DEVELOPMENT AND CHARACTERIZATION OF *PINUS WALLICHIANA* BASED NANOEMULSION USED AGAINST FUNGAL INFECTION

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ABSTRACT

The nano emulsion of ethanolic leaves extract of *P. wallichiana* has not been formulated and tested for anti-fungal activity using prescribed protocols and methods. Therefore, this research focuses on development and characterization of *P. wallichiana* based nano emulsion used against fungal infection. Ethanolic leaves extract of *P. wallichiana* was obtained from the Uttarakhand region and other chemicals from Sigma Aldrich, India. It was identified and authenticated by a botanist. The powder of *P. wallichiana* were weighed and extracted through Soxhlet apparatus using ethyl alcohol. Pre-formulation study was performed for estimation of better compatibility profile. After that total 6 formulations of nano emulsion were developed. Formulations were evaluated for parameters including physical appearance, droplet size & polydispersity index, in-vitro drug release, % drug content, viscosity, pH and stability. It results, *P. wallichiana* has more anti-fungal potential on *A. flavus* as compared to *A. niger.* It might be due to destruction of cell wall or nucleic acid of fungi. In terms of pH, % drug content, and in-vitro drug release, all stability criteria were nearly the same after 1 month as they had been in prior evaluations. In conclusion, NE 5 was most prominent nano emulsion among other preparations. It also showed improved stability, maintaining its pH and medication release rate during a month of storage. It showed a remarkable anti-fungal potential against both the fungal species used. After in-vivo clinical study, this formulation could be used to treat and distribute drugs for conditions including cancer. Dermatitis, pruritis, and other epidermal illnesses can be treated with this.

Keywords: *Pinus wallichiana*, formulation, herbal, nano emulsion, FTIR.

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INTRODUCTION

Nano emulsions, liposomes vesicles, NLCs & SLNs are examples of lipid-based colloidal systems employed in skin delivery. These nano systems have the strength to facilitate BA and efficacy, target distribution to skin areas and follicles, improve active stability, and make lipophilic, poorly water-soluble chemicals easier to formulate (Roberts et al. 2017). Colloidal dispersions are developed by oil, water, surfactant & co-surfactant that are clear, monophasic, and optically isotropic with droplet size lower than 100nm & low PD (Nastiti et al. 2017).

Plant Profile

The Pinus species that are native to the Hindu Kush Mountains, Karakoram Mountains, and Himalayan Ranges. With an altitude range of 1800 metres to 4300 metres, this plant can be found in extensive and luxuriant growth all along the Himalayan ranges, starting in eastern Afghanistan and continuing through Pakistan, India, Nepal, Bhutan, Myanmar, and China (Ghimire et al. 2010). The plant loves to thrive in colder climates and can be found in areas with high altitudes and little rainfall or low altitudes with a lot of rainfall, either in the form of pure or mixed forests (Rahman et al. 2017). *Pinus wallichiana* is second only to deodar in terms of commercial importance and is mostly utilised for lumber. The heartwood is light pink to deep red with dark striations; it is resinous; the grain is straight and regular; it is medium fine in texture; it is soft; and it is heavy. The sap wood appears white. The wood has a calorific value of 4995, making it an excellent fuel source, and it also makes excellent charcoal. Furniture, window frames, planks, and other interior fixtures are common uses for the wood (Bhat et al., 2015). Timber isn't the only thing this plant is good for; oleoresins extracted from the plant can also be used to create turpentine oil, needle oil, and camphor (Aslam et al., 2011).

Taxonomy

Subclass: Pinidae
Order: Pinales
Family: Pinaceae
Genus: *Pinus*
Species: *wallichiana*

With a straight trunk and small, downcurved limbs that are longer in solitary trees, Pinus wallichiana grows to a height of more than 50 metres, giving the tree a dome-like appearance. When young, the bark of the trunk is smooth and resinous. As it ages, the bark becomes corky and grey with shallow fissures. Five leaves or needles per fascicle, the leaves or needles range in length from 10 to 20 cm and are blue green in colour. At maturity, female cones are very resinous and pale brown. 20 cm to 30 cm long female cones are found in groups of one to six. The cone is upright when young, turning pendent as it matures, and it is light brown in colour. The colour of the apophysis is light brown. The pollen sacs on the lower surface of each of the male cones' ovoid or globose, short-lived scales or microsporophylls. The male cones have dimensions of 8–10 x 3 mm. Ovoid and 6-10 x 5-6 mm in size, seeds have membrane wings that are 1.5 to 3 cm long (Sharma et al.2018).

