Formulation and Characterization of Nanosuspension of Ethanolic Extracts of Malaxis Acuminata

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Abstract
A nanosuspension is a submicron colloidal dispersion of drug particles which are stabilized by surfactants. The particle size distribution of the solid particles in nanosuspensions is usually less than one micron with an average particle size ranging between 200 and 300 nm. The present study was designed to formulate and characterized the nanosuspensions of *Malaxis Acuminata* plant extract. The nanosuspension of plants extract will be formulated using nano precipitation technique followed by its lyophilization using mannitol as a cryoprotectant. The nanosuspension was evaluated for their particle size, zeta potential, SEM and TEM. The particle size of formulations were in a range of 200-300 nm and PDI is 0.247 . The formulation was physically and chemically stable when stored at the 4-8°C and 40 ± 2°C at 75 ± 5 % RH for a period of one month. The nature of the nanoparticles synthesized from bulbs extract was analyzed by X-ray diffraction analysis an intensive peaks at 20.3022 and 28.5995 degrees of 2-theta (deg) on the XRD spectrum. Morphology study of drug particles in the *Malaxis Acuminata* nano suspension shows that it exhibit a spherical shape within the a size range. These particles are discrete, uniform in size, and show no indications of agglomeration. It can be concluded that Nanosuspension of Ethanolic Extracts of *Malaxis Acuminata* is stable.

Keywords : Nano-suspension, *Malaxis Acuminata* , Nano precipitation technique, Scanning electron microscopy, Transmission electron microscopy.

Introduction :
A nanosuspension is a submicron colloidal dispersion of drug particles which are stabilized by surfactants. A pharmaceutical nanosuspension is defined as very finely dispersed solid drug particles in an aqueous vehicle for either oral and topical use or parenteral and pulmonary administration. The particle size distribution of the solid particles in nanosuspensions is usually less than one micron with an average particle size ranging between 200 and 600 nm [1,2].
The stability of particles within nanosuspensions is credited to the consistent particle size they possess, characterized by achieved through various manufacturing processes. The absence of particles with significant differences in size prevents the existence of different saturation solubilities and concentration gradients, thereby preventing the Oswald ripening effect. Ostwald ripening is a process responsible for crystal growth and the creation of micro-particles, driven by differences in dissolution pressure/saturation solubility between small and large particles.
In Ostwald ripening, molecules diffuse from the higher concentration area around tiny particles exhibiting increased saturation solubility to the vicinity of larger particles with lower drug concentration. This results during the creation of a supersaturated solution around the large particles, leading to drug crystallization and the expansion of large particles.
Nanosuspensions are sub-micron colloidal dispersions of pure drug particles in an outer liquid phase. Nanoparticle engineering enables poorly soluble drugs to be formulated as nanosuspensions alone, or with a combination of pharmaceutical excipients. Nanosuspension engineering processes currently used are precipitation , high pressure homogenization and pearl milling , either in water or in mixtures of water and water miscible liquids or non-aqueous media [3-8].
Nanoprecipitation method presents numerous advantages, in that it is a straightforward technique, rapid and easy to perform. In this method, the drug is dissolved in an organic solvent such as acetone, acetonitrile, methanol or ethyl acetate. The organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type of stabilizer, concentrations of stabilizer, and homogenizer speed. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed. The super saturation is further accentuated by evaporation of drug solvent. This yields to the precipitation of the drug. High shear force prevents nucleus growth and Oswald's ripening [9].
It is important to focus on the methods. For the improvement of nano suspension to have more applicable, areas like dissolution velocity, solubility, bioadhesivity, versatility in surface modification are to be enhanced. The applications of nanosuspensions in parentral and oral routes have been very well investigated and applications in pulmonary and ocular

delivery have been realized. The present study was designed to formulate and characterized the nanosuspensions of *Malaxis Acuminata*[10].

MALAXIS ACUMINATA

Malaxis Acuminata is a worldwide soil loving plant belongs to the family Orchedaceae, commonly named as Jeevak. This species grow in colonies and one colony may contain 5-25 individuals. *Malaxis Acuminata* forms colonies in shady places, moist ground and in the area that are wet & mossy.

Malaxis Acuminata is an important medicinal plant having immense ethnomedicinal potential. The dried pseudobulbs known as 'jeevak' are important ingredients of 'Chyavanprash' which is a polyherbal immune booster known to restore vigour, vitality and youthfulness. [11,12]

MATERIALS & METHODS

Collection of plants material:

Plant of Jeevak (*Malaxis Acuminata*) were collected. After the gathering of plant their authentication was done by Department of Botany at RTMNU, Nagpur. A specimen number assigned to the authenticated sample sheet was 10371.

Extraction:

After the collection, the bulbs were separated from the plants and dried in shade and was powdered mechanically to induce the coarse powder. Weighed quantity of coarse powder (1kg) was extracted with petroleum ether at 50° - 60° C for 72 hrs by hot percolation employing a Soxhlet apparatus. The residual material remaining after the petroleum ether extract will to be the dried material was later subjected to extraction ethanol 95 % at (60° - 70° C) up to 72 hours in Soxhlet apparatus was done. A residue obtains after concentrating the alcoholic extract will kept in a desiccator.

