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Research Article

FORMULATION AND EVALUATION OF MICROEMULSION-BASED SUBLINGUAL LIQUID CONTAINING VALERIAN ROOT EXTRACT

VIRASHRI M NORAJE, VIJAYANAND PUJARI*

Department of Pharmaceutics, Rani Chennamma College of Pharmacy, Belagavi, Karnataka, India. Email: viranoraje7698@gmail.com

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ABSTRACT

Objective: The objective of this work was to perform pre-formulation studies, prepare, and optimize microemulsion containing valerian root extract, and evaluate the prepared microemulsion liquid containing valerian root extract.

Methods: Valerian roots were subjected to extraction by maceration and percolation methods. The microemulsion formulation was prepared using different concentrations of peanut oil as an oil phase, Tween 20 as a surfactant, and span 80 as a cosurfactant. Pseudoternary phase diagrams were constructed to identify the microemulsion region, and a suitable composition was identified to formulate the microemulsion. The microemulsion was evaluated for viscosity, pH, staining test, globule size, zeta potential, and transmission electron microscopy.

Results: The extractive value of valerian root in 70% ethanol was the maximum (27.56±1.95% w/w). Based on the thin-layer chromatography experiment, the reported Rf value for valerenic acid is 0.48. Peanut oil showed the highest solubilization capacity for the drug, i.e., 7.00±0.02 mg/mL. The optimized F4 formulation showed viscosity 110±7.9cP, pH 6.22±2.00, globule size 96.78±10.9, and zeta potential +67.5±1.9. The staining test for the optimized formulation (F4) indicated that the emulsion is an zo/w type of microemulsion.

Conclusion: This novel delivery of the drug in the oral cavity may ensure the quick and full release of the drug without interfering with the food, pH, enzymatic degradation, and gastric motility. This formulation containing herbal drug may offer an alternative natural solution for the treatment of insomnia.

Keywords: Microemulsion, Valerian root extract, Insomnia, Sublingual liquid.

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INTRODUCTION

Microemulsions (MEs) are a novel drug delivery system that is thermodynamically stable and a clear isotropic mixture of oil, surfactant, and co-surfactant. Microemulsion plays an important role in improving the solubility as well as the bioavailability of a poorly soluble drug. The microemulsion can enhance oral bioavailability due to its capacity to increase the permeability and solubility of the drug in the whole area of the gastrointestinal tract. It also improves the delivery of the drug through dermal transport because of its better permeation rate. The droplet size of ME ranges from 10 to 100 nm [1-3].

Valerian continues to be a safe sedative/hypnotic choice for patients with mild-to-moderate insomnia [4]. The effective dosage of valerian root extract for the treatment of insomnia ranges from 300 to 600 mg. The product should be ingested 30 min to 2 h before bedtime [5-7].

Drug delivery through the oral mucous membrane is considered to be a promising alternative to the oral route. The sublingual dosage form is to be placed under the tongue and produces an immediate systemic effect by enabling the drug to be absorbed directly through the mucosal lining of the mouth beneath the tongue which is very rich in vascular blood supply. The sublingual route is useful when a rapid onset of action is desired with better patient compliance than orally ingested tablets. In terms of permeability, the sublingual area is more permeable than the vocal area and hence also than the palatal area. The absorption of drugs through the sublingual route is 3–10 times larger than that presented by the oral route and hence can be employed for emergency weather [8-10].

Nowadays, insomnia is a conspicuous problem or disease in our restless society. Recent statistics show that 10–30% of people across the world have insomnia, with being as high as 50–60% in some countries. Based on the current global population, up to 237 million people are affected [1]. In the clinical setting, one-fifth of patients attending general practitioners have been reported to be suffering from insomnia [2,3]. Insomnia is often defined by sleeping problems. People who suffer from insomnia may encounter difficulty getting to sleep or staying asleep, or having non-refreshing sleep, to some degree. Poor quality of sleep is naturally followed by functional impairment while awake [11,12].

Insomnia can be treated by synthetic medicines. Many use synthetic drugs such as benzodiazepines and newer non-benzodiazepines to get rest. These medicines have several problems and result in addiction after long-term use. Daytime fatigue and cognitive impairment are common side effects associated with these sedatives. On the other hand, insomnia can be treated with herbal remedies. Some insomnia patients are inclined to take medicinal plants owing to the low frequency of side effects. Several medicinal herbs have been used traditionally to induce sleep throughout the world [12,13].

METHODS

Plant material

Valerian wallichii roots were purchased from Girirajii collections in Chhattisgarh and authenticated from Agharkar Research Institute, Pune.

Other materials

Peanut oil (Adani Wilmar Ltd, Ahmedabad), Tween 20 (Visso Trading Company, Belgaum), Span 80 (Bugoyne Burbidges and Co. Mumbai), Ethanol (Seema chemicals, Bengaluru), Stevia extract (Krusexim, Ahmedabad), Peppermint oil (Indus Pvt. Ltd, New Delhi), Sodium benzoate, Methyl paraben, Ethyl acetate, Sulfuric acid, Dil hydrochloric

acid, Nitric acid, Ferric chloride, Chloroform, Lead acetate (Spectrum Reagents and Chemicals Pvt. Ltd.), Methanol (Finare Limited, Chacharwadi), Silica gel G (Nice chemicals [p]), Hexane (Visso Trading Company, Belgaum), Acetic anhydride (Universal Scientific Work, Bangalore),

Pre-formulation study

Macroscopic/organoleptic study

The macromorphological description refers to an evaluation of a drug by color, odor, taste, size, and shape. It is a technique of qualitative evaluation based on the study of morphology. The macroscopic study of the medicinal plant plays an important role in the standardization of drugs and is also helpful in the rapid identification of plant material [14-17].

Microscopical study

Microscopy mainly helps in the detection of adulterated substances and also helps in the confirmation of the purity of crude drugs. Powder microscopy is done to determine the type of cell present in the drug. The dried root was powdered and subjected to microscopic studies [14,18,19].

