alternative approaches for developing transition metal-based anti-cancer agents. The cytotoxic potential of nickel nanoparticles (Ni-NPs) has been thoroughly investigated using cervical and lung cancer cell lines. In this study, the utilization of Horse Gram seed extract (*Macrotyloma uniflorum*) has revealed its richness in phenolic and flavonoid compounds, well-documented for their potent antioxidant activity in existing literature. The experiments were structured to investigate the impacts of diverse factors, and the utilization of Response Surface Methodology proved effective for optimization. Following computation, the regression coefficients were incorporated into a second-order polynomial equation for fitting. The experimental and predicted values agree closely which validates the model. Moreover, the application of *Macrotyloma uniflorum*-mediated Ni-NPs has demonstrated an enhanced anticancer effect against HeLa and A549 cell lines responded in a manner dependent on the concentration. The mechanism underlying the deactivation of cancer cell lines aligns with existing literature, attributing it to intra-cellular

nucleus damage. An additional noteworthy observation indicates that Nickel metal NPs with 2,4-dicarboxylic acid (ML₂) as a ligand exhibit a more significant impact on antioxidant and anticancer activities compared to their counterparts with 3,4-dicarboxylic acid (ML₁) as the ligand moiety. This difference in impact may be attributed to the method employed to convert metal complexes into metal-NPs. The current research underscores the possibility of refining the bio-toxicity and biochemical properties of Ni-NPs to develop a new generation of versatile anticancer agents with clinically proven efficiency. Remarkably, the predicted data aligns closely with experimental results, affirming the reliability and credibility of RSM (Response Surface Methodology) model for studying anticancer and antioxidant properties in this context.

Abbreviations

- ML₁ Nickel metal NPs with 2, 4-dicarboxylic acid (ML₂) as a ligand
- ML₂ Nickel metal NPs with 3, 4-dicarboxylic acid (ML₂) as a ligand
- DPPH 2, 2-Diphenyl-1-picrylhydrazyl
- MTT 3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
- SEM Scanning electron microscope
- RSM Response surface methodology

Supplementary Information

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Additional file 1. Four tables of statistical data are being provided for Anti-cancer studies.

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Author contributions

SA conducted a comprehensive literature review focusing on organically Nano-Fabricated Ni metal complexes, exploring their antioxidant and anti-cancer activities, and conducted characterization and activity assessments. SB coordinated the development of the initial draft of the article. MVM enhanced the writing style and participated in the proofreading process. The final manuscript was reviewed and approved by all authors.

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Declarations

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Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

Author details

¹Department of Chemistry, Anurag University, Hyderabad, Telangana, India.

²Department of Chemical Engineering, Chaitanya Bharathi Institute of Technology, Hyderabad, Telangana, India.

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