Preliminary Phytochemical screening of ethanolic extracts of Jeevak (*Malaxis Acuminata*)

The plants may be considered as biosynthetic laboratory for multitude of compounds like alkaloids, glycosides, volatile oils, tannins, saponins, flavonoids, sugar etc. that exerted physiological effect. These compounds are termed as secondary metabolites. To check the presence or absence of primary and secondary metabolites all the extracts were subjected to a various chemical tests. [13,14]

Formulation of nanosuspension from plants extract:

The formulation of the nanosuspension of the plant extract will involve the use of the Nano precipitation technique, followed by lyophilization with mannitol as a cryoprotectant. Optimization of formulation and process parameters was undertaken to achieve the desired particle size and saturation solubility. The formulation of the nanosuspensions was conducted through the nano-precipitation method with slight modifications.

1. A solution was prepared by dissolving 2.5g of plant extract in 15 ml of acetone and ethanol (3:1) through sonication for 60 seconds.
2. The prepared solution was then slowly injected (1 ml/min) using a syringe connected to a thin Teflon tube into 25 ml water containing 1.5% w/v PVA, under continuous magnetic stirring at 1000 rpm.
3. The resulting emulsion was further diluted in 50 ml PVA solution (0.2% w/v in water) to minimize coalescence.
4. The mixture was stirred continuously (500 rpm) for 6 hours at room temperature to facilitate solvent evaporation and the formation of nanoparticles.
5. The resulting nanosuspension was then be cooled to -18°C and subjected to lyophilized to obtain dry powder[15].

Characterization of Nanosuspension of *Malaxis Acuminata* Plant Extract:

The characterization of the formulated nanosuspension from the plant extract was encompass assessments related to analyze Particle size, Polydispersity index (PDI), Zeta potential, Sedimentation volume, Viscosity, Stability study and Morphology through SEM (Scanning Electron Microscopy) and TEM (Transmission Electron Microscopy).To evaluate the crystalline state of formulation, Powder X-ray diffraction (PXRD)analysis was conducted.

Particle Size and PDI Determination:

The Mean particle size and size distribution, poldispersity index (PDI) of *Malaxis Acuminata* Nanosuspension was measured by photon correlation spectroscopy using Nanophox Symphatech GmbH(NX0088), Germany at room temperature. Before measurement sample were diluted with filtered double distilled water to avoid multi-scattering events. Nanosuspension of *Malaxis Acuminata* was placed in transparent polystyrene cuvette (path length = 1 cm) and placed in thermostatic sample chamber. Detection was carried out at a scattering angle of 90º. The results obtained for particle size distributions were used to confirm the formation of nano sized particles.

Zeta Potential Determination:

The zeta potential is the electric potential of a particle in a system. It is a parameter which is very useful for the assessment of the physical stability of the colloidal dispersions. The particle charge of colloidal system was measured as zeta potential via the electrophoretic mobility of the particles in an electrical field. Zeta potential of *Malaxis Acuminata* nanosuspension was measured on zeta meter (Desla Nano C,Beckman Coulter, Japan) with parameters configured for a temperature of 25.1ºC and a refractive index of 1.33.

Sedimentation Volume:
The rate of separation of formulation was determined by pouring 100 ml portion of nanosuspension in measuring cylinder and stored undisturbed at room temperature. The separation of clear liquid was noted and subsequently the sedimentation volume measurements were taken at intervals of one month for a total duration of 6 month.

The results acquired for formulation was utilized for sedimentation volume calculations using the equation:
F=Vu/ Vo
Where, F represents the sedimentation volume,
Vu denotes the ultimate volume after sediment, and
Vo signifies the initial volume of the total suspension.

Viscosity determination:
The settling behavior of a suspension can be characterized using an viscometer (LMDV-60, Labman, India) equipped with a spindle, mounted on a stand. Turn on the instrument and select the desired spindle speed and deep the temperature probe in the sample. Let the viscometer run until the readings stabilize. Record the viscosity reading, displayed on the screen of viscometer in MilliPascal-seconds (mPa.s).

Short term stability study:
The nanosuspension was undergo a short-term stability study in accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines over a one-month period. This study is essential to assess the nanosuspension's stability under various storage conditions and to ensure its viability for pharmaceutical applications. Following ICH guidelines, the stability study was involved monitoring key characteristics of the nanosuspension, including sedimentation volume, physical appearance, viscosity, redispersibility over the specified one-month duration. These assessments are crucial in determining the formulation's ability to maintain its intended properties and performance over time.

The study was conducted under controlled environmental conditions, considering factors such as temperature, humidity, and light exposure, as outlined in the ICH guidelines.
The nanosuspension was placed at room temperature for twelve month and was analyzed after twelve months for the zeta potential measurement.

Redispersibility study:
The method for redispersibility essentially consisted of holding the sample vial straight in upright position between two fingers with thumb at the bottom and the index finger at the top followed by almost uniform rotation through 180º and brought back to same path. The pair of successive upward and down ward movement each of approximately equal force, constituted one complete shake. The endpoint of this process is determined when the bottom of the vial becomes clear of sediment, indicating the achievement of uniformity in the suspension and redispersibility of drug particles. This method ensures a standardized approach to evaluating the redispersibility characteristics of the suspension. By subjecting it to controlled rotational conditions with hands, the process allows for a systematic comparison of different formulations and provides valuable insights into the stability and performance of the suspended particles.