Extraction process

The root of the valerian was cleaned, and the dust particles were removed. It was dried and made into a coarse powder with the help of an electronic grinder, and the dried powder of the root material was further subjected to an extraction process using the percolation method. Valerian root is filled with a thimble. Then, a round bottom flask (RBF) is filled with solvent and placed on a heating mantle at ≤50°C. The solvent evaporates and condenses. Condensed solvents absorb the phytoconstituents from the powdered drug. Then, the solvent passed from the syphon tube to the RBF after 48 h. The solvent is collected and evaporated to dryness [14,20-22].

Physicochemical investigation

Determination of extractive value: valerian root in different solvents (70% ethanol, water, and methanol) was exhaustively subjected to extraction, and the obtained extract is being calculated for %yield [16,22].

$$\%\ \text{yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Determination of moisture content

The percentage of active chemical constituents in the crude drug is mentioned on an air-dried basis. Hence, the moisture content of a drug should be determined and should also be controlled. The moisture content of a drug should be minimized to prevent the decomposition of crude drugs, either due to chemical change or microbial contamination. The powdered root extract of valerian (W2) was placed in a weighed china dish (W1). The china dish was kept in a hot air oven at 105°C until the constant weight (W3) was achieved. Then, the sample was placed in a desiccator after it had achieved a constant weight and then weighed to determine the moisture content [14,15].

$$\text{Moisture content}\% = \frac{(W1 + W2) - W3}{W2} \times 100$$

W1 = Weight of china dish; W2 = Weight of sample; W3 = Weight of dried sample + Weight of china dish

Preliminary phytochemical screening

The plant contains a wide range of characteristic therapeutically important molecules in the form of secondary metabolites. Thus, a systematic preliminary phytochemical screening of plant material is essential for identifying plant constituents and establishing a chemical profile of the crude drug for its proper evaluation. Phytochemical extracts were subjected to preliminary screening using the standard procedure for identifying various phytoconstituents. Phytochemical screening tests were performed to determine the various phytoconstituents present in the valerian [21].

Identification of valerenic acid in the extract by thin layer chromatography (TLC) method

Applied the silica gel G as a stationary phase to the plate and dried it at room temperature. Then applied the test solution to the stationary plate and kept it in a mobile phase, then allowed the mobile phase to rise over the plate and then dried the plate at room temperature. Then spray with the anisaldehyde reagent solution prepared by mixing 0.5 mL of anisaldehyde, 10 mL of glacial acetic acid, 85 mL of methanol, and 5 mL of sulfuric acid added in the specified sequence. Heat the plate at 105°C for 10 min and examine it in daylight [14,15,22,23].

Stationary phase: Silica gel G, Mobile phase: a mixture of 65 volumes of hexane, 35 volumes of ethyl acetate, and 0.5 volumes of glacial acetic acid. Test solution: 0.5 g of coarse powder in 5 mL methanol sonicate for 10 min and filtered.

Screening of oil

Oils such as peanut oil, sunflower oil, coconut oil, and soybean oil were screened for their potential to solubilize the extract. The oil in which the solubility of the drug was more that oil is selected for further study.

Pseudoternary phase diagram

Pseudoternary phase diagrams were constructed using Ternaryplot.* The method used for the study was the water titration method. The surfactant and cosurfactant were mixed in a ratio of 1:1, 2:1, and 3:1, keeping the co-surfactant concentration constant and varying the surfactant concentration. The surfactant and cosurfactant were mixed in a ratio and vortexed for 5 min. Then, the oil was added to the surfactant and cosurfactant in a ratio ranging from 1:9 to 9:1, respectively. The water was added to the mixture drop by drop by burette, and then, the mixture was observed for any turbidity or gel formation. The point at which turbidity or gel formation is observed is considered as the end point of the titration. The data obtained were fed into the Ternary plot to obtain the area of emulsification [1,24,25]. *https://www.ternaryplot.com

Formulation of microemulsion (preparation of drug-loaded microemulsion)

The water titration method was employed for the preparation of the microemulsion. The concentrations of oil, water, surfactant, and cosurfactant were varied in each case, keeping the concentration of the drug constant. Surfactant and cosurfactant were added to an oily solution of the drug and mechanically stirred (with a magnetic stirrer) to form an emulsion. Water was added dropwise to the emulsion until it formed a transparent mixture. The formation of a transparent solution indicates the formation of a microemulsion [24,26]. The formulation chart for microemulsion is presented in Table 2.

Evaluation of microemulsion

Viscosity measurement

The viscosity of the microemulsion formulation was determined using a Brookfield Viscometer (RVDVE230). The Brookfield Viscometer

Table 1: Random chosen ratio for 1:1, 2:1, 3:1 for determination of droplet size

Sr. no	Ratio	Oil (mL)	Surfactant (mL)	Cosurfactant (mL)	Water (mL)
1	1:1	20.90	25.48	25.48	28.13
2	2:1	20.62	37.82	18.91	22.65
3	3:1	20.75	42.36	14.12	22.77

Table 2: Formulation of microemulsion

Sr. no	Concentration of oil (%)	Concentration of surfactant (%)	Concentration of cosurfactant (%)	Concentration of water (%)	Concentration of drug (%) per mL
F1	12.24	52.51	17.50	17.74	30 mg
F2	14.10	49.88	16.62	26.47	30 mg
F3	15.28	47.43	15.81	23.47	30 mg
F4	17.28	44.99	14.99	21.13	30 mg

consists of a cup, which is stationary, and a spindle, which is rotating. Different-sized rotating spindles are used and immersed in the test material. Rotate the spindle in the microemulsion till we get a constant reading on the display of the viscometer. This procedure is repeated 3 times for reproducible results [26].

Measurement of pH

The pH values of the microemulsion formulation were measured by immersing the electrode directly into the dispersion using a calibrated pH meter [27].

Droplet size determination

The size analysis of the microemulsion was carried out by dynamic light scattering with a Zetasizer (Nanotrac Particle Size Analysis). Samples were placed in the sample cell, and droplet size analysis was carried out for optimized microemulsion formulation [25,26].

Zeta potential

Zeta potential is the charge that develops at the interface between a solid surface and its liquid medium. This potential, which is measured in millivolts (mV). The zeta potential is mostly used to check the dispersion stability of a sample. The surface charge of the ME is measured by the zeta sizer [26,28].