Fig 1. Leaves and fruits of *Pinus wallichiana* plant

The plant is also extremely valuable medicinally to the various ethnic groups residing in the Himalayan region. The plant's the resin and burned wood known as Kaalo are both used as antiseptics. 7The resin is applied topically to treat cuts and wounds, and it is combined with honey to treat gonorrhoea, abscesses, and wound healing. The resin can also be used to treat cuts and wounds and is combined with onion paste to cure chafing of the heels (Balodi et al. 2018).

Chemical constituents

Researchers from diverse groups have thoroughly analysed the chemical components of different Pinus wallichiana plant parts using various extraction and detection methods and solvents. The primary chemical identified in turpentine and pine needle essential oils is a terpene (Coppen et al., 1988). The alcoholic extract of various plant parts contains a wide variety of chemicals, such as hydrocarbons, terpene acids, organic acids, flavonoids, flavonoid glycosides, and terpene alcohols.

It has proved for following chemical constituents-

- α-Pinene

- β- Pinene
- Myrcene
- A- Terpineol
- Caryophyllene Oxide
- Trans Caryophyllene
- Limonene
- α- Cadinol
- Camphene
- α- Terpinyl Acetate
- Geranyl acetate
- Isorhamnetin
- Quercetin
- Kaempferol
- Rhamnetin
- Myricetin

Since above literature survey, I found that nano emulsion of ethanolic leaves extract of *P. wallichiana* has not been formulated and tested for anti-fungal activity using prescribed protocols and methods. Therefore, this research focuses on development and characterization of *P. wallichiana* based nano emulsion used against fungal infection.

MATERIALS AND METHODS

Experimental requirements

Ethanolic leaves extract of *P. wallichiana*, surfactant- Tween 80 (Sigma Aldrich, India), ethanol, Isopropyl alcohol, iso propyl myristate, pot. Bromide, distilled water, phosphate buffered solution and pH 7.2.

Collection, Identification & Authentication of plant

The leaves of *P. wallichiana* were collect from the local region in Uttarakhand. It was identified and authenticated by a botanist. It was washed, dried under shade, and sieved for making dust-free and kept at room temperature or shade.

Extraction of plant

The powder of *P. wallichiana* were weighed and extracted through Soxhlet apparatus using ethyl alcohol. After, it was filtered with whatman filter paper to get the extract in homogenous manner. A rotating evaporator was used to dry the brownish, semisolid extract obtained under partial vacuum. The yield of the extract was calculated in percentage (Khan et al. 2028).

Pre-formulation study

In order to formulate a stable, safe, and therapeutically effective and efficacious dosage forms, pre-formulation investigations are conducted prior to formulation development and focus primarily on characterizing the pharmacological substance.

Following objectives of the pre-formulation studies before product development are:

- To check the important physicochemical nature of the drug.
- To determine the drug compatibility with different excipients used in the formulation.

❖ Extract and excipients compatibility

To check for any changes in the *P. wallichiana* chemical composition following its combination with the other polymers. The herbal extract mixed with KBr was applied and pressed into the shape of a disc. The disc was examined using Shimadzu FTIR spectroscopy (4000-400/cm).

❖ Solubility

The solubility of the herbal extract was determined by placing a small amount of it (about 1-2mg) individually in a test tube, adding 5ml of solvent (water, ethanol, PEG, 0.1N HCl, chloroform & phosphate buffer), shaking vigorously, and holding for a while. Take note of the product's solubility in various solvents when it is at room temperature.

Preparation of formulation

The nano emulsion was prepared by dissolving *P. wallichiana* extract in distilled water at different quantity. Tween 80, Isopropyl alcohol, iso propyl myristate at different % w/v) is added drop-wise to the obtained mixture with continuous stirring on vortex mixer. Heat is avoided during formulation. The produced nano emulsion was maintained in an airtight container at room temperature and its physical stability was determined by monitoring for the occurrence of phase separation at regular intervals.

Table 1. Ingredients of herbal nano emulsion

Formulation	Ingredients (% w/v)				
	Herbal extract	Tween 80	Isopropyl alcohol	iso propyl myristate	Distilled water
NE 1	0.0	25	25	44	5.5
NE 2	0.5	25	25	44	5.5
NE 3	0.5	25	40	16	25
NE 4	0.5	15	40	1	15
NE 5	0.5	30	15	50	5
NE 6	0.5	25	20	45	10

Characterization parameters

Physical appearance

There will be a total of six different Nanoemulsions created (designated NE1 through NE6), and they will all be evaluated based on how they look to the naked eye. A superior nanoemulsion formulation will result in a homogenous nanoemulsion.