Morphological characterization of the nanoparticles by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM):
The assessment of the nanosuspension's morphology was conducted using advanced imaging techniques, including Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The SEM analysis was performed using a Carl Zeiss ,Supra 55, Oberkochen, Germany with Zeiss SEM Software and tungsten filament as source with accelerating voltage of 5 kV. For sample preparation, a small amount of the nanosuspension was carefully was placed on a SEM-stub using double-sided adhesive tape and dry it under room temperature for a day. Sample coated with thin layer of gold under vacuum. Sample stub kept in SEM chamber, operate at 5 kV. Run the sample and capture the images as different magnification.

In parallel, the dimensions and detailed morphology of the nanosuspension was characterized through TEM using an Tecnai T20,FEI,USA with Tecnai imaging & Analysis software and tungsten filament as source with accelerating voltage of 80 kV. The TECNAI T20 typically operates in a high-vacuum environment, suitable for nanosuspensions once properly dried. The sample preparation for TEM involves depositing a droplet of the nanosuspension onto carbon-coated mesh copper grids as substrate and were dried at room temperature for TEM measurement. Both SEM and TEM techniques offer high-resolution imaging capabilities, allowing for the visualization of nanoscale features. SEM provides surface topography details, while TEM offers insights into the internal structure of particles. The combined use of these microscopy techniques, along with meticulous sample preparation methods, enables a comprehensive understanding of the nanosuspension's morphology, including particle size, shape, and distribution. This information is crucial for assessing the formulation's quality, homogeneity, and overall suitability for its intended application.

Powder X-ray diffraction analysis of the nanoparticle:
To conduct X-ray diffraction analysis, the water content in the nanosuspension was eliminated through lyophilization using an lyophilizer (Freeze Dryer, MSW-137, Nutronics). Following this process, the resulting lyophilized samples was subjected to analysis using a X- ray diffractometer (Rigaku, Smartlab Cu 1.5 KV,Japan) equipped with a Cu Kα radiation source. The diffractometer will operate at an electrical potential of 40 kV and a current of 40 mA. During the analysis, scans was recorded with a detector rate set at 0.2°/min, covering a 2θ range from 5 to 50°. The use of Cu Kα radiation as the X-ray source and the specific instrument parameters contribute to the accuracy and precision of the diffraction patterns obtained.

The X-ray diffraction analysis provides valuable information about the crystalline structure of the nanosuspension after lyophilization. The diffraction patterns that ensue will reveal details about the configuration of atoms within the sample, helping to identify the presence of crystalline phases, assess the degree of crystallinity, and detect any potential changes in the material's structure due to the lyophilization process. This analysis is instrumental in understanding the solid-state characteristics of the nanosuspension and is vital for ensuring the stability and integrity of the formulation for its intended application. [16,17,18]

Results and Discussion :
Extraction of plants :
Following concentration of the alcoholic extract, a brown residue was obtained for *Malaxis Acuminata* and stored in a desiccator.

Table.No.1 Extractive Value of *Malaxis Acuminata*

Extractive Value (% w/w)	**Alcohol Soluble (% w/w)**	**6.1**

Preliminary phytochemical screening :

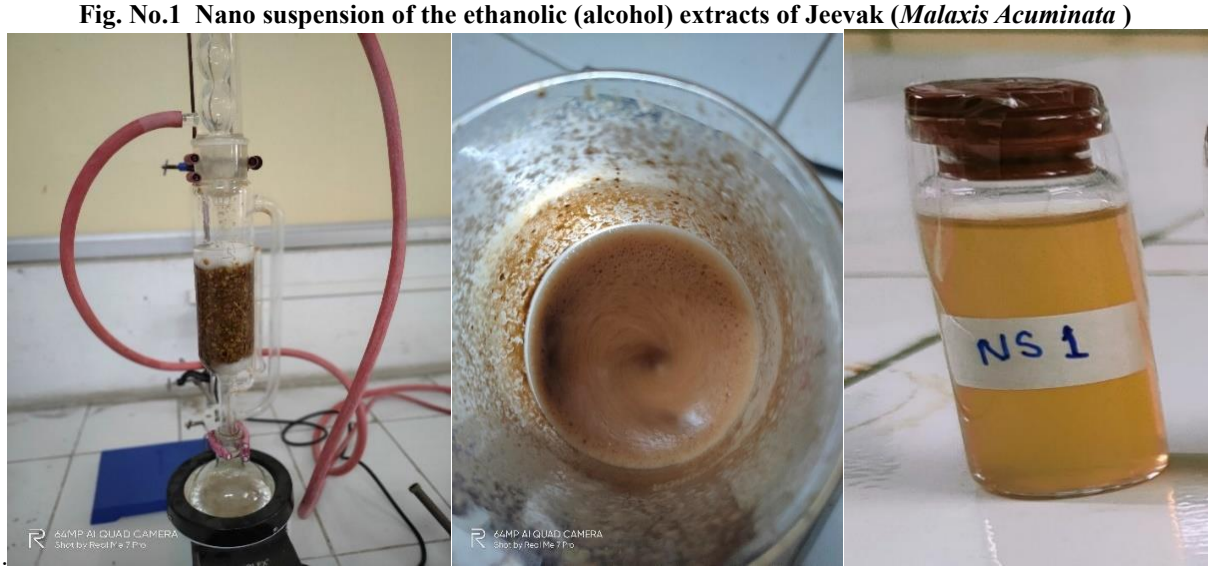
Table.No.2 Preliminary phytochemical screening of the alcoholic extract of Jeevak (*Malaxis Acuminata*)