Phase separation test

Microemulsion systems were subjected to centrifugation at 3000 rpm for a period of 2 h and examined for any evidence of phase separation [27,28].

Transmission electron microscopy (TEM)

The morphology of the valerian extract microemulsion was also observed by transmission electron microscopy. One drop of the sample was deposited on a polyvinyl acetate-coated copper specimen grid and allowed to stand for 10 min, after which any excess fluid was removed with filter paper. The grid was later stained with one drop of 3% phosphotungstic acid and allowed to dry for 5 min before the examination [29-31].

Staining test/dye solubility test

A water soluble dye and a methylene blue solution of 10 μL were added to the microemulsion. If the continuous phase is water (o/w emulsion), the dye will dissolve uniformly throughout the system. If the continuous phase is oil (w/o emulsion), the dye will remain as a cluster on the surface of the system [27,32].

Stability study

The optimized formulation (F4) was stored for 3 months in a tightly closed container at room temperature away from light. At the end of the 1st, 2nd, and 3rd months, the formulation was subjected to various tests like globule size, viscosity, and pH. The procedures employed for the study were identical to those described before. The results are shown in Table 16.

RESULTS

Macroscopic/organoleptic study

Macroscopic and organoleptic studies were performed on the raw root of valerian. The results are shown in Table 3.

Table 3: Macroscopic/organoleptic properties of the valerian root

S. no	Characters	Observation
1	Color	Root-Yellowish brown
2	Odor	Characteristics, Bitter
3	Taste	Bitter
4	Size	1mm in diameter and 3–5 cm in length
5	Shape	cylindrical

Table 4: Extractive values

S. no	Parameters	% yield (%W/W) (± S.D)
1	70% Ethanol soluble extractive value	27.56±1.92
2	Water soluble extractive value	20.79±1.42
3	Methanol soluble extractive value	13.97±1.56

Table 5: Phytoconstituents

S. no	Phytoconstituents	Result
1	Alkaloids	+
2	Reducing sugar	+
3	Tannins	+
4	Flavonoids	+
5	Terpenoids	+

+ = positive

Microscopic studies

Microscopic studies were conducted on powdered valerian root. The results of the study showed the presence of (A) scleroids, (B) round starch granules, (C) lignified large polygonal cells, (D) scalariform vessels, and (E) brown content. The results are shown in Fig. 1.

Determination of extractive value

After the extraction process, the obtained extract was calculated for %yield. The %yields were reported in Table 4 and Fig. 2.

Determination of moisture content

Humidity is the main factor responsible for the degradation of samples. Low moisture content is always desirable for greater drug stability. In the present study, the moisture content of valerian root extract was determined and calculated. The standard limit for the moisture content of valerian root extract should not be more than 12% w/w, and in the present study, the moisture content of the valerian root extract, in the sample, was found to be in the range of 9% w/w.

Preliminary phytochemical screening

The extract contains alkaloids, reducing sugar, tannins, flavonoids, and terpenoids. The results of the preliminary phytochemical screening are expressed in Table 5. The root of Valeriana wallichii contains alkaloids, tannins, flavonoids, terpenoids, and glycosides in the 70% ethanolic, extract and it was used for pharmacognostical, phytochemical evaluation.

Identification of valerenic acid in the extract by the TLC method

On the basis of the experiment, hexane, ethyl acetate, and glacial acetic acid in the ratio of 65:35:0.5 were selected as solvent systems for the

preparation of TLC. Based on the experiment, the sample observed Rf values are 0.16, 0.48, 0.65, and 0.84.

Screening of oil

To screen appropriate oils for the preparation of microemulsions, the solubility of valerian extract in various oils was carried out. The oils used for the study were peanut oil, sunflower oil, coconut oil, and soyabean oil. The solubility was found to be 7.00±0.02 mg/mL in peanut oil, 6.48±0.16 mg/mL in sunflower oil, 4.44±0.21 mg/mL in coconut oil, and 2.38±0.05 mg/mL in soya bean oil. From Table 6, it was clear that peanut oil solubilizes the maximum amount of the drug. The screening oils are represented in Table 6 and Fig. 3.

