Sustainable Synthesis of MoO_3 Nanostructures Employing Nyctanthes Extract and Assessment of Their Biological Properties

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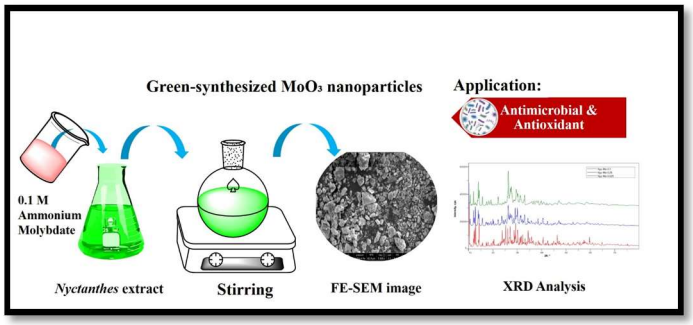


ABSTRACT:

The green synthesis approach adopted in the synthesis of molybdenum trioxide nanoparticles using the extract of *Nyctanthes arbor-tristis* flowers was based on the observation that precursor concentration influences the physical and biological behavior of the resulting nanoparticles. The UV-visible analysis established that there is a unique absorption peak of about 398 nm, which underwent bathochromic shift at higher precursor concentration, a sign of increased optical absorbance. XRD analysis indicated that the phase obtained is orthorhombic with crystallite sizes of 30 to 50 nm. The FESEM analysis confirmed the formation of semi-spherical nanoparticles with sizes ranging from 22 to 48 nm and with even distribution and uniform shape. FTIR analysis indicated the formation of Mo-O and Mo=O bonds and with sharper peaks at higher precursor concentrations indicating improvement in crystallization. Biological testing indicated that the best antibacterial effect was shown by the nanoparticles synthesized with precursor concentrations of 0.1 M, with inhibition zone widths of 11 mm and 22 mm against *Bacillus* and *Candida albicans* strains, respectively. The highest percentage of radical scavenging of approximately 30% was recorded with precursor concentration of 0.1 M.

Keywords: *Nyctanthes arbor-tristis*, Molybdenum trioxide (MoO_3), bioactive compounds, physicochemical properties, antimicrobial activity, biological activity.

How to cite this article: Mohite S, Shukla S, Gadekar-Shinde S, Gadale S. Sustainable synthesis of MoO_3 nanostructures employing Nyctanthes extract and assessment of their biological properties. Int J Drug Deliv Technol. 2026;16(75s): 1173-1180. DOI: 10.25258/ijddt.16.75s122

1. INTRODUCTION:

In recent years, the field of nanotechnology has become one of the driving forces behind innovations in medicine, agriculture, and environment because of the ability to fabricate materials on the nanoscale level with unique properties. Among these materials, nanomaterials based on metal oxides such as molybdenum trioxide (MoO_3) nanoparticles have gained significant interest due to their catalytic capabilities, high-level electronics, and antimicrobial properties [i]. Traditional methods of synthesizing such nanoparticles involve toxic chemicals and energy-intensive conditions and produce waste products harmful to the environment and human health. Green synthesis that uses biological sources as natural reducing and stabilizing agents has become an environmentally-friendly alternative to conventional processes. Phytochemicals contained in these natural resources can facilitate nanoparticle formation and increase their biocompatibility, making them applicable in biomedical and ecological areas [ii, iii].

In this respect, *Nyctanthes arbor-tristis* (Night-flowering jasmine or Parijat) is worth considering because of its rich history and valuable properties. The plant itself has antioxidant, antimicrobial, anti-inflammatory, and anticancer properties due to a wide spectrum of bioactive substances contained in its flowers, leaves, and bark. The plant extract can be used in the green synthesis of nanoparticles of silver, gold, copper oxide, zinc ferrite, and selenium with good

antimicrobial, antioxidant, and photocatalytic activity [iii, iv]. In addition to these qualities, nanoparticles produced from the extracts of the plant exhibit increased conductivity, catalytic activity, and antimicrobial action along with low toxicity. Due to these characteristics, they may be potentially used for biomedical applications such as targeted drug delivery and antibacterial coatings; however, there are only a few reports concerning the synthesis of MoO_3 nanoparticles using Nyctanthes extract [v].

In the current research, MoO_3 nanoparticles synthesized using Nyctanthes arbor-tristis flower extract were characterized with UV-Vis spectroscopy, X-ray diffraction, Fourier-transform infrared spectroscopy, and field-emission scanning electron microscopy. The samples' biocompatibility was tested with DPPH assay. Antimicrobial and catalytic activities were also investigated to evaluate the potential of MoO_3 nanoparticles produced with the help of a green synthesis method in various biomedical and ecological areas [v, vi].