S. No.	Plant Constituent	Test/ Reagent	Inference
01.	Sugars	Molisch Test Fehling's Test	+ +
02.	Proteins	Millon's Test Xanthoprotein Test	- -
03.	Amino acids	Ninhydrin Test	-
04.	Alkaloids	Dragendorff's reagent Mayer's reagent Wagner's reagent	+ + +
05.	Glycosides	Legal's Test Borntrager's Test	+ +
06.	Tannins	Ferric chloride Test Lead acetate Test Potassium dichromate Test	+ + +
07.	Flavonoids	Shinoda Test	-
08.	Phytosterols	Salkowaski Test Liebermann Test	+ +
09.	Saponins	Foam Test	+

'+' indicates: present; '-' indicates: absent

Formulation of nanosuspension from extract of *Malaxis Acuminata*:
The nano-precipitation technique was utilized with a minor adjustment in the preparation of nanosuspensions for *Malaxis Acuminata*. The Concentration of nanosuspension was found to be 33 mg/ml.

Fig. No.1 Nano suspension of the ethanolic (alcohol) extracts of Jeevak (*Malaxis Acuminata*)



Characterization of prepared nanosuspension of *Malaxis Acuminata* :

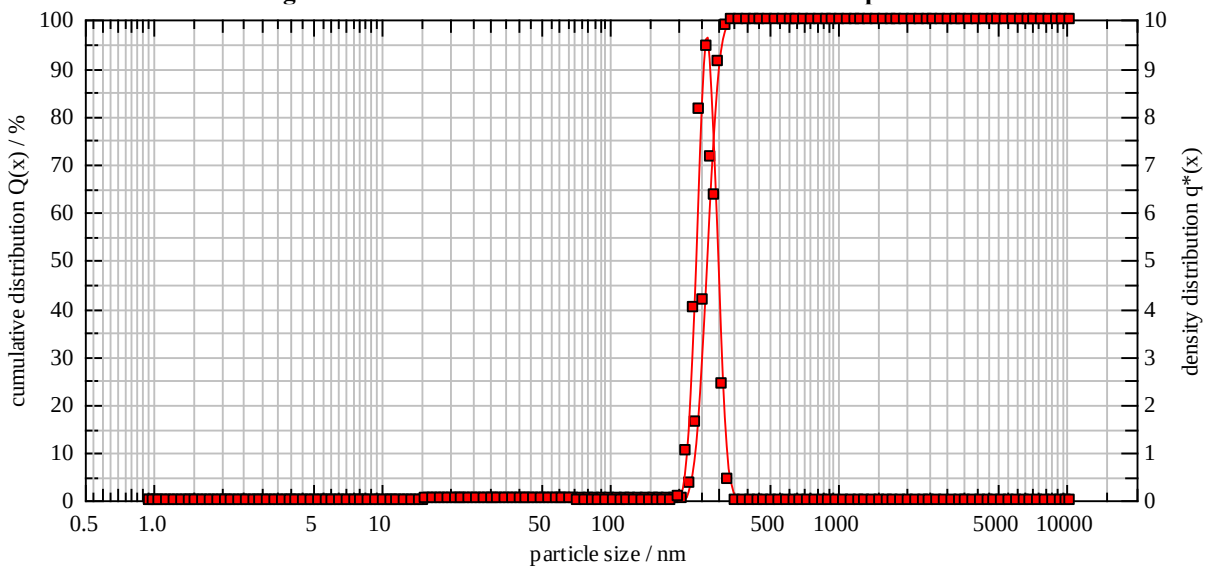
Particle Size Measurement :

The Mean particle size, poldispersity index (PDI) of *Malaxis Acuminata* Nanosuspension was measured by photon correlation spectroscopy using Nanophox Symphatech GmbH (NX0088), Germany. The choice of suitable stabilizers and its concentration are the most important factors to control the size and stability. Nanosuspension with PVA shows acceptable particle size and poly dispersity index and formed a homogenous suspension on reconstitution and this value will indicate good stability of the nanosuspension. PDI value indicates good uniformity of particle size distribution. A low PDI (typically <0.3) means the uniform particle size distribution.