Droplet size & Polydispersity index (PDI)

Zetasizer Nano-ZS (Malvern Instrument, UK) was be used to determine the typical globule size of the nanoemulsion. Taken at 25 degrees Celsius, measurements are at a 90-degree angle. Light scattering intensity was kept within the instrument's detection range by diluting the nanoemulsion with twice-distilled water. A constant 25 degrees Celsius is used for all measurements. The formulation's polydispersity index can be determined with the same tool. Polydispersity index (Samadhan et al., 2019) shows how broad the size distribution is.

In vitro drug release

A little quantity of the nanoemulsion can be dissolved in a PBS solution with a pH of 7.4. The polymer is made soluble (pH 7.4) by adding the solvent ethanol, and the resulting volume is then raised up to 100 ml with PBS. After then, 1 ml is removed from the solution and it is diluted again till there is 10 ml total. The absorbance of the solution is measured at 270 nm in order to calculate its concentration.

For the in vitro diffusion study, a modified Franz diffusion cell was utilized. A glass cylinder 10 cm in height, 3.7 cm in diameter at the top, and 3.1 cm in diameter at the bottom was used as the difusion cell. One end of the cylinder was fitted with shep mucosa to act as a diffusion cell. One milliliter of the nanoemulsion was added to the cell, and the cell was then put in the receptor section of a beaker containing one hundred milliliters of phosphate buffer with a pH of 6.8. While being magnetically churned, the receptor compartment was in contact with the entire cell surface and kept at 37 degrees Celsius. The sink condition was maintained by removing 10 ml from the receptor compartment and replacing it with the same volume.

% Drug content

A measured volume of the formulation is transferred to a 10 ml volumetric flask and diluted with ethanol. For maximal absorbance at 240 nm, the resultant solution is sonicated for three minutes at room temperature before being compared to a blank (Samadhan et al. 2019).

Viscosity

A nanoemulsion's viscosity is measured by a Brookfield viscometer at 23 degrees Celsius to 2 degrees Celsius room temperature. In order to get an accurate reading of the viscosity, it is necessary to perform three experiments at two different spindle speeds (Makhmalzadeh et al., 2012).

pH

Nanoemulsion evaluation is incomplete without determining pH. The excipients included in the formulation determine the pH of the final preparation and, by extension, the route of administration. The pH of the compound is measured with a digital pH meter. The results were recorded in triplicate to cut down on inaccuracies (Derie et al., 2008).

Stability

The nanoemulsion's stability is tested by keeping it at 37 degrees Celsius for one month. The sample is used to evaluate the degree of openness. The medication content and pH should be monitored monthly for three months (Jufri & Natalia, 2014).

Screening of anti-fungal activity

For 24 hours, various fungal species were kept at 37 degrees Celsius in an incubator. They were then placed in the fridge at 4 degrees centigrade for storage. In this study, we used the cylinder plate or cup-plate method to conduct qualitative screening for antifungal susceptibility.

Cup-plate or cylinder-plate technique: The aseptic area with the Ultra-Violet laminar air flow was where all the sanitized materials were stored. The petri dishes were then inoculated with fungus suspensions of 3ml. When the nutrient agar reached 50 degrees Celsius, 20 milliliters of media were placed into the petri dishes holding the bacterial or fungal suspension, and the dishes were rotated to thoroughly combine the suspension with the media. A sterile 8-mm borer was used to create holes in the plate once the agar had set. Each plate had four holes drilled into it. One is for adding the standard, another is for adding the control, and the two remaining holes are for adding samples of the same concentration. In each tube, 0.1 mL of the material was deposited. After a three-hour period of room temperature incubation to promote diffusion, the plates were placed upright in a 37° C incubator to promote fungal growth for a further twenty-one hours. Each treated plate had its zone of inhibition for bacterial growth precisely determined. At each concentration, the test solution's ability to inhibit fungal growth was compared to that of the standard (Rahman et al. 2016).

RESULTS AND DISCUSSION

Pre-formulation studies

Solubility

The following solvents were tried to see if the herbal extract will dissolve in them. Distilled water was found to have low solubility for P. wallichiana. Isopropyl alcohol, ethanol, Tween 80, and isopropyl myristate were all found to dissolve it without any difficulty. Therefore, it may provide further evidence that it is more soluble in amphoteric solvents in a lipoidal environment.

