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Research Article

Enhancing Herbal Efficacy: Synthesis and Evaluation of Nanosuspension from Withania somnifera Root Extract

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Abstract

Background: Ayurveda emphasizes the balance of mind, body, and spirit, drawing on ancient texts that highlight the medicinal properties of herbs like *Withania somnifera*, known for its wide range of therapeutic applications and pharmacological benefits. Commonly referred to as Indian ginseng or Ashwagandha, *Withania somnifera,* demonstrates a variety of therapeutic effect , including anti-inflammatory, antitumor, sedative, hypnotic, deobstruent effects, narcotic, antioxidant, tonic, antistress, diuretic, aphrodisiac, immunomodulatory, and rejuvenating properties. Nanotechnology, particularly through the development of nanosuspensions, offers a significant advancement in medicine, addressing challenges like poor solubility and low bioavailability of lipophilic drugs. Nanosuspensions can enhance drug safety and efficacy by improving solubility and altering pharmacokinetics.

Methods: This study focuses on formulating and characterizing a nanosuspension using an ethanolic extract of *Withania somnifera* roots. The nanosuspension (NSWS) underwent comprehensive characterization, utilizing techniques such as FT-IR spectroscopy, particle size analysis, zeta potential measurement, polydispersity index (PDI) assessment, Field emission scanning electron microscopy (FESEM), X-ray diffraction analysis, pH measurement, and stability testing. This study seeks to explore the potential of nanosuspensions, aiming to integrate nanotechnology with herbal medicine to improve the safety and efficacy of herbal formulations.

Results: The nanosuspension (NSWS) shown particle size about 133.09 nm, and its uniform particle distribution was indicated by a Polydispersity Index (PDI) about 0.27. Stability and uniformity were further supported by the zeta potential, particle size, and PDI values.

Conclusion: Nanosuspension formulations represents a versatile strategy to improve the therapeutic efficacy of hydrophobic drugs across various routes of administration.

Keywords: *Withania somnifera*, Nanosuspension, Characterization, FT-IR, PDI, FESEM

INTRODUCTION

Integrating Ayurveda with modern medicine aims to enhance personalized and effective healthcare, emphasizing the balance of mind, body, and spirit that Ayurveda promotes[1]. Ayurveda with modern medicine seeks to leverage the strengths of both systems, highlighting natural remedies with a rich historical background. Studies have explored the effectiveness of Ayurvedic treatments for various diseases, indicating that incorporating these principles can improve patient care[2]. Ayurveda has limitations, and its integration with modern medicine encounters challenges like insufficient scientific research, standardization issues, regulatory hurdles, cultural biases, and a shortage of trained practitioners in the West[3].

In Ayurvedic literatures like the Astanga Hridaya, Charaka Samhita, and Susruta Samhita, ashwagandha is renowned for its diverse therapeutic applications and is included in many formulations. These ancient writings highlight its medicinal properties and importance in various remedies. The phytochemicals in ashwagandha offer multiple pharmacological benefits, including anti-cancer, antioxidant, immunomodulatory, anti-diabetic, cardioprotective, anti-aging, anti-inflammatory and adaptogenic effects[4]. For more than 3,000 years, *Withania somnifera* (L.) Dunal also recognised as Ashwagandha in Sanskrit, Winter cherry, or Indian