Table.No.3 Particle size of Nanosuspension

Sample Name	Particle Size	PDI	VMD
MANS	260.67 nm	0.247	263.23 nm

Fig. No.2 Particle Size of *Malaxis Acuminata* Nanosuspension



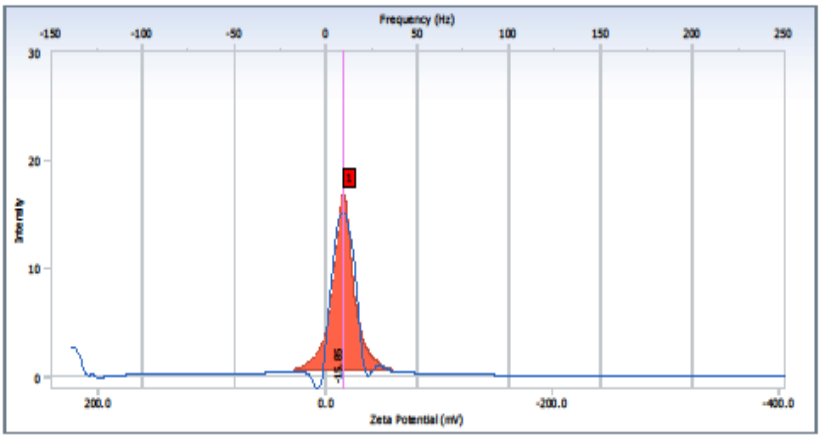
Zeta Potential Measurement :

The zeta potential had a foremost effect on the storage stability of colloid dispersion system and it reflected the electrostatic barriers which could intercept the nanoparticles from aggregation and agglomeration. It provide an idea about the physical stability of nanosuspension. In the present study, the zeta potential of *Malaxis Acuminata* nanosuspension was found to possess negative value and the measured zeta potential was -15.85 mV, indicating good stability of the nanoformulation. A negative zeta potential is particularly beneficial as it hinders particle aggregation and supports dispersion.

Fig.No. 3 Zeta potential of *Malaxis Acuminata* Nanosuspension

Version 1.34 / 2.00

Mobility Distribution



Measurement Results

Zeta Potential	: -15.85	(mV)	Doppler shift	: 9.81	(Hz)
Mobility	: -1.239e-004	(cm²/Vs)	Base Frequency	: 128.1	(Hz)
Conductivity	: 0.1564	(mS/cm)			

Zeta Potential of Cell			Diluent Properties		
Upper Surface	: -30.19	(mV)	Diluent Name	: WATER	
Lower Surface	: -19.67	(mV)	Temperature	: 25.1	(°C)
Cell Condition			Refractive Index	: 1.3328	
Cell Type	: Flow Cell		Viscosity	: 0.8858	(cP)
Avg. Electric Field	: -16.40	(V/cm)	Dielectric Constant	: 78.3	
Avg. Current	: -0.13	(mA)			

Peak Data Table of Distribution Graph

Sedimentation Volume:

The results acquired for formulation was utilized for sedimentation volume calculations using the equation:

F=Vu/ Vo

In the case of *Malaxis Acuminata*, the zeta potential of -15.85 mV implies a negative charge. Negative indicates that the nanosuspensions experience electrostatic repulsion between particles, contributing to stability and favourable sedimentation behaviour.

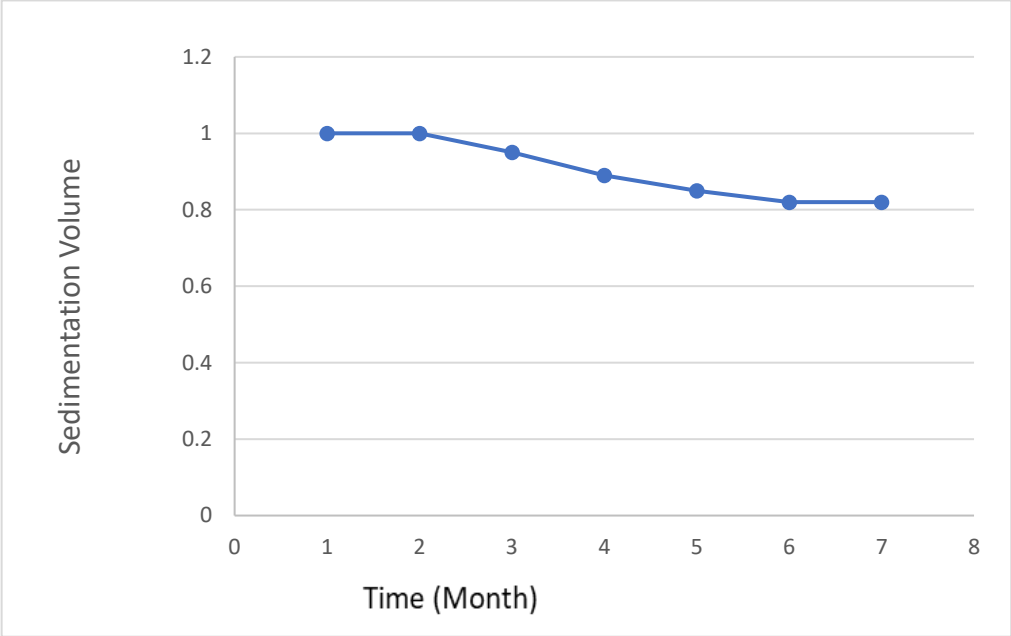
In conclusion, the information provided suggests that the nanosuspensions exhibit good stability, as their negative zeta potential values work to prevent particle aggregation and promote effective dispersion and sedimentation.