Table 2. Solubility of herbal extract

Solvent	*P. wallichiana* extract
Distilled water	Poor Soluble
Isopropyl alcohol	Freely Soluble
Ethanol	Freely Soluble
Iso propyl myristate	Freely Soluble
Tween 80	Freely Soluble

Extract-polymers compatibility

P. wallichiana extract was also tested for drug-excipients compatibility studies using FTIR spectrum as single once and in formulation to compare.

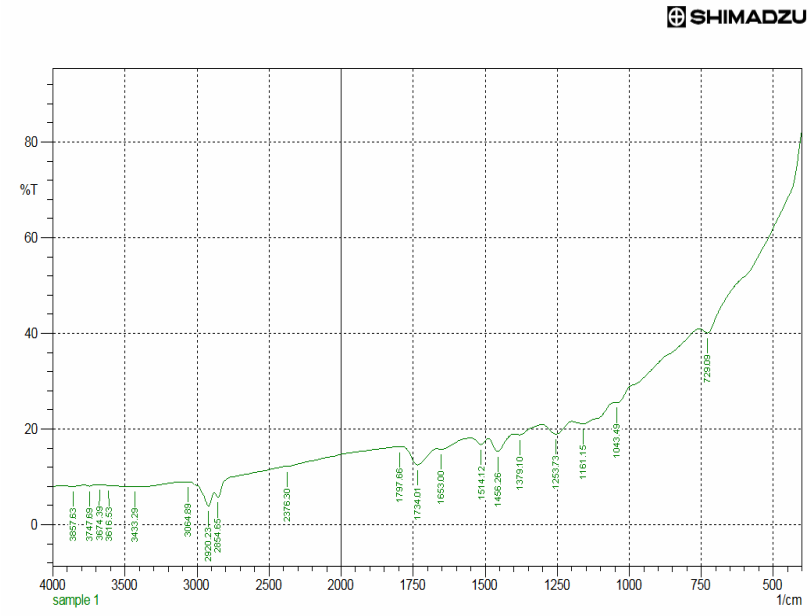

Fig 2. FTIR representation of *P. wallichiana* extract

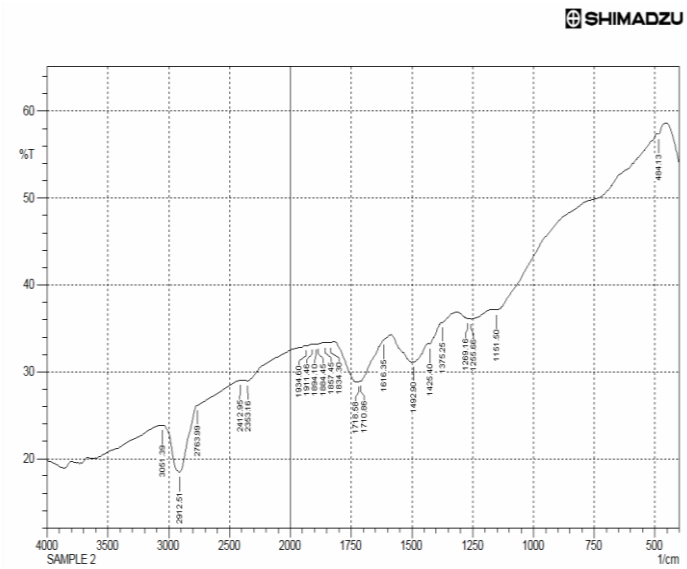

Fig 3. FTIR representation of herbal extract + Tween 80

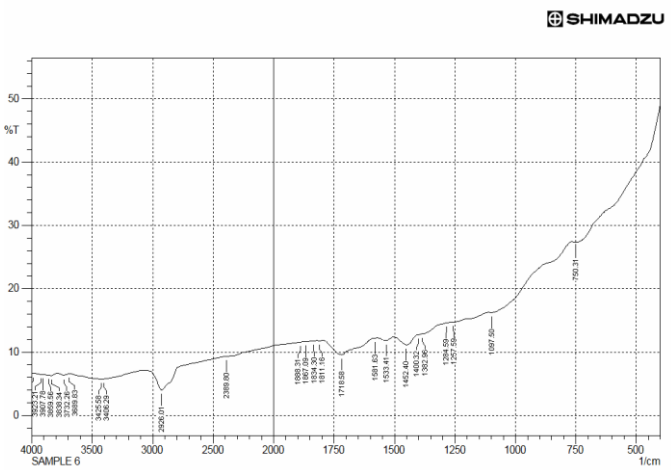

Fig 4. FTIR representation of herbal extract + Isopropyl alcohol

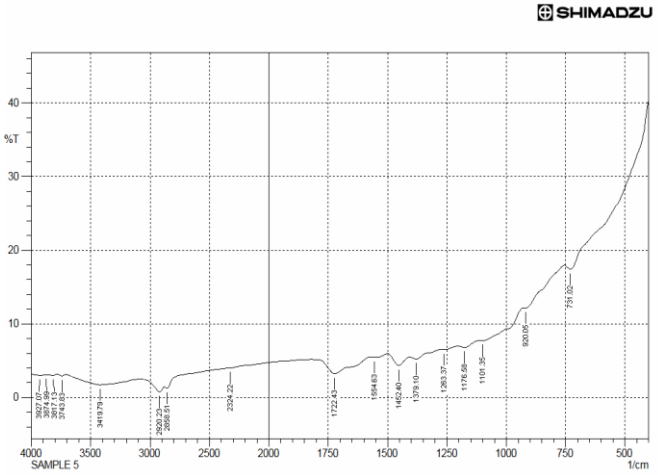

Fig 5. FTIR representation of herbal extract + Iso propyl myristate

Better drug-excipient compatibility has been established due to the lack of spectral shifts or functional peak losses between the drug and herbal nano emulsion.

5.2 Standard calibration curve- herbal extract

UV Spectrophotometry was used for the study of P. wallichiana. The absorbance of the medication in phosphate buffered saline (pH 7.4) containing a small amount of methanol was measured at 274 nm. The standard curve for the herbal extract in PBS at pH 7.4 was linear between 2 and 10 g/ml, starting at the origin. It is clear that the curve follows Beer-Lambert's law.

Table 3. Standard calibration curve- *P. wallichiana* extract

Conc. (µg/ml)	Absorbance
10	0.14
20	0.23
30	0.29