ginseng in English has been an extensively utilised plant in traditional medicine[5, 6]. This small, woody shrub from the Solanaceae family, known as *Withania somnifera*, has been valued in Ayurvedic and indigenous medicine for its active compounds, including alkaloids, withanolides, saponins, flavonoids, and tannins. Research has identified over 45 withanolides, 5 unidentified alkaloids, various free amino acids, chlorogenic acid, glycosides, condensed tannins, glucose, and flavonoids in the plant[7]. Herbal formulations in India have long offered therapeutic benefits for various health issues. Medicinal plants and their phytoconstituents are generally negligible toxic and adverse than modern drugs, but their low solubility can hinder formulation. Nanotechnology provides a promising solution to enhance the solubility of herbal drugs. For example, a puerarin nanosuspension significantly increased cell inhibition rates compared to same concentrations of puerarin-free solution[8]. When compared to their crude extracts, the antiradical capability of plants such as *S. marianum, C. sativum,* and *E. cardamomum* was greatly enhanced by their nanosuspensions[9]. *Psidium guajava* Linn's flavonoid-rich fraction's ability to treat type 2 diabetes was improved by its nanosuspension[10]. In Sprague-Dawley rats, *Phyllanthus amarus* extract nanosuspension improves oral bioavailability and helps avoid hepatotoxicity brought on by paracetamol[11].

Nanotechnology through the development of nanosuspensions, represents a significant advancement in medicine. Nanosuspensions effectively address challenges poor solubility and limited bioavailability of lipophilic drugs[12]. Nanotechnology enables the creation of disintegrable medicinal formulations, enhancing drug safety and efficacy by improving solubility, addressing bioavailability, and alters pharmacokinetics[13]. Nanosuspensions enhance drug delivery by addressing solubility and bioavailability to improve overall safety and effectiveness[14]. Compared to conventional dose forms, they offer a number of benefits, including better stability, higher pharmacodynamic activity, decreased systemic toxicity, and tailored effects[15]. Integrating nanotechnology with herbal drugs is increasingly encouraged, as nanostructured systems can enhance the efficacy of herbal extracts, reduce dosage needs, minimize side effects, and improve bioactivity[16]. Additionally, polyherbal formulations are valued for their safety and effectiveness, which can enhance patient compliance[17]. The application of ashwagandha in the environmentally friendly synthesis of copper oxide (CuO), zinc oxide (ZnO), titanium dioxide (TiO_2), selenium (Se), and silver (Ag) has been explored in earlier research[18]. However, the application of nanosuspensions derived from *Withania somnifera* root extract remains unexplored. This study aims to fill that gap by synthesizing nanosuspensions from W. somnifera roots and evaluating their potentials.

MATERIALS AND METHODS

Collection of Plant Material, Extraction and Phytochemical Screening

Withania somnifera (Ashwagandha) roots were sourced and subsequently authenticated. A weighed quantity of dried *Withania somnifera* root powder (1kg) was extracted with petroleum ether at 50°C - 60°C for 72 hours using a Soxhlet apparatus followed by extraction with 95% ethanol at 60°C - 70°C for an additional 72 hours in the same apparatus. The concentrated ethanol extract was stored in a desiccator. A preliminary phytochemical analysis was conducted to identify secondary bioactive metabolites. Standard methods were used to detect various compounds, including sugar, protein amino acids, alkaloids, flavonoids, saponins, glycosides tannins, glycosides, phenols, and steroids[19, 20].

Formulation of Nanosuspension (NSWS) from Plant Extract

The nanosuspension was prepared using a media milling technique followed by lyophilisation. Various formulation process are optimised to attend desirable size and saturation solubility. First, 1.5 gram of the plant extract was dispersed in a solvent mixture made up of acetone and ethanol in ratio of 3:1 and sonicated for 60 seconds to ensure thorough mixing. After that, a magnetic stirrer was used to continuously mix the solution at 1000 rpm while it was gradually injected in 25 ml of water with 1.5% (w/v) PVA (polyvinyl alcohol) at the speed of 1 ml per minute. To minimize coalescence, the emulsion was diluted with 50 ml of a 0.2% (w/v) polyvinyl alcohol (PVA) solution in water. The mixture was stirred continuously at 600 rpm for six hours at room temperature to facilitate solvent evaporation and promote nanoparticle formation. After that, the nanosuspension was chilled to -18°C and then lyophilised in a lyophilizer to produce a dry powder suitable for further analysis and application for further analysis and application[21, 22].

