IJPSR (2020), Volume 11, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 August 2019; received in revised form, 27 April 2020; accepted, 22 July 2020; published 01 August 2020

FORMULATION DEVELOPMENT AND CHARACTERIZATION OF MICROEMULSION OF EXTRACT OF *E. NUDA*

Dhara Bhatt *, Disha Suthar, Khushboo Jethva, Shrikalp Deshpande and Maitreyi Zaveri

Department of Pharmacognosy, K. B. Institute of Pharmaceutical Education and Research, Sector - 23, Gandhinagar - 382023, Gujarat, India.

Keywords:

Eulophia nuda, Microemulsion, Anti-cancer, MTT assay, MCF7 cell line

Correspondence to Author:
Dhara Bhatt

Department of Pharmacognosy, K. B. Institute of Pharmaceutical Education and Research, Sector - 23, Gandhinagar - 382023, Gujarat, India.

E-mail: dharabhatt88@gmail.com

ABSTRACT: The compounds that are derived from the natural sources have been used for the treatment of cancer since ancient times. The tubers of *Eulophia nuda*, belonging to family Orchidaceae, have been reported for its anti-cancer use in the literature. There are many disadvantages to the conventional herbal drug delivery system. These disadvantages could be overcome by formulating these herbs using novel drug delivery systems. Microemulsions are the novel formulations that improve the solubility and bioavailability of herbal bioactive bio-active compounds. In the present study, microemulsion loaded with alcoholic extract of tubers of *E. nuda* has been formulated by the water titration method using oil (Oleic acid), surfactant: cosurfactant (S_{mix}) (Tween 80 and PEG400) and water. The prepared formulations of microemulsions were subjected physicochemical characterization parameters such as globule size, percentage transmission, refractive index, viscosity, pH, drug loading, and percentage drug permeated. The optimized microemulsion was further subjected to an *in-vitro* cytotoxicity study by MTT assay using the MCF7 breast cancer cell line. The optimized microemulsion loaded with alcoholic extract of tubers of *E. nuda* showed enhanced permeation and increased cytotoxicity as compared to the alcoholic extract of tubers of *E. nuda*. Based on this study, it can be concluded the solubility and permeability of alcoholic extract of tubers of *E. nuda* can be increased by formulating into microemulsion.

INTRODUCTION: The anti-cancer drugs that are discovered from herbal medicines have a long history. The compounds derived from plants have served as an important source of very useful clinically used anti-cancer agents like vinblastine, vincristine, camptothecin derivatives, taxol derivatives, etoposide, topotecan, and irinotecan. A number of scientific evidence at molecular mechanisms and the clinical trial showed they have anti-cancer potential.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(8).3709-16

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(8).3709-16

One such perennial herb having underground tubers, *Eulophia nuda*, belonging to family Orchidaceae, has been reported to have many medical uses. The tubers are used against tumors, bronchitis, scrofulous glands of the neck, skin rash, rheumatoid arthritis, acidity, piles, stomach ailments, and also they are reported to be used as an aphrodisiac, anthelmintic and demulcent [1-8].

In recent years, traditional medicine has become very popular for the treatment of cancer worldwide, and these approaches are being accepted worldwide as a part of complementary and alternative medicines [9]. But there are many disadvantages of the current drug delivery system used in herbal medicines such as bulk dosing, decreased bioavailability and decreased absorption and lack of target specificity [10].

The herbal medicines can be administered with increased efficacy by formulating them into a modern dosage form. This can be achieved by designing novel drug delivery systems for herbal constituents. Novel drug delivery systems have many advantages like decreased frequency of dosing to overcome patient non-compliance, increased therapeutic value, decreased toxicity, and enhanced bioavailability [11]. The usage of the colloidal systems like emulsions and microemulsions as drug delivery systems in cancer chemotherapy has been trending in both academic as well as industrial research in recent years [12]. The use of colloidal systems like emulsions and microemulsions is a very popular research interest in the fields of academic and industrial cancer research in today's time. These systems have the ability to affect the drug's biodistribution and pharmacokinetics. It not only reduces the toxicity of the drug but also increases the drug's response of the tumor.

Microemulsions offer several potential advantages due to their unique physicochemical properties in cancer therapy [12]. Microemulsions are defined as a colloidal, optically isotropic, transparent, or slightly opalescent formulations, consisting of a surfactant, co-surfactant, oil, and water. Microemulsions have several advantages for pharmaceutical use, such as ease of preparation, long-term stability, and high drug solubilization capacity. They are suitable for the incorporation of poorly water-soluble drugs to improve oral absorption [13]. Thus, in the present study, the development and characterization of microemulsion loaded with alcoholic extract of *E. nuda* was done.