40	0.34
50	0.41
60	0.49

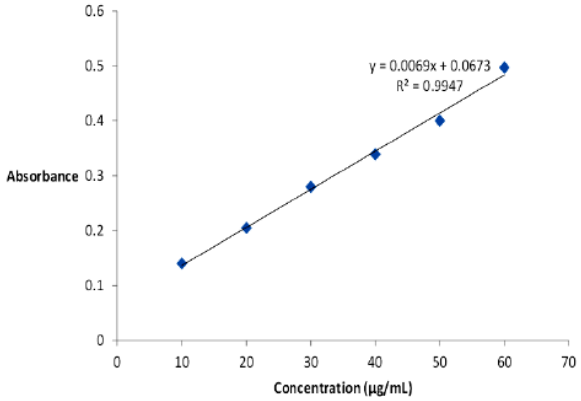

Fig 6. Standard calibration curve of *P. wallichiana* extract at pH 7.4

EVALUATION

Physical appearance

In order to characterize total 6 forms of Nano emulsion, NE 0- NE 6 were evaluated for their physical appearance i.e., homogenous/heterogenous or colour. It showed that NE 1- NE 6 found as homogenous in appearance. They all were brown in colour that might be due plant.

Table 4. Physical appearance of formulated nano emulsions

Nano emulsion	Physical appearance
NE 1	Brown & homogenous
NE 2	Brown & homogenous
NE 3	Brown & homogenous
NE 4	Brown & homogenous
NE 5	Brown & homogenous
NE 6	Brown & homogenous

Determination of droplet size

Nano emulsion's droplet size was estimated using nanodroplet analyser. Formulation NE 1, NE2 showed almost identical droplet size as 21.39±0.21nm, 22.18±0.17nm, respectively. However, NE3, NE4 exhibited droplet size as 23.22±0.15nm and 23.12±0.42nm, respectively. Nano emulsion NE5 showed optimum droplet size as 24.31±0.21nm.

Table 5. Determination of droplet size (nm)

Nano emulsion	Droplet size (nm)
NE 1	21.39±0.21
NE 2	22.18±0.17
NE 3	23.22±0.15
NE 4	23.12±0.42
NE 5	24.31±0.21
NE 6	22.51±2.13

Determination of Polydispersity Index

When assessing the particle homogeneity of topical dosage forms, polydispersity index is a crucial metric to consider. In NE1, NE2, NE 3, NE4 and NE6 the PDI was estimated as 0.47±0.04, 0.49±0.03, 0.46±0.07, 0.48±0.01 and 0.45±0.02, respectively.