Lyophilization of Nanosuspension

The prepared nanosuspension was frozen and lyophilized using a MAC lyophilizer (Model MSW-137, Serial No. 2511) for 8 cycles at -35°C.

Characterization of Nanosuspension

FT-IR Spectroscopy

The nanosuspension (NSWS) was subjected to Fourier-transform infrared (FT-IR) spectroscopy using a Shimadzu DRS-8400 IR spectrophotometer, employing the KBr pellet method for sample preparation. This procedure involved pressing a tiny portion of the nanosuspension into the form of a pellet after mixing it with potassium bromide (KBr). This pellet, then placed in the spectrophotometer to record the infrared spectrum. In addition to the nanosuspension, FT-IR spectra were also obtained for the ethanolic extract of *Withania somnifera* and the stabilizer, Polyvinyl Alcohol (PVA), following established protocols. For these samples, similar preparation methods were used: the ethanolic extract and PVA were mixed with KBr to create pellets, which were then analyzed under the same spectroscopic conditions. The obtained spectra were compared in order to determine the distinctive functional groups and interactions present in the nanosuspension, to assess the extract's particular bioactive components, and to validate the stabilizer's activity[23].

Particle Size Analysis and PDI

The NANOPHOX-SympaTec instrument was used to measure the nanosuspension's (NSWS) particle size, which utilizes polarization-separated backscattering technology combined with photon cross-correlation spectroscopy (PCCS) for high-resolution nanoparticle size characterization. To calculate the average particle size and evaluate the distribution of particle sizes, which is measured by the Polydispersity Index (PDI), photon correlation spectroscopy was utilised. This method provides precise measurements that are crucial for evaluating the quality and uniformity of the nanosuspension[24].

Zeta Potential

A Beckman Coulter Delsa Nano C was employed to measure the zeta potential, assessing the stability of the nanosuspension (NSWS). To achieve the optimal concentration for measurement, the sample was diluted tenfold with distilled water prior to analysis. This dilution reduces sample viscosity, improving the accuracy of the zeta potential measurements[25].

FESEM

The surface morphology of the nanosuspension (NSWS) was characterized using a Carl Zeiss Supra 55 field emission scanning electron microscope (FESEM). First, the sample was attached using double-sided sticky tape to a SEM stub. To enhance imaging quality, After that, it was vacuum-coated with a thin coating of 1.5–3.0 nm gold, which improves conductivity and reduces charging effects during observation. Imaging was conducted at various magnifications, with the SEM operated at 10 kV[26].

XRD and pH Measurement

X-ray diffraction (XRD) with a Rigaku Smartlab diffractometer was used to assess the crystalline phase and purity of the nanosuspension (NSWS), employing radiation of Cu Kα at 1.5 kV. This technique allows for the identification of crystalline structures and the assessment of sample purity. A digital pH meter was used to measure the pH of the nanosuspension (NSWS). The pH readings were taken three times to ensure accuracy, and the average value was calculated from these measurements. This pH assessment is essential for determining the stability and suitability of the nanosuspension for various applications[27, 28].

Stability Testing

The physical stability of the optimized nanosuspension (NSWS) was assessed over a three-month period at two different temperatures: 4°C (in a refrigerator) and 25–30°C. This evaluation helps determine the formulation's stability under varying storage conditions[29].

RESULTS

Phytochemical Analysis and Formulation of Nanosuspension (NSWS)

Withania somnifera roots were collected from local market and was authenticated by Dr. N. M. Dongarwar, Department of Botany at RTMNU, Nagpur (Specimen no. 10374). The collected roots were thoroughly washed air-dried under shade. The material was then divided into coarse powder. The weighed quantity of coarse powder undergoes extraction with petroleum ether followed by 95% ethanol in Soxhlet apparatus. A brown residue was obtained after concentrating the ethanolic extract and kept in the desiccator. Preliminary phytochemical screening of *Withania somnifera* indicated the presence of several secondary metabolites, including alkaloids, saponins, tannins, glycosides, phenols, steroids, and flavonoids. The nanosuspension was prepared from the ethanolic extract of the *Withania somnifera* root, with optimization achieved by adjusting various formulation and process parameters.