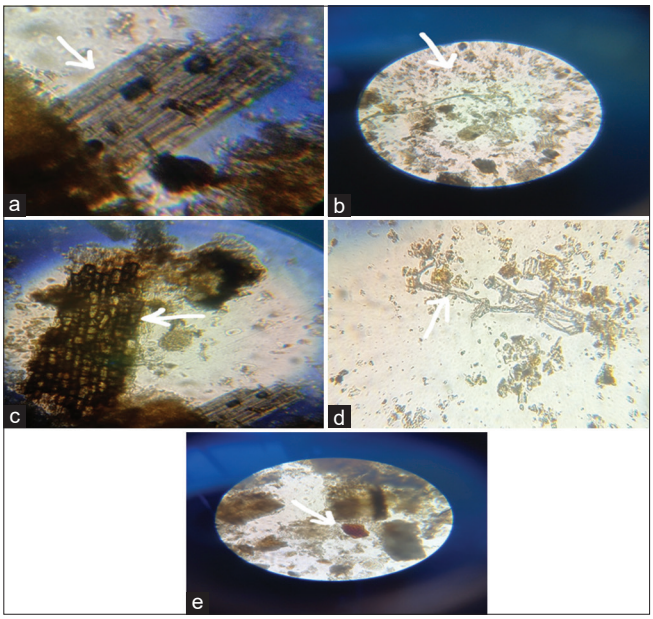

Fig. 1: (a-e) microscopic studies

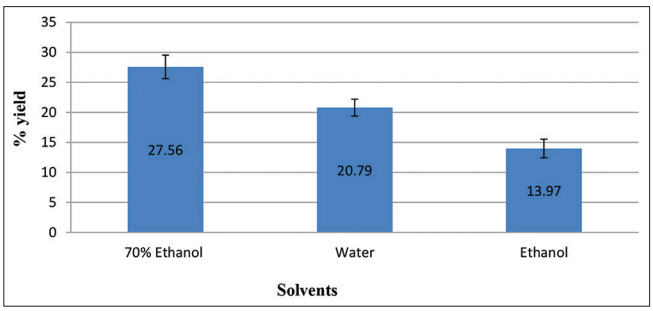

Fig. 2: Extractive value

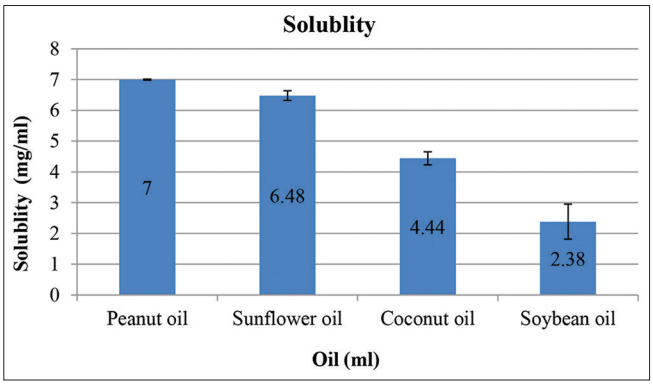

Fig. 3: Screening of oil

Pseudoternary phase diagram

The construction of phase diagrams makes it easy to find the concentration range of components for the existence range of microemulsions. The selected oil, surfactant, and cosurfactant were used to construct the phase diagram. The method used for microemulsion is the water titration method. All possible regions for emulsion formation at all possible ratios of surfactant: cosurfactant:oil was represented in Tables 7-9. Based on the solubility study, the oil with the highest solubility was selected, and then for the construction of the pseudoternary phase diagram, the ratio of cosurfactant was kept constant, but the ratio of surfactant varied from 1:1, 2:1, and 3:1. The oil was added to each ratio of surfactant and cosurfactant in varying quantities from 1:9 to 9:1. The water was added drop-wise through the burette until turbidity was observed. The microemulsion region in the phase diagram is the region where a clear and transparent formulation was produced. The results of the pseudoternary phase diagram are shown in Figs. 4-6.

Table 6: Screening of oils

S. no	Oil	Solubility (mg/mL)
1	Peanut oil	7.00±0.02
2	Sunflower oil	6.48±0.16
3	Coconut oil	4.44±0.21
4	Soybean oil	2.38±0.57

Table 7: Pseudoternary phase diagram for surfactant and cosurfactant ratio 1:1

Oil (%)	Smix (%)	Water (%)
10	90	34.4
20	80	33.8
30	70	33.1
40	60	32.8
50	50	32.3
60	40	31.9
70	30	31.1
80	20	30.8
90	10	30.1

Table 8: Pseudoternary phase diagram for surfactant and cosurfactant ratio 2:1

Oil (%)	Smix (%)	Water (%)
10	90	26.4
20	80	26.1
30	70	25.7
40	60	25.1
50	50	24.9
60	40	24.3
70	30	23.7
80	20	22.9
90	10	22.1

Table 9: Pseudoternary phase diagram for surfactant and cosurfactant ratio 3:1

Oil (%)	Smix (%)	Water (%)
10	90	20.2
20	80	19.7
30	70	19.3
40	60	18.9
50	50	18.2
60	40	17.7
70	30	17.1
80	20	16.5
90	10	16.3