2. MATERIAL AND METHODS

2.1 MATERIALS:

Fresh Nyctanthes arbor-tristis leaves were sourced from the botanical garden of Yashwantrao Mohite College of Arts, Science and Commerce, Pune. Ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$) of analytical grade purchased from Sigma-Aldrich was used as the starting material for the formation of molybdenum trioxide (MoO_3) nanoparticles. All chemicals used in this study were of superior quality and did not require any further purification. To maintain consistency and accuracy throughout the experiment, all the steps were performed using double distilled water as a solvent. The ATCC reference strains were used in the antimicrobial and antifungal assays under controlled laboratory conditions to ascertain reproducibility in Bio Cyte Research and Development Pvt. Ltd., Sangli, Pune [vii].

2.2 PREPARATION OF *NYCTANTHES LEAF* EXTRACT:

To prepare extract, freshly harvested leaves were initially washed in running tap water then distilled water to wash away remaining impurities. The dried leaves were dried by air at room temperature over 5-7 days and then milled to fine powder using a mechanical grinder. This powdered material, 10 grams, was combined with 100 mL of double-distilled water and allowed to heat to 60ºC during 30 minutes to release bioactive compounds. The mixture was allowed to cool to room temperature, and the mixture was filtered using Whatman No.1 filter paper, then the extract was stored at 4°C until it was used [viii, ix].

2.3 SYNTHESIS AND OPTIMIZATION OF SYNTHESIZED MOO_3 NANOPARTICLES PARAMETERS:

Nanoparticles of MoO_3 were synthesized by a green synthesis process based on the use of the flower extract of *Nyctanthes arbor-tristis* as the reducing and stabilizing agent. In a standard process, 50 mL of the extract of the plant was put into 100 mL of 0.1 M ammonium molybdate solution with constant stirring at 60°C. The change in color of the solution gradually to deep orange was an indication of the formation of the nanoparticles. The mixture reaction was kept 24 h where the product was centrifuged at 8000-10,000 rpm during 15-20 min. Systematic variation of parameters like extract concentration (5-15%), precursor concentration (0.025 M-0.1 M), pH (5-9), reaction temperature (50-70°C) and reaction time (12-48 h) were varied in order to optimize the yield of nanoparticles, their stability, and morphology [x, xi].

2.4 CHARACTERIZATION STUDY:

Comprehensive physicochemical characterization of the synthesized nanoparticles was done. The spectrophotometer used was Shimadzu UV-1800 in the wavelength range 200-800 nm with quartz cuvettes (length of path 1cm) having distilled water as reference blank. X-ray diffraction (XRD) of a Bruker D8 Advance diffractometer using 40 kV and 30 mA of Cu-Kα radiation (1.5406 A°) was used to analyze crystallinity and phase composition. The patterns of diffusion were observed at various 2θ ranges between 10ºC and 80ºC at a scan rate of 2ºC/min and the functional groups related to the stabilization of nanoparticle were identified through the Fourier-transform infrared (FTIR) spectroscopy at the mid-infrared region (4000-400 cm^{-1}) by mixing nanoparticle powder with Field Emission Scanning Electron Microscopy (FESEM) was used to examine morphological features on a Zeiss Sigma 500. Before imaging the samples were mounted on carbon-coated stubs and sputter-coated with a thin layer of gold to facilitate conductivity. The analyses were all performed under standardized operating conditions to make them reproducible and accurate [xii, xiii, xiv].

2.5 ANTIBACTERIAL ACTIVITY:

Agar well diffusion test was used to evaluate the antimicrobial activity of the green-synthesized MoO_3 nanoparticles with bacterial and fungal strains. Gram-positive (*Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633) and Gram-negative (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 15442) cultures were inoculated with 10^6 CFU/mL on nutrient agar, and *Candida albicans* ATCC 14053 were inoculated at 10^4 CFU/mL. Nanoparticles (1mg/mL) as 50 µl of suspension were placed in wells of 6 mm diameter. Positive controls included standard antibiotics and antifungal agents and sterile water was

used as the negative control. After incubation (bacteria: 37ºC 24 h; fungi: 28ºC 48 h), the points of inhibition were determined, which proved the high antimicrobial potential of the synthesized nanoparticles [xv, xvi].