Characterization of Nanosuspension (NSWS)

Fourier Transform Infrared Spectroscopy (FT-IR)

As illustrated in Fig. 1, FT-IR spectroscopy of the nanosuspension (NSWS) indicated distinct peaks at 3603.15 cm^{-1} (–OH stretching of alcohol), 3549.14 cm^{-1} (–NH stretching of amine), 3271.38 cm^{-1} (–CH stretching of alkyne), 2939.61 cm^{-1} (–NH stretching of amine salt), 2901.04 cm^{-1} (–CH stretching of alkane), 1658.84 cm^{-1} (–C=C– stretching of alkene), 1435.09 cm^{-1} (–OH bending of carboxylic acid), 1064.74 cm^{-1} (–CN stretching of amine), 709.83 cm^{-1} (–C=C–bending of alkene), and 563.23 cm^{-1} (C–Br stretching of halo compounds). The Withania somnifera extract and the nanosuspension's FT-IR spectra showed similar peak positions with little to no changes, suggesting that the functional groups from the plant extract were preserved in the nanosuspension. This implies that the extract and stabiliser are effectively compatible (Fig. 2, 3).

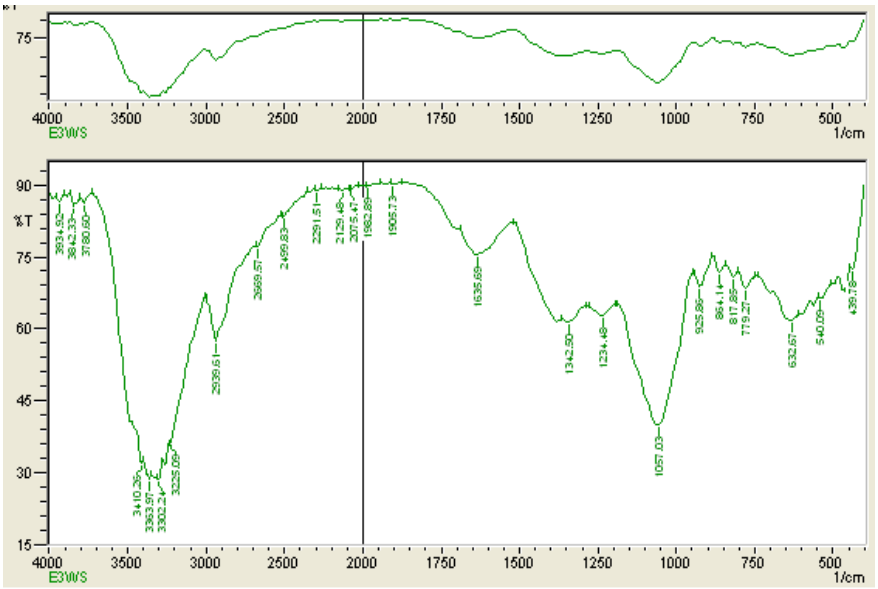

Figure 1: FTIR of ethanolic extract of *Withania somnifera*

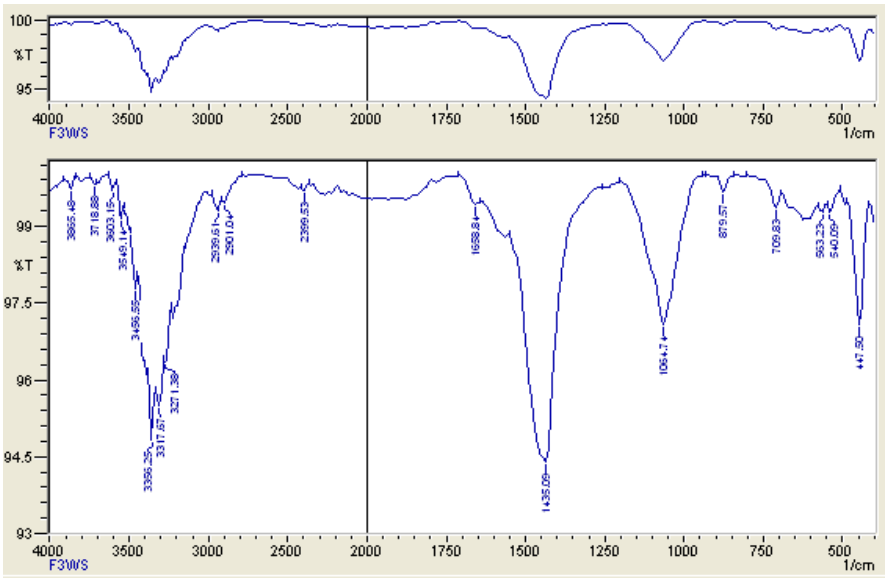

Figure 2: FTIR of Nanosuspension (NSWS)

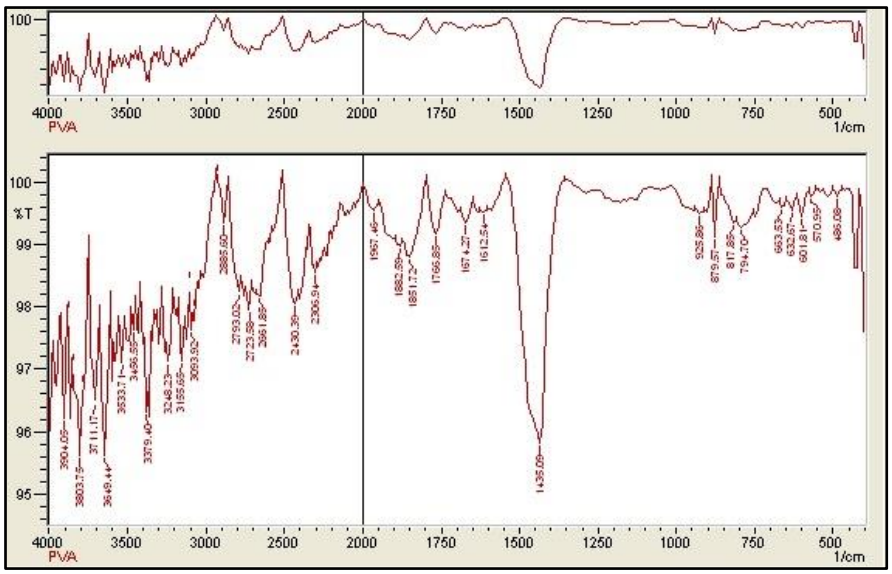

Figure 3: FTIR of Stabiliser used in formulation of nanosuspension (NSWS)

Particle Size Analysis

The nanosuspension (NSWS) had an average particle size of about 133.09 nm, according to particle size analysis performed with the NANOPHOX-SympaTec device. The size distribution of the NSWS ranged from 118.81 nm (at x10) to 154.46 nm (at x90). The mean volume diameter (VMD) was found to be 133.90 nm, and the Sauter mean diameter (SMD) was measured to be 132.10 nm. According to calculations, the true surface area (Sv) is 45.42 m^2/cm^3 (Fig. 4). A prominent peak that was seen between 110 and 160 nm attested to the nanosuspension's homogeneity.