The Sedimentation volume of *Malaxis Acuminata* nanosuspension was calculated and depicted below.

Table.No.4 Sedimentation volume of *Malaxis Acuminata* nanosuspension

Time (Month)	Sedimentation Volume
0	1
After 1 Month	1
After 2 Month	0.95
After 3 Month	0.89
After 4 Month	0.85
After 5 Month	0.82
After 6 Month	0.82

Fig. No. 4 Sedimentation volume of *Malaxis Acuminata* nanosuspension



Viscosity determination:

The viscosity of Nano suspension of the ethanolic (alcohol) extracts of *Malaxis Acuminata* was determined at room temperature using Brookfield viscometer at laboratory scale. The Nano suspension of ethanolic (alcohol) extract of Jeevak (*Malaxis Acuminata*) shows viscosity of 12.35 mPa.s.

Short-term accelerated stability study:

Stability study was carried out for *Malaxis Acuminata* nanosuspension to assess its stability study for a duration of one month as per ICH guidelines. In the present investigation, a stability assessment was conducted at 4-8°C& 40 °C ± 2°C and 75% ± 5% relative humidity (RH). Nanosuspension was aassessed for physical appearance, viscosity, sedimentation rate, redispersibility study. To evaluate the physical appearance, nanosuspension samples were observed for agglomeration and color change for one month at room temperature. Visually identification of nanosuspension was done on base of color change and agglomeration. There was not found any colored change after preparation of nanosuspensions. After one month there was no changes in above parameters at room temperature.

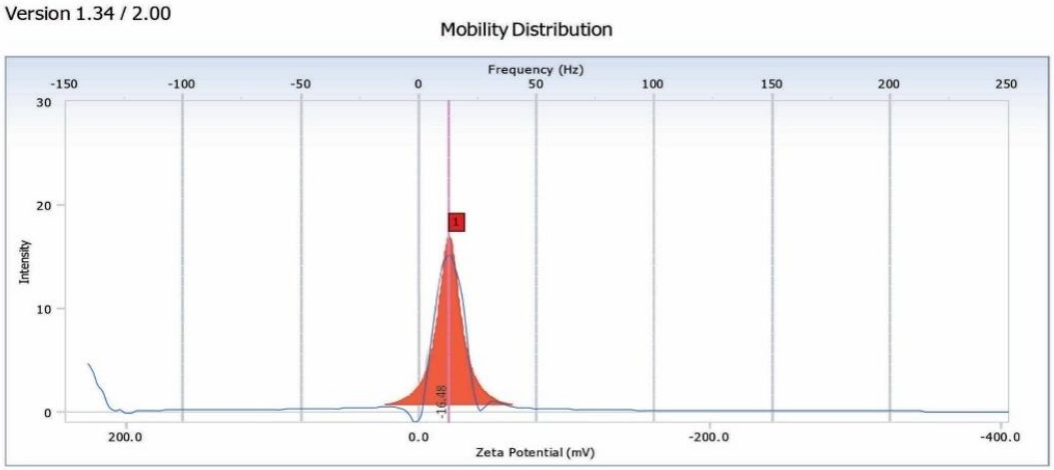
Stability study suggested that the formulation was physically and chemically stable when stored at 4-8°C and at the 40 ± 2°C and 75 ± 5 % RH for a period of one month. It was noted that there was a slight change in all optimization parameters which have less than ±5% bias which was significance.

Table.No. 5 Measurement of Stability parameters of *Malaxis Acuminata* Nanosuspension after one month at 4-8°C and 40 ± 2°C and 75 ± 5 % RH

Parameters	Sedimentation Volume	Viscosity (mpa)	Redispersibility	Physical appearance
After one Month (4-8°C)	01	12.29	Easily Redispersible	No sign of colour change and agglomeration
After one Month (40 ± 2°C and 75 ± 5 % RH)	0.98	12.75	Easily Redispersible	No sign of colour change and agglomeration

The nanosuspension was analyzed after twelve month for the zeta potential measurement as stability parameter. In this study, the zeta potential of *Malaxis Acuminata* nanosuspension was found to possess negative value and the measured zeta potential was -16.48 mV, which indicate negligible difference and a stable nanoformulation.

Fig. No.5 Zeta Potential of *Malaxis Acuminata* nanosuspension after 12 Month at room temperature



Measurement Results

Zeta Potential	: -16.48	(mV)	Doppler shift	: 10.22	(Hz)
Mobility	: -1.289e-004	(cm²/Vs)	Base Frequency	: 128.1	(Hz)
Conductivity	: 0.1629	(mS/cm)			

Zeta Potential of Cell			Diluent Properties		
Upper Surface	: -31.34	(mV)	Diluent Name	: WATER	
Lower Surface	: -20.47	(mV)	Temperature	: 25.1	(°C)
Cell Condition			Refractive Index	: 1.3328	
Cell Type	: Flow Cell		Viscosity	: 0.8898	(cP)
Avg. Electric Field	: -17.04	(V/cm)	Dielectric Constant	: 78.4	
Avg. Current	: -0.14	(mA)			

Peak Data Table of Distribution Graph

Redispersibility study:

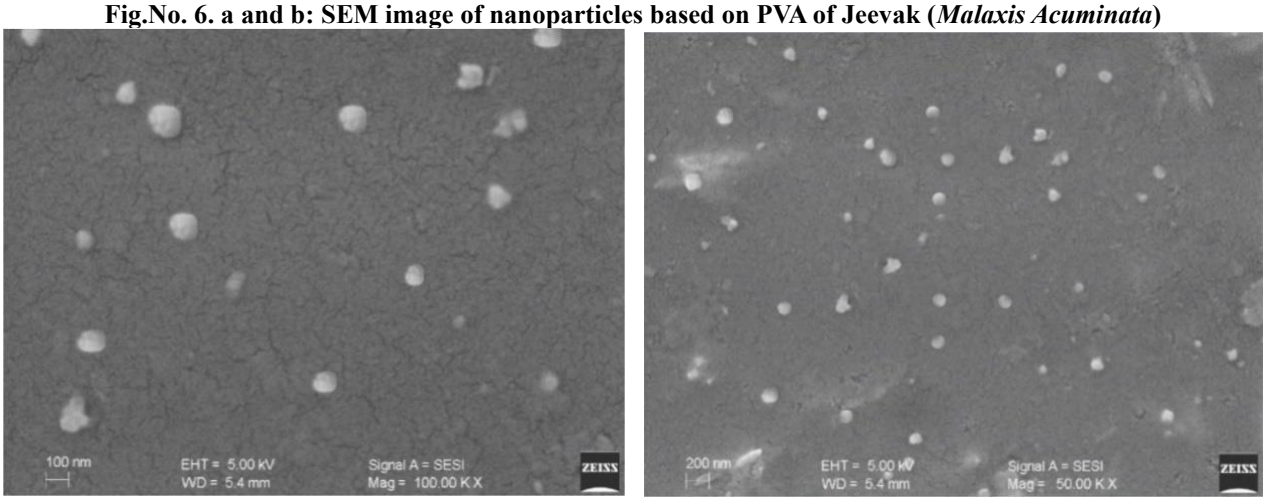
It was determined by tilting the vial containing nanosuspension up and down with finger till the sediment was uniformly dispersed in the liquid phase. Nanosuspension of the ethanolic (alcohol) extract of Jeevak *(Malaxis Acuminata)* was easily redispersed with soft agitation.

Morphological characterization of the nanoparticles by SEM:

The formulated nanosuspension of Jeevak (*Malaxis Acuminata*) was characterized for their particle size and morphological features, which was observed using SEM. The SEM images demonstrated that the size of the particles the nano suspension was within the accepted range for nanoparticles (10 -1000 nm).

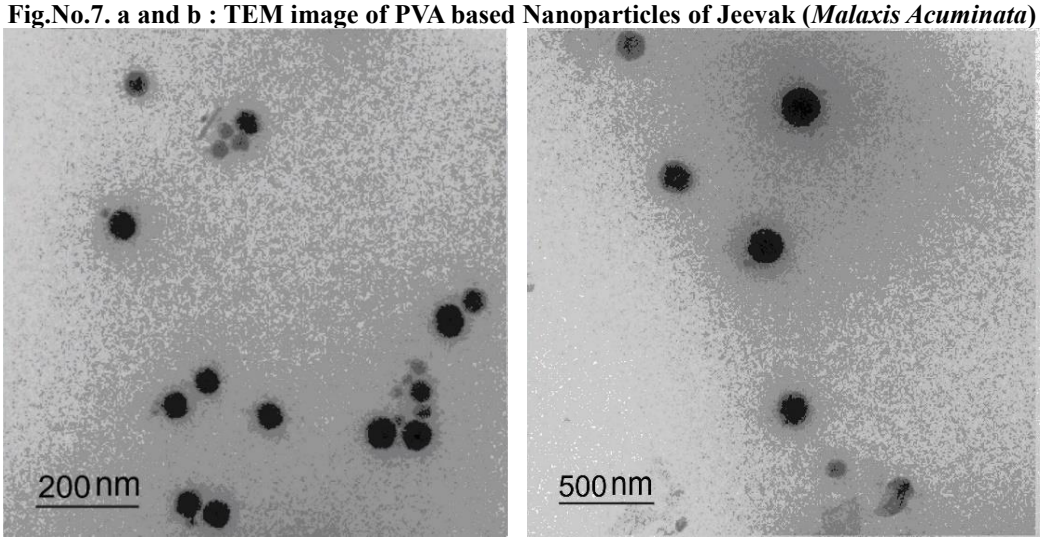
Morphology of drug particles in the Jeevak (*Malaxis Acuminata*) nano suspension is shown in Fig.6.a & b.The drug particles generated with PVA as a stabilizer, exhibit a spherical shape within the a size range. These particles are discrete, uniform in size, and show no indications of agglomeration.TEM image (Fig.7.a & b) showed that the nanoparticles were approximately spherical and uniform distribution.