2.6 ANTIOXIDANT ACTIVITY:

The DPPH radical scavenging assay was used to measure the antioxidant ability of MoO_3 nanoparticles. At the same concentration levels, 20-100 µg/mL nanoparticle suspensions were added to 0.1 mM DPPH solution in volumes of 1:1 and allowed to mix in the dark during 30 minutes at room temperature. The absorbance of the solution was measured at 517nm with a UV-Vis spectrophotometer using ascorbic acid as the reference standard. The nanoparticles had a dose-dependent radical scavenging activity with a maximum of ~30% inhibition and hence moderate antioxidant activity [xvii, xviii].

3. RESULTS AND DISCUSSION:

3.1 UV-VIS SPECTROSCOPY:

The presence of the typical absorption peak at 398nm MoO_3 nanoparticles was confirmed by UV-Visible spectroscopy that matches the bandgap of the MoO_3 nanoparticles. The effect of change in the synthesis parameter on this peak was apparent in the strength and location of the peak, which was associated with the change in particle size and distribution. The concentration of extract increased the redshift in the absorption band, which indicates the presence of a larger particle size, and higher concentrations of the precursors brought about more extensive peaks, which indicates heterogeneous size distribution. Optimization of optical characteristics was obtained under the conditions 10% extract concentration, 0.1 M precursor, pH 7, reaction temperature 60°C, and reaction time 24 h to produce nanoparticles with equal particle size and low level of aggregation. These results indicate the importance of synthesis conditions to the optical characteristics of MoO_3 nanoparticles and are in line with previous studies that have shown how minor variations in the precursors and extract concentrations can easily change the morphology and optical activity of the particle. In general, the UV-Vis analysis confirms the effective production of MoO_3 nanoparticles in green conditions and reveals the ability to adjust the optical characteristics of nanoparticles with the help of the reaction parameters [xix, xx].

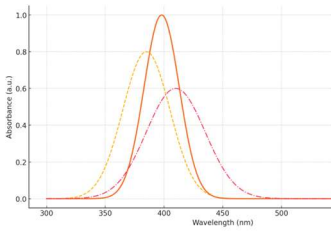


Fig. 1: UV-Vis Absorption Spectra of MoO_3 Nanoparticles under varying extract concentrations

3.2 X-RAY DIFFRACTION ANALYSIS:

X-ray diffraction (XRD) was conducted to identify the crystalline structure of MoO_3 nanoparticles produced through the green route with the help of *Nyctanthes arbor-tristis* extract. The diffraction pattern exhibited sharp peaks with 2 theta values of around 12.8°, 23.3°, 25.7°, 27.3°, and that of (020), (110), (040), and (021) planes respectively, which confirms the orthorhombic state of MoO_3 in line with JCPDS card no. 05- The clear reflections were a sign of the high level of crystallinity and the mean size of crystallites was estimated to be 30-50 nm based on the Debye-Scherrer equation. The lack of secondary peaks was evidence of the purity of the product in terms of phase. The phytochemicals in the Nyctanthes extract served to play a reducing role as well as a capping role which helped in the stabilization of the nanoparticles and even controlled growth of the crystals. These results are not new since previous studies of plant-mediated synthesis have emphasized the importance of bioactive compounds in guiding the refinement of the structure. All in all, XRD data confirmed the effective preparation of high crystallinity, pure, and nanosized orthorhombic MoO_3 nanoparticles using an environmentally friendly synthesis process, and their future use in catalysis, sensors, optoelectronic gadgets, and energy storage devices [xxi, xxii].

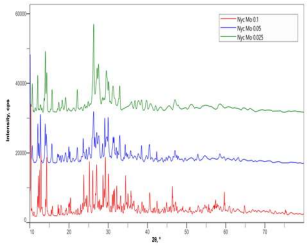
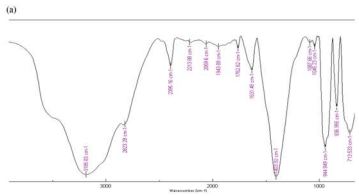


Fig. 2: X-Ray Diffraction analysis of MoO_3 Nanoparticles under varying extract concentrations

3.3 FESEM OF MoO_3 NANOPARTICLES:

Morphology and surface properties of MoO_3 nanoparticles obtained through the green synthesis method employing Nyctanthes arbor-tristis flower extract were examined using Field Emission Scanning Electron Microscope (FESEM). FESEM images revealed the presence of nano-size particles having an irregular shape, such as semi-spherical and plate-like



structures, usually forming clusters due to the effect of phytochemicals present in flowers acting as both natural reducing and stabilizing agents. The size of the particles ranged between 22 nm to 48 nm, which is in line with the data provided about the biological production of nanomaterials. The small size and homogeneity of the particles enable them to have high surface area-to-volume ratios, thereby making them suitable for catalysis, antibacterial activity, and antioxidants applications. Rough surface texture and closely packed arrangement of the particles clearly suggest that phytochemical capping has taken place effectively. Images with low magnification also revealed sheet-like structures which are typical of MoO_3 nanostructures synthesized under gentle, environmentally friendly conditions. High crystallinity and phase purity were supported by XRD results by the absence of porosity and compactness of the particles. In general, FESEM analysis revealed the successful green synthesis of uniform MoO_3 nanoparticles with nanoscale dimensions and bio-capped surfaces, highlighting their application in catalysis, biosensing and environmental remediation [xxiv, xxv].