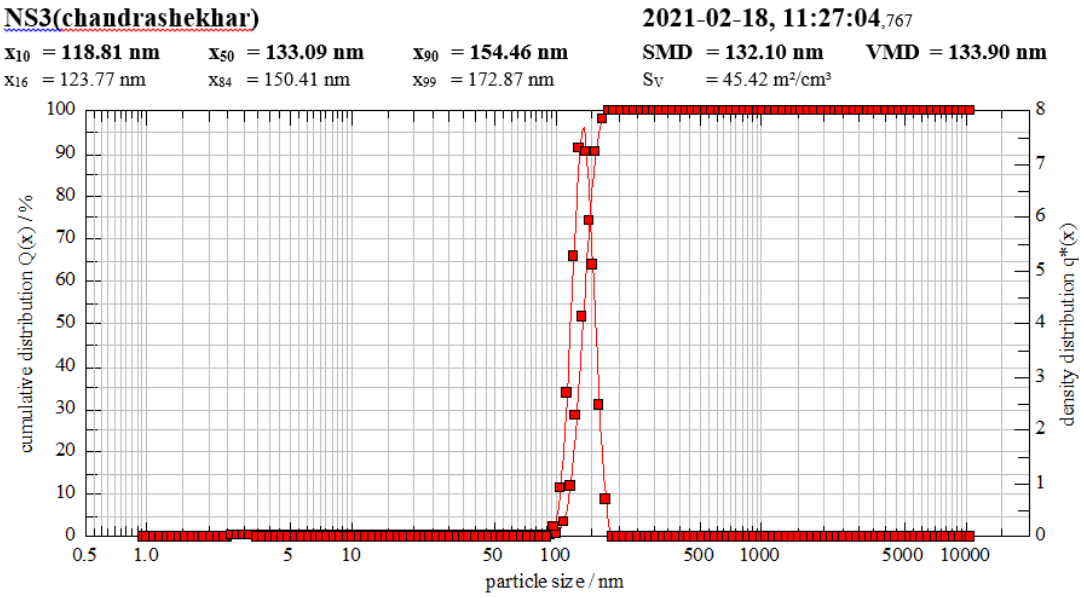

Figure 4: Particle size analysis of nanosuspension (NSWS)

Polydispersity Index (PDI)

The nanosuspension's (NSWS) Polydispersity Index (PDI) obtain was 0.27, represents a high level of homogeneity in the particle size distribution. This low PDI value suggests that there was minimal polymeric aggregation during the nanoprecipitation process.

Zeta Potential

As seen in Fig. 5, the nanosuspension (NSWS) zeta potential was determined at -0.13 mV. Zeta potential levels between +30 mV and -30 mV often reflect the stability of drug-loaded nanoparticles. The negative zeta potential suggests that the nanosuspension is stable and effectively prevents particle aggregation.