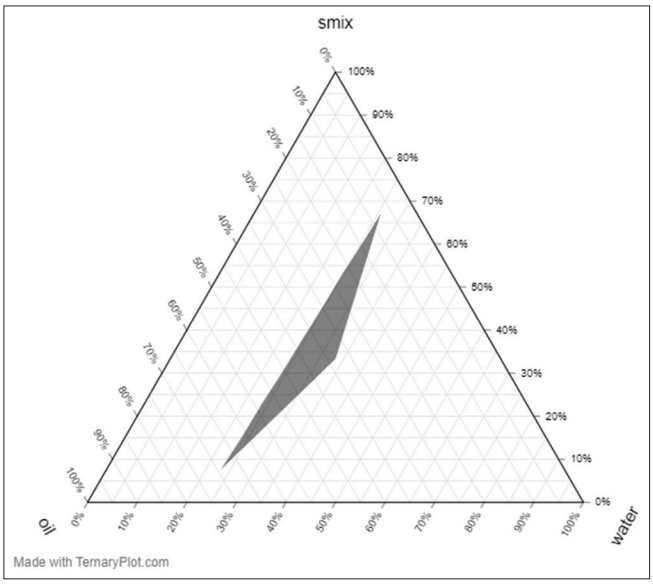

Fig. 4: Pseudoternary phase diagram for Smix 1:1

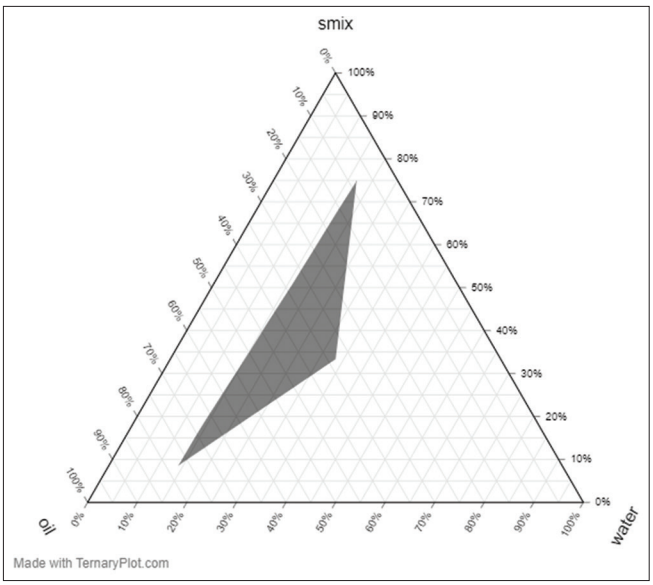

Fig. 6: Pseudoternary phase diagram for Smix 3:1

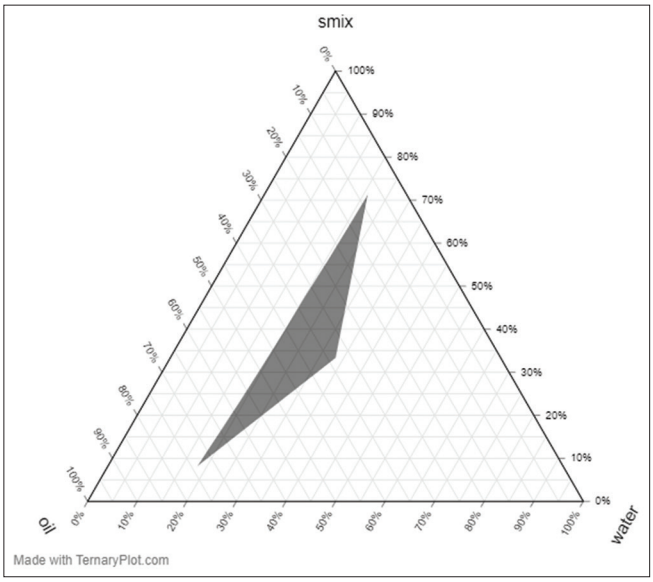

Fig. 5: Pseudoternary phase diagram for Smix 2:1

The region shaded black in the phase diagrams was considered the microemulsion region, as shown in Figs. 4-6. The rest-phase diagram region corresponds to turbid and conventional emulsion systems. The effect of water concentration on the area of isotropic regions was evident in the given phase diagrams. The area of microemulsion increased as the concentration of surfactant increased, possibly because tween 20 (polysorbate 20) is a nonionic solvent that forms a clear solution in water, so the area of o/w ME increased. The largest microemulsion region was obtained for the surfactant: cosurfactant ratio of 3:1, and the smaller microemulsion area was obtained for the ratios of 1:1 and 2:1.

The ratio from the pseudo-ternary phase diagram Smix (1:1), (2:1), and (3:1) was used for the determination of droplet size using a compound microscope (10×) and stage micrometer, as shown in (Figs. 7-9). The size of the 100 droplets was noted, and the standard deviation was calculated.

Average droplet sizes obtained for the 1:1, 2:1, and 3:1 ratios are presented in Table 10 and Fig. 10.

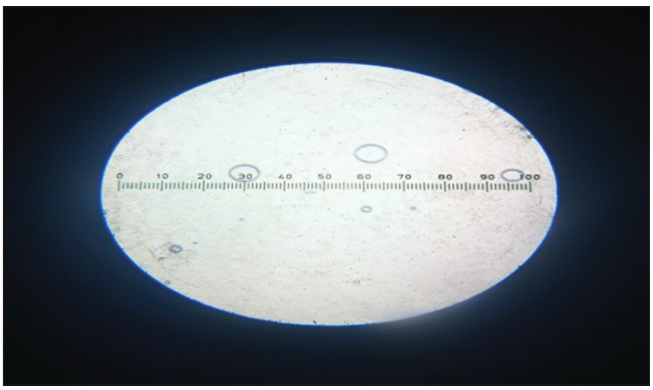

Fig. 7: Determination of droplet size for Smix 1:1

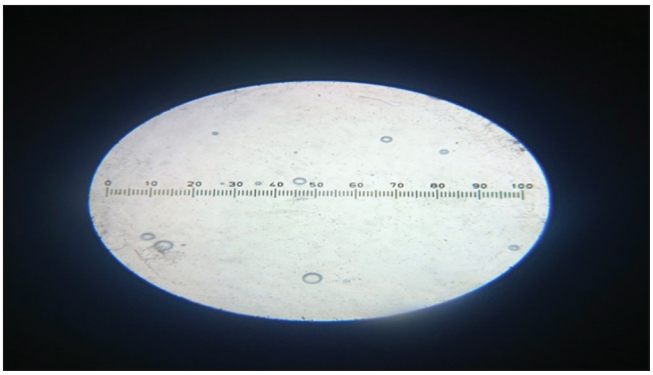

Fig. 8: Determination of droplet size for Smix 2:1

The ratio of 3:1 showed the largest microemulsion region in the pseudoternary phase diagram and had the smallest droplet size (4.92±1.45). Hence, formulation 3:1 was selected for further study.

Formulation of microemulsion (preparation of drug-loaded microemulsion)

In a ratio of 3:1, four different concentrations of oil, Smix, and water were chosen to formulate the microemulsion. 30 mg/mL of the drug was added to all the formulations (F1-F4). The mixture was kept for stirring on a magnetic stirrer till the drug completely solubilized to

attain equilibrium. Then water was added dropwise. The formed ME were then evaluated for globule size (Table 2).

Viscosity measurement

The viscosity of the o/w microemulsion was studied using a Brookfield viscometer (CP-4 spindle) at 50 revolutions per min at room temperature. The reported value is shown in Table 11 and Fig. 11. The viscosity of formulations F1, F2, F3, and F4 were found to be 135±6.82cP, 127±8.5cP, 117±9.8cP, and 110±7.9cP, respectively. All the formulations show that they are visually acceptable and adequate for sublingual delivery. Formulation F4 showed comparatively less viscosity and hence is considered the best formulation. It has been reported that the viscosity of sublingual application plays a commanding role in the pharmacokinetic profile of the drug. The viscosity values in centipoises (cP) are shown in Table 11. The viscosity of each formulation was taken in triplicate, and the mean ±SD was reported.