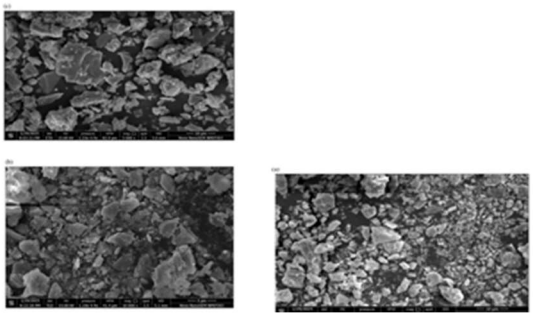


Fig. 3: FESEM of Nyctanthes-mediated MoO_3 Nanoparticles under varying extract concentrations (a) 0.025M, (b) 0.05M, and (c) 0.1M

3.4 FTIR ANALYSIS OF MoO_3 NANOPARTICLES: To examine the structural characteristics of MoO_3 nanoparticles synthesized using Nyctanthes arbor-tristis flower extract under different concentrations of the precursor (0.025 M, 0.05 M and 0.1 M), Fourier Transform Infrared (FTIR) spectroscopy was used. The spectra showed typical absorption at 565 cm^{-1} Mo-O stretching vibrations, and prominent peaks at 810 and 849 cm^{-1} which were ascribed to Mo=O stretching vibrations. The presence of other signals at approximately 978 cm^{-1} (C-H bending) and 2349 cm^{-1} (O=C=O stretching) were indications of the inclusion of plant-derived organic compounds which helped stabilize the nanoparticles. An apparent concentration-dependent variation was present: smaller concentration of precursors yielded medium peaks, and higher concentrations led to sharper and stronger bands, which indicated higher crystallinity and better yield of nanoparticles. The spectral characteristics were most pronounced at 0.1 M, which was used to suggest the presence of highly crystalline and homogenous nanoparticles of MoO_3. These results confirm the dual mechanism of phytochemicals as reducing and capping agents as well as other reports on the synthesis of the phytochemicals through plants, where precursor concentration is a significant factor in structural refinement. In general, the successful eco-friendly production of MoO_3 nanoparticles was determined by FTIR analysis, but the concentration of the precursor seems to be the crucial element that defines their quality and stability [xxvi, xxvii].

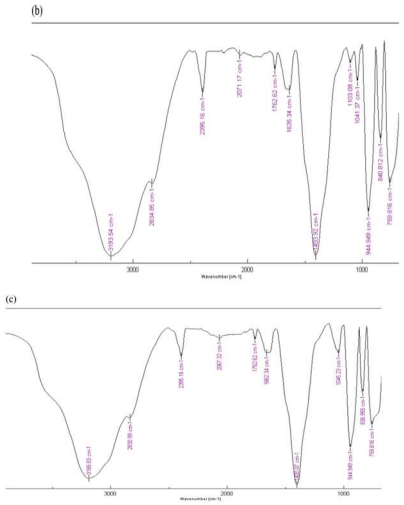


Fig. 4: FTIR Analysis of MoO_3 Nanoparticles under varying extract concentrations A) 0.025 M, B) 0.05 M, and C) 0.1 M

3.4 ANTIMICROBIAL ACTIVITY:

The antimicrobial activity of MoO_3 nanoparticles prepared using Nyctanthes arbor-tristis flower extract with precursor concentration of 0.025 M, 0.05 M and 0.1 M was evaluated systematically against bacterial and fungal pathogens. FTIR and FESEM structural analysis confirmed an increase in crystallinity and particle definition with precursor concentration. Antibacterial testing on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus spp.*, and *Pseudomonas spp.* showed that the nanoparticles were made at low concentrations (0.025 M and 0.05 M) had little inhibition zones (1-4 mm), whereas the nanoparticles synthesized at A concentration-dependent increase in efficacy was also observed in antifungal studies in the presence of *Candida albicans* as the test organism with 0.1 M nanoparticles producing an inhibition zone of 22 mm at a dosage of 10 mg and 16 mm at a dosage of 5 mg in comparison to Fluconazole (30 mm) as the reference standard. These results demonstrate that the increased precursor concentration produces nanoparticles with enhanced crystallinity, increased surface reactivity, and efficient phytochemical capping, which are ultimately beneficial in antimicrobial activity. The findings highlight the promise of the present green synthesis method as a green pathway to the synthesis of functional MoO_3 nanomaterials of great biomedical interest [xxviii, xxix].