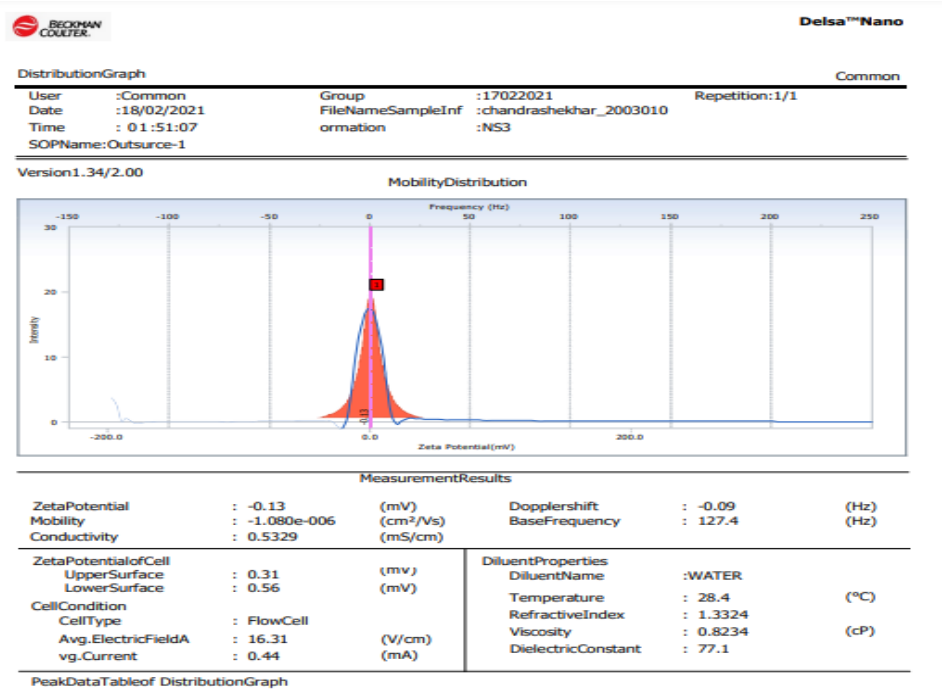

Figure 5: Zeta potential value of nanosuspension (NSWS)

Field Emission Scanning Electron Microscopy (FESEM)

The particles in the nanosuspension (NSWS) were mostly spherical in shape and had average diameters of about 160 nm, according to FESEM analysis. Figure 6 shows that the particles textures were smooth and in cluster form throughout the photographs.

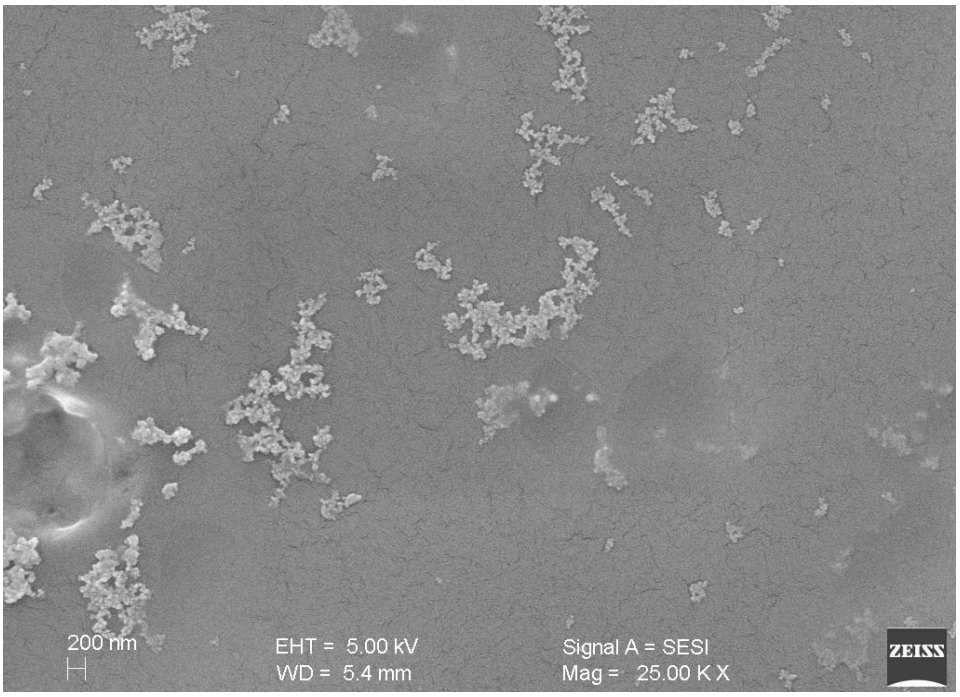

Figure 6: FESEM image of nanosuspension (NSWS)

Powder X-Ray Diffraction (XRD)

XRD analysis of the nanosuspension (NSWS) revealed intense peaks at 19.71°, 28.30°, and 31.85° 2θ. These Bragg reflections confirm the crystalline nature of the nanoparticles, consistent with the expected spherical crystalline structure.

pH Measurement

The pH of the nanosuspension (NSWS) was measured at 7.2, indicating suitability for the formulation.

Stability Studies

The physical appearance of the nanosuspension (NSWS) remained stable when stored at 4°C for three months. At room temperature, slight sedimentation was observed, but this was easily resolved with gentle shaking. However, there was an increase in particle size to 165 nm and a rise in PDI to 0.3 at room temperature, compared to 120 nm and a PDI of 0.27 at 4°C. Overall, refrigeration proved to be the optimal condition for maintaining the stability of the nanosuspension.