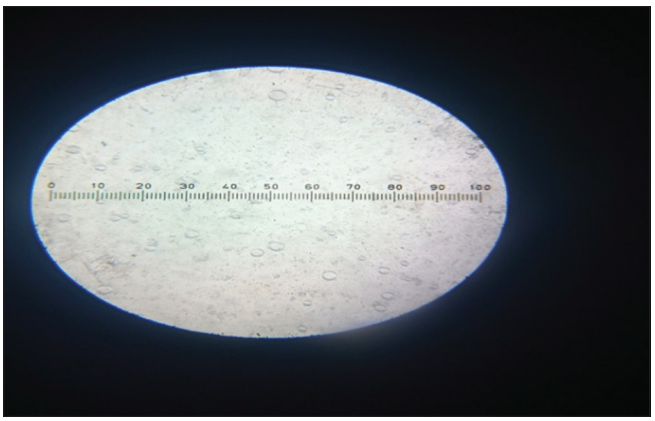

Fig. 9: Determination of droplet size for Smix 3:1

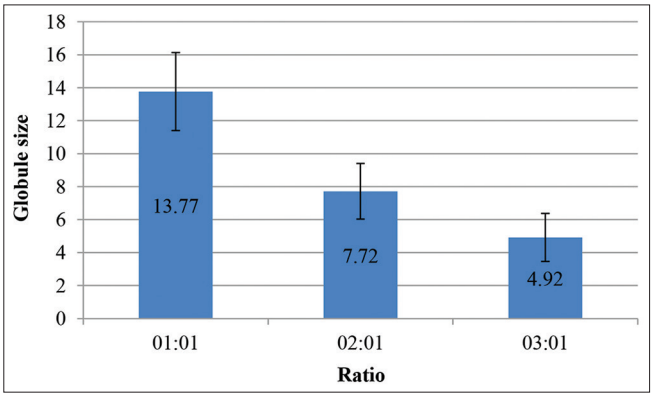

Fig. 10: Average globule size (µm) for 1:1, 2:1, 3:1

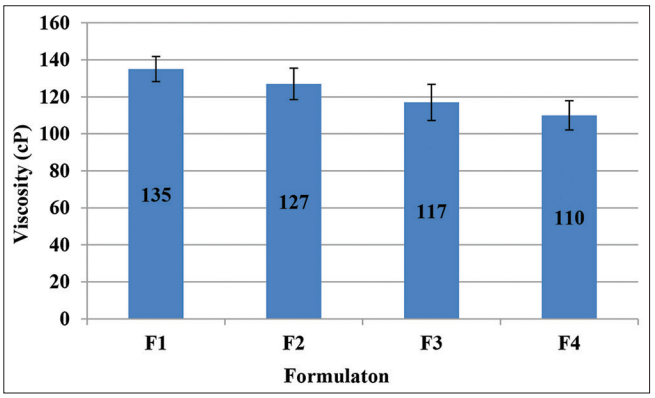

Fig. 11: Viscosity measurement

Measurement of pH

The pH of the prepared valerian microemulsion formulations was determined using a digital pH meter. Each formulation was tested in triplicate. The mean ± standard deviation (±SD) was reported. The pH of all formulations is very close to the pH of the sublingual mucosa which is in the range of (6.2–7.6). It can be concluded that these formulations are suitable for sublingual administration. The reported values are shown in Table 12 and Fig. 12.

Globule size determination

The globule size of all prepared ME was evaluated on a zeta sizer (malvern). The results of globule size were reported in Table 13 and Fig. 13.

Based on the result, it was found that the globule sizes of F1, F2, F3, and F4 were 133.19±12.5 nm, 112.26±11.9 nm, 105.13±11.6 nm, and 96.78±10.9 nm, respectively. The lowest globule size was obtained for the microemulsion formulation F4, and the globule size was found to be 96.78±10.9 nm. The globule size of the formulation F4 falls within the range of microemulsion and is the smallest among all tested formulation. Hence, formulation F4 is considered the best formulation and selected for further studies.

Zeta potential

The zeta potential of all prepared ME was evaluated on nanotrac (malvern). The zeta potential was reported in Table 14 and Fig. 15.

Table 10: Average globule size (µm) for 1:1, 2:1, 3:1

S.no	Ratio	Avg. globule size (µM) (±S.D)
1	1:1	13.77±2.37
2	2:1	7.72±1.69
3	3:1	4.92±1.45

Table 11: Viscosity measurement

S.no	Formulations	Viscosity (cP) ± S.D
1	F1	135±6.82
2	F2	127±8.5
3	F3	117±9.8
4	F4	110±7.9

Data are expressed as mean±S.D (n=3)

Table 12: Measurement of pH

S.no	Formulation	Ph
1	F1	6.25±0.01
2	F2	6.28±1.00
3	F3	6.24±2.00
4	F4	6.22±2.00

Table 13: Globule size determination

S.no	Formulations	Globule size (nm) ± S.D
1	F1	133.19±12.5
2	F2	112.26±11.9
3	F3	105.13±11.6
4	F4	96.78±10.9

Table 14: Zeta potential

S.no	Formulations	Zeta potential (mV)± S.D.
1	F1	+63.4±3.7
2	F2	+62.9±3.6
3	F3	+66.2±2.3
4	F4	+67.5±1.9