But, PDI was observed highest in the formulation NE5 as 0.51±0.03. Possible explanation: more surfactants were utilized than usual in the formulation of the nano emulsion.

Below table depicts the PDI of NE as below-

Table 6. Determination of Polydispersity Index (PDI)

Formulation	PDI
NE 1	0.47±0.04
NE 2	0.49±0.03
NE 3	0.46±0.07
NE 4	0.48±0.01
NE 5	0.51±0.03
NE 6	0.45±0.02

***In-vitro* drug release**

In vitro drug release was measured, and NE1 had the lowest value at 82.170.15. Drug release was measured in vitro and found to be 83.470.35, 85.120.37, 86.390.58, and 84.530.72 for NE 2, NE3, N34, and NE 6.

NE5 showed the highest drug release, at 89.190.21. When compared to other formulations, its in vitro drug release is the most effective.

Table 7. *In-vitro* drug release

Formulation	*In-vitro* drug release (%)
NE 1	82.17±0.15
NE 2	84.62±0.61
NE 3	83.17±0.20
NE 4	86.20±0.29
NE 5	89.19±0.21
NE 6	87.10±0.25

Determination of % Drug content

Better medication homogeneity and concentration were observed in the generated nano emulsions. The formulation NE1, NE2, NE3, NE4 and NE 6 were estimated as 97.12±0.30%, 96.29±0.16%, 95.43±0.19%, 96.50±0.32% and 97.21±0.39%, respectively.

But nano emulsion F5 exhibited the drug content % as 98.18±0.16% that was greatest in every formulation. A facilitated % drug release is the sign of better uniformity of the formulation.

Table 8. Determination of % Drug content

Formulation	% Drug content
NE 1	97.12±0.30
NE 2	96.29±0.16
NE 3	95.43±0.19
NE 4	96.50±0.32
NE 5	98.18±0.16
NE 6	97.21±0.39

Determination of viscosity

Viscosity demonstrates as strength of formulations that facilitates the absorption and thus bioavailability of drugs incorporated in dosage forms. The formulation NE 1, NE 2, NE 3, NE 4 and NE 6 showed increased viscosity as 517.19±023, 524.21±0.34, 527.49±0.16, 532.17±0.32 and 535.18±0.21, respectively. Highest viscosity was estimated in NE 5 as 536.42±0.20.

Table 9. Estimation of viscosity

Formulation	Viscosity
NE 1	517.19±023
NE 2	524.21±0.34
NE 3	527.49±0.16
NE 4	532.17±0.32
NE 5	536.42±0.20
NE 6	535.18±0.21

Measurements of pH

The pH was measured for better tolerability and absorption property. The pH range for each formulation was in slight acidic environment. The NE1, NE3 and NE5 exhibited pH ranges of 6.3±0.2, 6.4±0.3 and 6.2±0.1, respectively.

Table 10. Measurements of pH range

Formulation	pH range
NE 1	6.3± 0.2
NE 2	6.6± 0.2
NE 3	6.4± 0.3
NE 4	6.8± 0.4
NE 5	6.2± 0.1
NE 6	6.8± 0.2

Estimation of % Drug release

At 6 hours, the % drug release from formulations F1, F2, F5, and F6 was 92.30.7, 86.70.4, 73.90.8, and 74.50.8, respectively. F3 and F4 demonstrated the lowest drug release percentages (80.4% and 75.8%, respectively) among the six formulations tested.

Table 11. Estimation of % Drug release

Time (hr)	% drug release					
	NE 1	NE 2	NE 3	NE 4	NE 5	NE 6
1	24.3±0.1	25.1±0.3	23.1±0.3	26.5±0.2	29.1±0.4	32.1±0.7
2	28.3±0.2	30.1±0.4	33.6±0.2	29.3±0.2	31.4±0.7	33.3±0.8
3	35.1±0.5	37.8±0.2	39.1±0.4	37.2±0.6	34.2±0.5	49.1±0.4
4	45.1±0.6	47.8±0.3	46.1±0.5	43.3±0.4	47.2±0.3	48.1±0.7
5	69.2±0.5	65.2±0.3	66.2±0.6	68.1±0.5	71.8±0.2	73.3±0.4
6	79.4±0.4	83.4±0.2	85.2±0.6	83.2±0.9	86.4±0.1	87.1±0.3