DISCUSSION

Specialised a submicron colloidal dispersion that consist of nanoscale drug particles stabilised by polymeric stabilisers or surfactants are known as nanosuspensions. These nanosuspensions, which are typically defined by a mean particle size in the nanometre range, are essential to their stability and efficacy[30]. The use of plant extracts, particularly *Withania somnifera* known for its numerous traditional medicinal applications—has gained significant attention in nanotechnology. Root extracts of *Withania somnifera* contain several bioactive compounds, including alkaloids, saponins, tannins, glycosides, phenols, and steroids[31]. These compounds contribute to the plant's therapeutic properties and are essential for formulating effective nanosuspensions. The nanoprecipitation method was employed to create the nanosuspension (NSWS) due to its simplicity, reproducibility, and effectiveness. The resulting nanosuspension exhibited the desired particle size and polydispersity index (PDI), confirming its uniformity. The small particle size (less than 200 nm) and low PDI (less than 0.3) are critical for maintaining physical stability, dispersibility, and homogeneity. A narrow particle size distribution is vital to prevent issues like Ostwald ripening, which can lead to particle growth and instability[32]. The zeta potential of the formulated nanosuspension (NSWS) was measured at -13 mV. While ideally, nanosuspensions should have a zeta potential of at least ±30 mV for electrostatic stability or ±20 mV for steric stabilization to prevent aggregation[33]. The -13 mV zeta potential still indicates reasonable stability, as supported by particle size and PDI results. According to stability experiments, the nanosuspension maintained its stability at room temperature and under refrigeration, with only slight variations in particle size and PDI that were still within tolerable bounds for use in medicinal applications. The increase in particle size and PDI at room temperature may be attributed to Ostwald ripening, suggesting that refrigeration is recommended for optimal stability[34]. FT-IR analysis revealed

absorption peaks ranging from 564 to 3604 cm^{-1}, indicating various functional groups in the nanosuspension (NSWS). The FT-IR spectra for both the nanosuspension and the *Withania somnifera* extract showed consistent peak positions with minimal shifts, suggesting effective integration of the extract with the stabilizer[35]. FESEM analysis demonstrated that the particles in the nanosuspension were predominantly spherical and clustered, with a size range of approximately 160 nm. This analysis confirms the nanoscale range and provides valuable insights into surface morphology[36]. XRD analysis affirmed the crystalline nature of the nanoparticles, with peaks corresponding to specific 2θ angles, consistent with Bragg's reflections, indicating a crystalline structure[37]. Overall, the formulation and characterization of the *Withania somnifera* nanosuspension successfully demonstrated the integration of plant extract with nanotechnology, yielding a stable and effective nanosuspension with desirable properties.

CONCLUSION

Nanosuspensions provide an effective solution to the poor bioavailability often seen with hydrophobic drugs, which typically face solubility challenges in both aqueous and organic environments. This study focused on preparing a nanosuspension using an ethanolic extract of *Withania somnifera* root, which is rich in active constituents such as alkaloids, flavonoids, phenolics, and tannins. Fourier Transform Infrared (FT-IR) analysis confirmed the retention of these functional groups in the nanosuspension (NSWS), indicating that the active components from the extract remained intact. The nanosuspension's particle size was about 133.09 nm, and its uniform particle distribution was indicated by a Polydispersity Index (PDI) about 0.27. Stability and uniformity were further supported by the zeta potential, particle size, and PDI values. Scanning Electron Microscopy (SEM) confirmed that the particles were nano-sized, while an examination using X-ray diffraction (XRD) revealed that they are crystalline. These results underscore the effectiveness of the nanosuspension in enhancing drug solubility and stability. Overall, employing drug nanocrystals in nanosuspension formulations represents a versatile strategy to improve the therapeutic efficacy of hydrophobic drugs across various routes of administration.

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Ethics approval and consent to participate: Not applicable

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