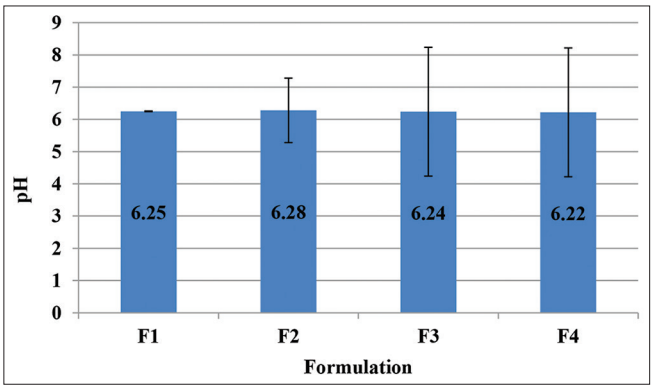

Fig. 12: pH measurement

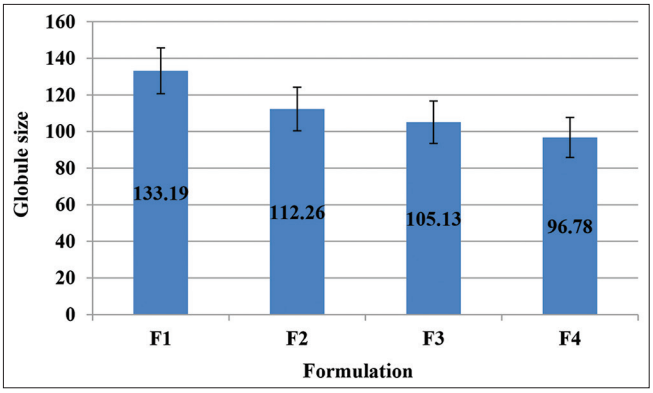

Fig. 14: Comparative reports of globule size

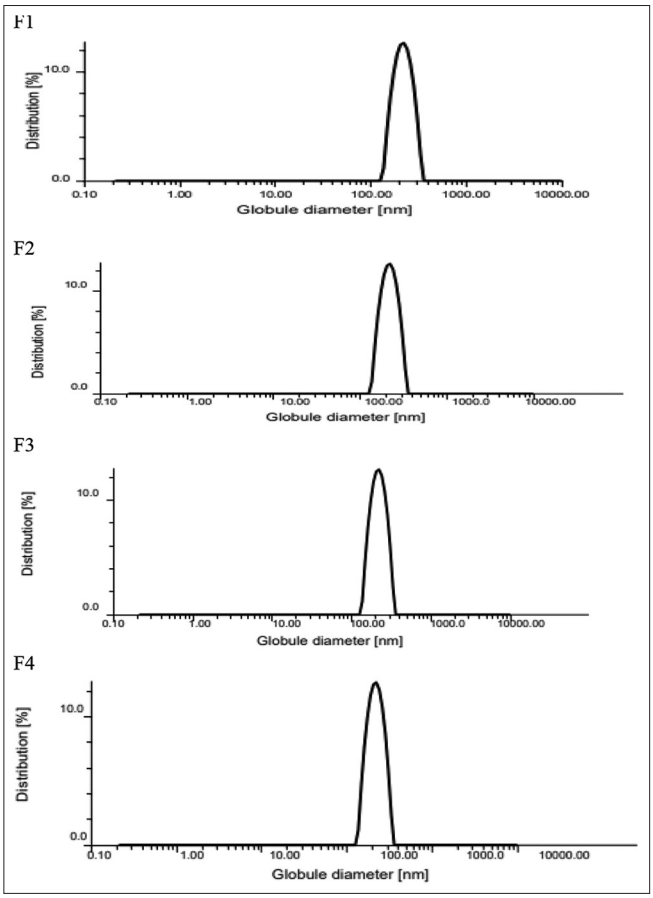

Fig. 13: Globule size of microemulsion formulation (F1-F4)

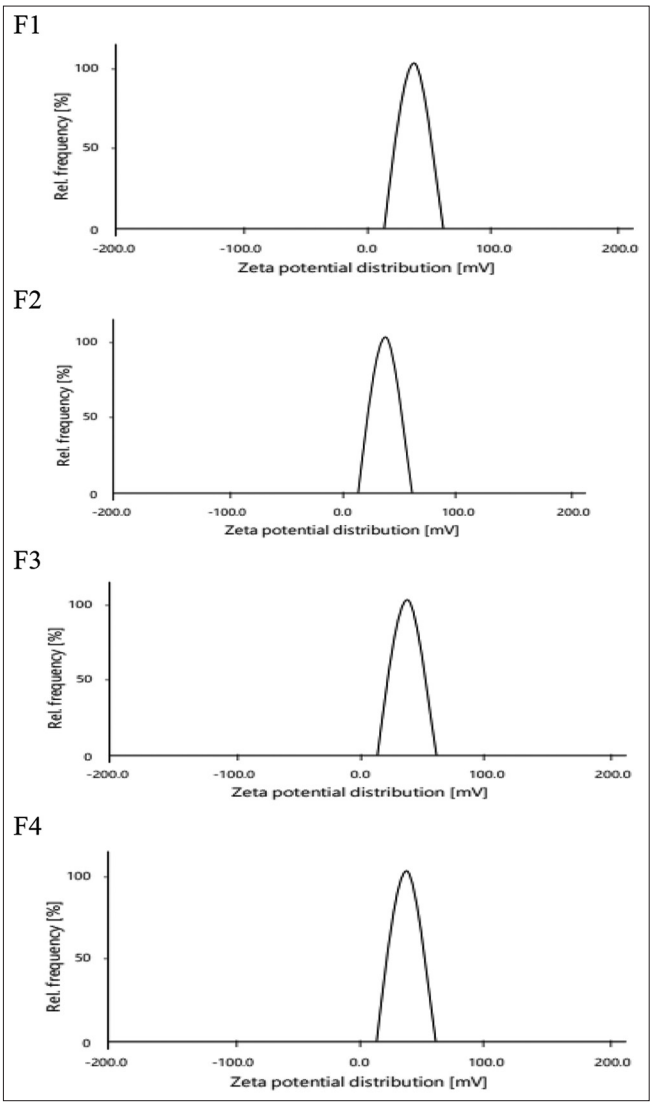

Fig. 15: Zeta potential of microemulsion formulation (F1-F4)

The zeta potential of all the formulations was above +60 mV. This indicates excellent stability of the microemulsion. The experimental measurements are indicated in Table 26, which shows that the F4 formulation has the highest zeta potential value (67.5±1.9), indicating the best stability compared to the other three formulations.

TEM

Transmission electron microscopy studies were carried out for the optimized formulation F4 at room temperature.

The TEM images showed the morphology of the microemulsion. All globules were round shape and the size of the globules was between 45.1 and 182 nm. These results matched the globule size range obtained from the zeta sizer (Malvern) instrument. The TEM images are depicted in Fig. 17.

Phase separation test

Microemulsion systems were subjected to centrifugation at 3000 rpm for a period of 2 h and examined for any evidence of phase separation, as shown in Table 15.