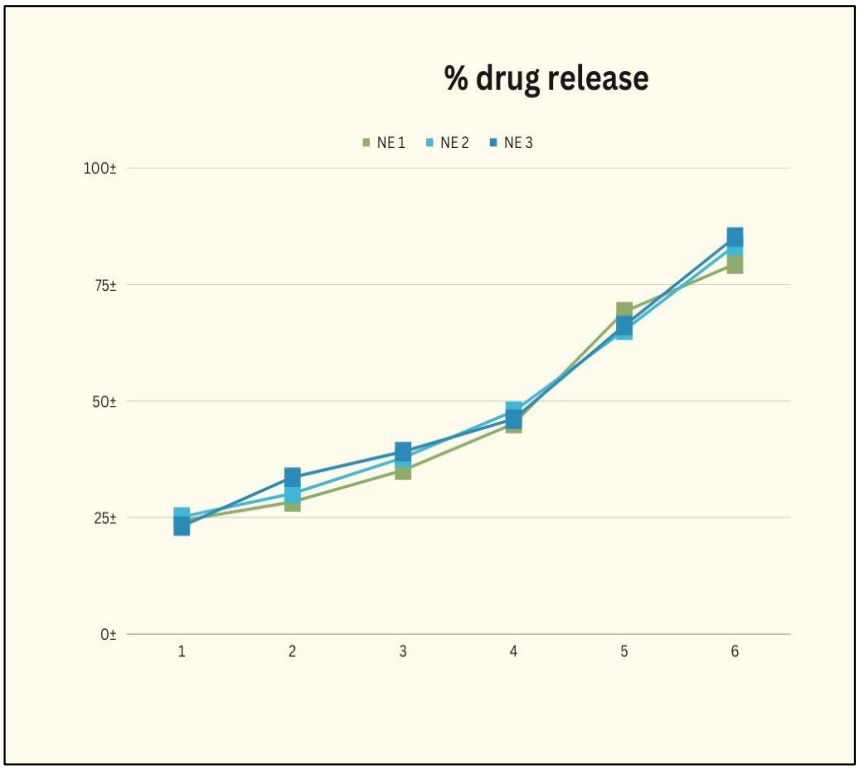

Fig 7. % drug release (NE 1, NE 2, NE 3)

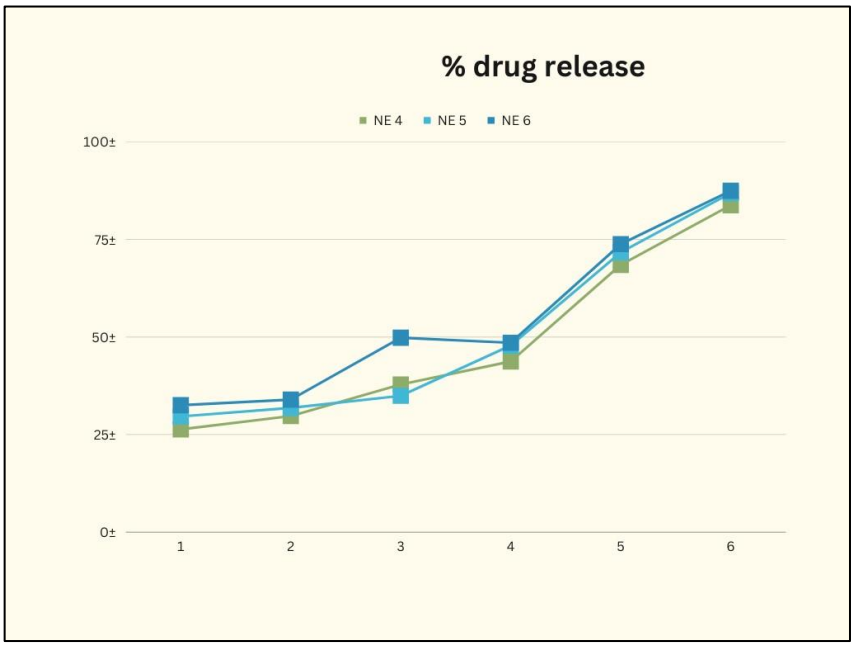

Fig 8. In-vitro drug release (NE 4, NE 5, NE 6)

Stability data

The stability tests were performed after 30 days to confirm its actual property in terms of % drug content, and pH range. The formulation NE1, NE2, NE3, NE4 and NE 6 were estimated as 97.12±0.30%, 96.29±0.16%, 95.43±0.19%, 96.50±0.32% and 97.21±0.39%, respectively. But nano emulsion F5 exhibited the drug content % as 98.18±0.16% that was greatest in every formulation.