Fig.No. 6. a and b: SEM image of nanoparticles based on PVA of Jeevak (*Malaxis Acuminata*)



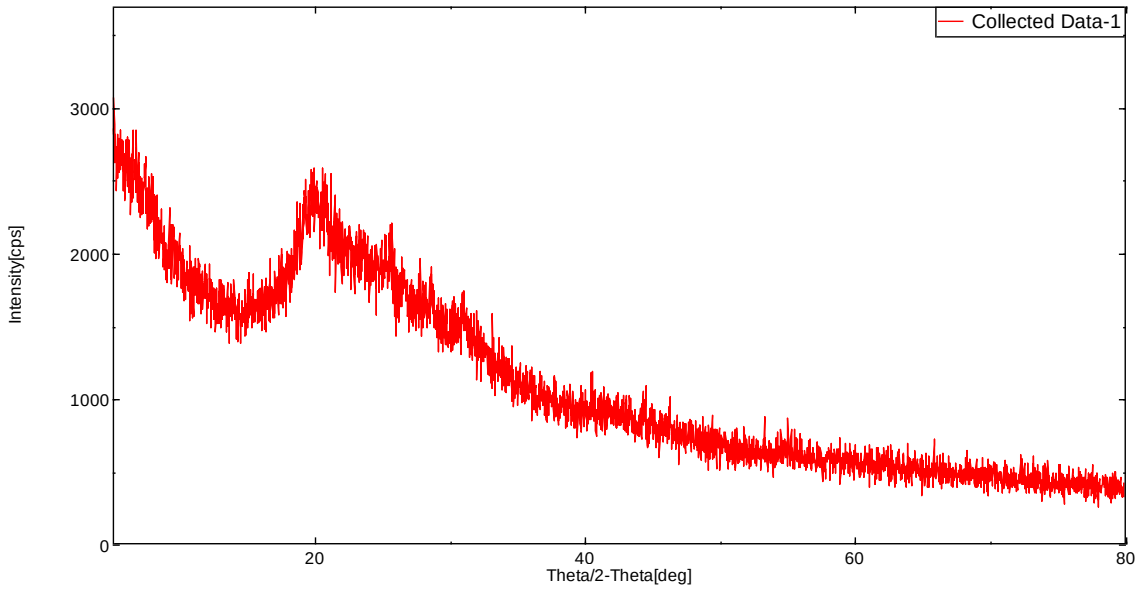
Morphological characterization of the nanoparticles by TEM:

Fig.No.7. a and b : TEM image of PVA based Nanoparticles of Jeevak (*Malaxis Acuminata*)



Powder X-ray diffraction analysis of the nanoparticle:
The samples patterns was scrutinized utilizing a diffractometer that incorporates a Cu Kα radiation source, functioning at a voltage of 40 kV and a current of 40 mA. Scans was documented at a detector rate of 0.2°/min, encompassing a 2θ range spanning from 5 to 50°.

Fig.No. 8XRD of Nanosuspension formulation of *Malaxis Acuminata* plant extract



The analysis of nanoparticles obtained from *Malaxis Acuminata* plant extract was conducted using X-ray diffraction (XRD). In the XRD spectrum of nanosuspensions derived from *Malaxis Acuminata* plant extracts, distinct peaks were observed at 20.3022 and 28.5995 degrees of 2-theta (deg) on the x-axis. The corresponding values of 903.29, 675.82 for these Bragg reflections provide compelling evidence that the nanoparticles possess a crystalline nature. The intensity and clarity of these peaks signify a well-ordered atomic arrangement within the nanoparticles. The specific angles of reflection further affirm the crystalline structure of the synthesized nanoparticles. Therefore, based on the XRD analysis, it can be unequivocally stated that the nanoparticles derived from *Malaxis Acuminata* plant extract exhibit crystalline characteristics, as evidenced by the distinct peaks at 20.3022 and 28.5995 degrees of 2-theta (deg) on the XRD spectrum.

CONCLUSION
The preliminary phytochemical screening of ethanolic extracts from Jeevak (*Malaxis Acuminata*) revealed the presence of carbohydrates, tannins, saponins, alkaloids, glycosides, phytosterols content. These constituents were identified through various phytochemical tests.

The calculated zeta potential values for the nanosuspensions of Jeevak (*Malaxis Acuminata*) was observed to be -15.85 mV indicating favourable stability of the formulations. These values signify favorable stability of the formulations, indicating positive sedimentation behaviour. Sedimentation volume of *Malaxis Acuminata* nanosuspension is near to 1 which indicates stable nanosuspension. The viscosity of the nanosuspension of the ethanolic extract of Jeevak (*Malaxis Acuminata*) was about 12.35 mPa. s.

The stability study revealed that the formulation remained physically and chemically stable when subjected to storage conditions Notably, there was a minor alteration in all optimization parameters, each exhibiting less than a ±5% bias.

The morphology analysis of by SEM and TEM of Nano suspension of Jeevak (*Malaxis Acuminata*), utilizing PVA as a stabilizer, revealed smooth, spherical, homogeneous nanosized particles. These particles exhibited a discrete and uniform size distribution, with no evidence of agglomeration. Peak of the XRD analysis indicate that the nanoparticles are crystalline in nature. The results of the present investigation clearly indicated that the preparation of Nanosuspensions by nano precipitation method greatly improved the stability of nano suspension of *Malaxis Acuminata* plant extract. Polyvinyl alcohol used as an inert stabilizer/surfactant to stabilize the Nanosuspension.

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