Staining test/Dye solubility test

Water-soluble dye, methylene blue solution, was added to the optimized microemulsion (F4). The dye will dissolve uniformly

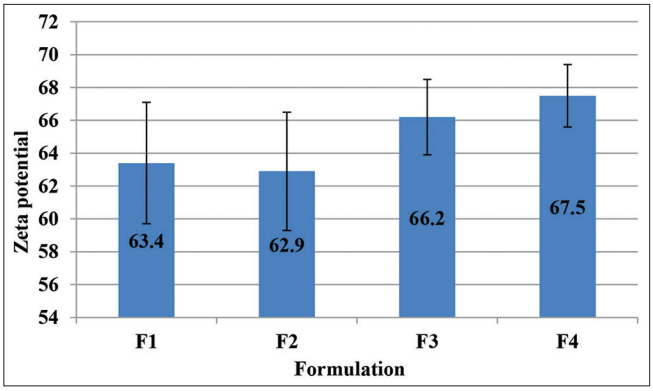

Fig. 16: Comparative reports of zeta potential

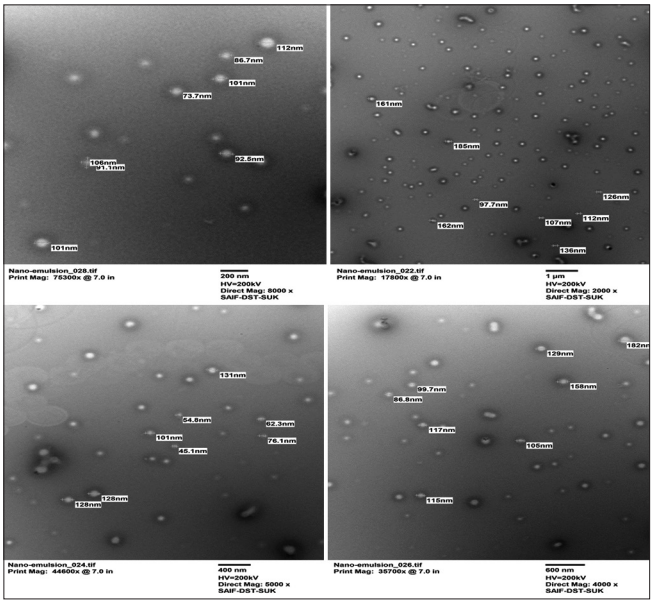

Fig. 17: Transmission electron microscopy

Table 15: Phase separation

Formulation	Phase separation
F1	No phase separation
F2	No phase separation
F3	No phase separation
F4	No phase separation

Table 16: Stability study

Evaluation parameters	Initial reading	Room temperature		
		1 month	2 month	3 month
Globule size (µm)	2.5 µm	2.9 µm	3.5 µm	3.8 µm
Viscosity (cP±SD)	110±2	112±5	113±7	115±2
pH±SD	6.22±2.00	6.25±1.00	6.31±1.00	6.33±2.00

throughout the system, identifying that the emulsion is of the o/w type.

Stability study

A short-term stability study of the optimized formulation was carried out for 3 months at room temperature. Insignificant changes are observed in globule size, viscosity, and pH. Hence, it can be concluded that the formulation F4 exhibited an acceptable stability profile at room temperature.

DISCUSSION

Insomnia affects approximately one-third of the adult population. Extracts of the roots of valerian are widely used for inducing sleep and improving sleep quality. The present research work aimed to formulate a microemulsion-based sublingual liquid containing valerian root extract for the treatment of insomnia. This liquid is to be used sublingually for a faster onset of action. The absorption of drugs through the sublingual route is 3–10 times larger than the oral route.

After collection and authentication of the plant material, the plant material was analyzed for pharmacognostic, phytochemical, and physicochemical parameters. Pre-formulation studies of valerian root extract were performed. The preliminary phytochemical study exhibited the presence of therapeutically important secondary metabolites. The physicochemical analysis, such as extractive value and moisture content of the sample, was studied. It was found that the extractive value of valerian root in 70% ethanol was maximum (27.56±1.95% w/w) as compared to water and methanol extract. The moisture content of valerian extract was found to be 9% w/w. The present study revealed that the valerian root extract contains alkaloids, reducing sugar, tannins, flavonoids, and terpenoids. A microscopy study showed the presence of scleroids, starch granules were rounded, lignified large polygonal cells, scalariform vessels, and brown content. Based on the TLC experiment, the reported Rf value for valerenic acid is 0.48, indicating the presence of valerenic acid in the sample.

The solubility of the valerian root extract in peanut oil was maximum (7±0.02 g/mL) compared to sunflower oil, coconut oil, and soybean oil. From the pseudoternary phase diagram, the largest microemulsion region was obtained for the surfactant: cosurfactant ratio of 3:1. It was also confirmed that the area of the microemulsion increased as the concentration of surfactant increased. The ratio of 3:1 shows the largest microemulsion region and the smallest droplet size (4.92±1.45). Hence, a 3:1 ratio was selected for the formulation of the microemulsion.

Formulation was characterized by viscosity, pH, globule size, zeta potential, TEM, and staining tests. The viscosities of F1, F2, F3, and F4 were found to be 135±6.82 cP, 127±8.5 cP, 117±9.8 cP, and 110±7.9 cP, respectively. Formulation F4 showed comparatively less viscosity. The pH of all formulations was between 6.22 and 6.28, which is well within the pH range of the oral cavity.

The globule sizes of F1, F2, F3, and F4 were observed as 133.19±12.5 nm, 112.26±11.9 nm, 105.13±11.6 nm, and 96.78±10.9 nm, respectively. The globule size of the formulation F4 was within the range of a microemulsion (1–100 nm) and was smallest among all tested formulations. Hence, formulation F4 is considered the best formulation and selected for further studies.

The zeta potential of all the formulations was above +60 mV, which indicates the excellent stability of the microemulsion.

The morphology of the valerian microemulsion (F4) characterized by TEM showed round particles. All globules were round in shape, and the size of the globules was between 45.1 and 182 nm.

No phase separation was observed when formulation (F4) was subjected to centrifugation at 3000 rpm for 2 h. The formulation F4 was found to be an o/w type of microemulsion in the staining test. The optimized formulation F4 was stored for 3 months at room temperature. It showed no change in the performance characteristics of the product during storage, indicating acceptable stability.

CONCLUSION

Microemulsion, as being one of the novel approaches, has gained importance due to its stability and physical properties it shows. The use of this novel approach can surely prepare a base for research for the

upcoming study, so we can see a concomitant commercial formulation in the near future.

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Informed consent: Written informed consent was obtained from all study participants.

AUTHORS CONTRIBUTION

Virashri M Noraje: Conceptualization, Investigation, writing-original draft and editing, Resources, Methodology, Software Visualization. Vijayanand Pujari: Conceptualization, Resources, Investigation, Supervision, Writing review and editing, Project administration.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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NA.

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