After 1 month, % drug content was partially changes in the F1 and F2 as 96.82±0.19% and 95.78±0.26%, respectively. Thus, it proves for its better stability.

Table 12. Stability of % drug content after 1 month

Formulation	**% Drug content**	
	before	***after***
NE 1	97.12±0.30	96.82±0.19
NE 2	96.29±0.16	95.78±0.26
NE 3	95.43±0.19	95.43±0.19
NE 4	96.50±0.32	96.50±0.32
NE 5	98.18±0.16	98.18±0.16
NE 6	97.21±0.39	97.21±0.39

At the time of preparation of formulation NE 1- NE 6, the pH was measured for better tolerability and absorption property. The pH was measured for better tolerability and absorption property. The pH range for each formulation was in slight acidic environment. The NE1, NE3 and NE5 exhibited pH ranges of 6.3±0.2, 6.4±0.3 and 6.2±0.1, respectively.

After 1 month, a negligible alteration was seen in formulation NE1 and NE3 as 6.4±0.2 and 6.6±0.3, respectively. It was found almost same that indicates for better stability profile.

Table 13. Stability of pH after 1 month

Formulation	**pH**	
	before	**after**
NE 1	6.3±0.2	6.4±0.2
NE 2	6.6±0.2	6.5±0.2
NE 3	6.4±0.3	6.6±0.3
NE 4	6.8±0.4	6.5±0.3
NE 5	6.2±0.1	6.7±0.2
NE 6	6.8±0.2	6.7±0.1

Screening of anti-fungal activity

Two species of fungus i.e., *A. niger*, *A. flavus* were taken in the study. In NE 1, NE 2 and NE 3 the antifungal response was recorded as 4.31, 4.63 and 5.42, respectively. Moreover, same formulations i.e., NE 1, NE 2 and NE 3 were showed anti-fungal potential as 6.21, 7.27 and 7.62, respectively.

It can be said that *P. wallichiana* has more anti-fungal potential on *A. flavus* as compared to *A. niger*. It might be due to destruction of cell wall or nucleic acid of fungi.

Table 14. Antifungal activity of nano emulsion

Formulation	Anti-fungal activity	
	A. niger	***A. flavus***
NE 1	4.31	6.21
NE 2	4.63	7.27
NE 3	5.42	7.62
NE 4	4.17	6.39
NE 5	5.42	7.10
NE 6	5.81	6.81

Nano emulsion was found to have remarkable properties as a kind of formulation across the board. The emulsions appeared to have improved clarity and transparency, which was a positive sign.

Better drug release and bioavailability of the integrated drug were observed, confirming the formulation's novel particle size and particle size index. Nano emulsion quality is mostly determined by its in-vitro drug release and viscosity. Both of these variables displayed the upbeat behaviour of NE 1-NE 6 in the same setting.

In terms of pH, % drug content, and in-vitro drug release, all stability criteria were nearly the same after 1 month as they had been in prior evaluations. As drug carriers for enhancing the delivery of pharmaceutical active ingredients, nano emulsions are gaining popularity because they provide a number of benefits for the delivery of pharmaceuticals. They are adaptable to practically all distribution methods and consequently show potential in a variety of industries, including biotechnology, cosmetics, and medicines. This novel technology might be created to overcome some phytopharmaceuticals' low absorption and poor miscibility with the lipids found in cell membrane linings.

CONCLUSION

To fight the late and general consequences on the lives of millions, it would be a tremendous step to go towards ayurvedic medications. It may also be improved so that manufacturing it would be affordable and have a considerable amount of utility. It would be very beneficial for the stability of the herbal nano emulsion. By inhibiting bacterial development and multiplication, it will be simpler to deliver the long-lasting impact.

In conclusion, NE 5 was most prominent nano emulsion among other preparations. It also showed improved stability, maintaining its pH and medication release rate during a month of storage. It showed a remarkable anti-fungal potential against both the fungal species used.

Future aspects

Herbal extract nanoemulsion development may have far-reaching implications for the treatment of bacterial infections and other stability criteria.

After in-vivo clinical study, this formulation could be used to treat and distribute drugs for conditions including cancer. Dermatitis, pruritis, and other epidermal illnesses can be treated with this.

FUNDING

Nil.

CONFLIT OF INTEREST

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