Table 1: Antimicrobial activity of MoO_3 nanoparticles through Zone of Inhibition

Sample	**Concentrations**	**ZOI of *E. coli* (mm)**	**ZOI of *S. aureus* (mm)**	**ZOI of *Bacillus* (mm)**	**ZOI of *Pseudomonas* (mm)**	**ZOI of *C. albicans* (mm)**
MoO_3 0.025 M	5 mg	01	01	03	02	02
	10 mg	03	03	10	04	04
MoO_3 0.05 M	5 mg	01	01	01	01	03
	10 mg	02	02	02	02	05
MoO_3 0.1 M	5 mg	01	05	04	05	16
	10 mg	09	08	09	11	22

3.5 ANTIOXIDANT ACTIVITY:

The scavenging capacity of MoO_3 nanoparticles fabricated using Nyctanthes arbor-tristis flower extract was assessed by measuring the scavenging capacity against the DPPH radical at precursor concentrations of 0.025 M, 0.05 M, and 0.1 M. It is evident that there is a concentration-dependent effect whereby the nanoparticles produced at 0.025 M concentration had moderate scavenging capacity with inhibition percentages ranging from 21.50% to 29.97%. The nanoparticles fabricated at 0.05 M concentration had low scavenging efficiency at ranges from 11.03% to 27.24%. Those formed at 0.1 M concentration demonstrated improved antioxidant effectiveness with inhibitions from 15.76% to 22.82%. These findings clearly show that the precursor concentration greatly affects the scavenging ability of the nanoparticles owing to the change in the physicochemical structure on the nanoparticle's surface through capping by phytochemicals. Comprehensively, the results affirm that MoO_3 nanoparticles produced by this environmentally friendly synthesis method have a quantifiable antioxidant property, and the concentration of the precursors is conducive to the improvement of antioxidant stability and bioactivity, which can be used in biomedical and environmental applications [xxx, xxxi].

Table 2: Effects of Antioxidant activity by DPPH of MoO_3 nanoparticles

Sample Code	Concentration	Absorbance			Mean	% Inhibition
MoO_3 0.025 M	250	0.876	0.875	0.976	0.875	11.30
	500	0.789	0.787	0.775	0.783	20.62
	1000	0.723	0.725	0.735	0.727	26.29
MoO_3 0.05 M	250	0.775	0.773	0.784	0.777	21.26
	500	0.712	0.768	0.764	0.748	24.24
	1000	0.668	0.0675	0.677	0.673	31.80
MoO_3 0.1 M	250	0.798	0.799	0.798	0.798	19.14
	500	0.645	0.644	0.0623	0.637	35.44
	1000	0.0625	0.635	0.633	0.631	36.09

4. CONCLUSION:

Finally, MoO_3 nanoparticles produced by a greener process with the aid of Nyctanthes arbor-tristis flower extract showed precursor-dependent differences in physicochemical and biological characteristics. Optimal optical performance was confirmed by the UV-Vis spectroscopy that showed a sharp absorbance peak at around 398 nm with 10% extract mixed with 0.1 M precursor, which was associated with well-dispersed particle sizes. The XRD and FESEM results showed that increasing the concentration of the precursor increased crystallinity and resulted in a homogeneously distributed nanoscale particle (2248 nm) with phytochemical capping. FTIR spectra also confirmed the existence of functional groups that were used to stabilize the nanoparticles with sharper peaks indicating a better structural order [xxxii]. Biological tests indicated a great deal of antimicrobial and antioxidant activity, and the nanoparticles produced at 0.1 M exhibited the highest antibacterial (8 -11 mm inhibition zones against *Bacillus* and *Pseudomonas*) and antifungal (22 mm inhibition zone against *Candida albicans*) activity, as well as radical scavenging activity (~30 This data provide a definitive relationship between a precursor concentration, nanostructure refinement, and bioactivity. Not only does the green synthesis methodology completely avoid the use of dangerous chemicals, but also structural robust and biologically active MoO_3 nanoparticles are produced, and these nanoparticles can be used in catalysis, sensing, antimicrobial coats, and environmental remediation in sustainable nanotechnology systems [xxxiii, xxxiv].

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