MATERIALS AND METHODS:

Chemicals and Reagents: Alcoholic extract of *E. nuda* was prepared in house. Capmul MCM was a kind gift by M/s Abitec Corporation, Janesville, WI. Isopropyl myristate, Tween 80 and Tween 20 were generously gifted by M/s Loba Chemie, Mumbai, India. Olive oil, sunflower oil and soya oil were purchased from M/s Hi-Media Pvt. Ltd., Mumbai, India. Labrasol®, Labrafac® was acquired as a gift sample from M/s Gattefosse, Hauptstrasse, Germany. PEG 400, PEG 200, and propylene glycol were purchased from M/s S.D. Fine Chemicals Ltd., Mumbai, India. Isopropyl alcohol was obtained from M/s Qualigens Fine Chemicals, India. Ultrapure water (Milli-Q® Integral system, Merck Millipore, Billerica, MA) was used throughout the study. All other reagents and chemical used were of analytical grade.

Screening of Components for Microemulsion: The saturation solubility study of alcoholic extract of *E. nuda* was determined in numerous saturated as well as unsaturated oils such as oleic acid, olive oil, sunflower oil, soya oil, and IPM, Capmul MCM, Labrafac®; surfactants like Tween 80, Tween 20 and Labrasol® and co-surfactants including Captex® 100, PEG 400, PEG 200, and PG. For solubility study, an excess amount of alcoholic extract of *E.nuda* was added individually in 3 ml of oil or surfactant or co-surfactant in 5 ml capacity borosilicate stoppered vials [14]. To achieve equilibrium, the resultant mixture was mixed initially by vortex mixture (Remi Cyclo, CM-101 plus, Mumbai India). The vials were then shaken on a magnetic stirrer (Remi Instruments, Mumbai, India) for 72 h followed by centrifugation (Remi Centrifuge, Mumbai, India) at 10,000 rpm for 15 min. The undissolved drug was allowed to settle at the bottom. The supernatant was filtered through a 0.45 µm membrane filter, and the concentration of *E. nuda* in the filtrate was determined by HPLC after appropriate dilution. The components that revealed the highest solubility of *E. nuda* extract were used for further studies. The procedure was performed in triplicate manner.

Compatibility Studies: The oil and surfactant that showed the highest solubility were checked for compatibility. The mixture of selected oil and surfactant were prepared at 1:1, 1:2, 1:3, 2:1, and 3:1, respectively. The blends were mixed for 5 min using a vortex mixture and were evaluated for physical compatibility (clarity and absence of opacity). Likewise, compatibility study between the selected co-surfactant and the mixture system of oil and surfactant were checked by preparing mixtures of a co-surfactant and mixed system of oil and surfactant at 1:1, 1:2, 1:3, 2:1 and 3:1, respectively. The blends were assessed for physical appearance [15]. Also, physicochemical compatibility between alcoholic extract of *E. nuda* and excipients used to prepare microemulsion was assessed using Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTs, Shimadzu, Japan). The sample was placed into a sample cup and analyzed.

The obtained spectra of alcoholic extract of *E. nuda* and microemulsion were compared to check compatibility.

Construction of Pseudo Ternary Phase Diagrams: The phase diagrams of oil, surfactant/co-surfactant (S_{mix}), and water were constructed to obtain a microemulsion region by water titration method [16-18]. A homogeneous liquid mixture of oil (Oleic acid), surfactant (Tween 80) and co-surfactant (PEG 400) was titrated with water phase at ambient temperature. The S_{mix} was altered at 1:1, 2:1, 3:1, and 4:1. Each of these ratios was mixed with an increasing amount of oil, *i.e.*, 1:9 to 9:1. The total amount of the microemulsion system was kept 100%. Mixtures are having a different proportion of oil, and S_{mix} were prepared and titrated with water until the endpoint. The homogeneous and transparent microemulsion was considered as the endpoint of aqueous titration method. The resultant data were fed into Chemix 3.0® (Arne Standnes, Bergen, Norway) software for mapping the triangular microemulsion region.

Preparation of Drug Loaded Microemulsion: From the pseudo ternary phase diagrams, S_{mix} ratio with maximum microemulsion region was selected for further studies. Oil and S_{mix} were mixed together in different proportions, as mentioned in **Table 1**. Alcoholic extract of *E.nuda* was dissolved in the mixture of oil and S_{mix} under magnetic stirring at ambient temperature. An appropriate amount of water was added dropwise to the above mixture until clear and transparent microemulsion was obtained. The mixture was allowed to stabilize and attain the equilibrium for the next 10-15 min. All extract loaded microemulsion formulations were then stored at ambient temperature till further evaluation.

Physico-Chemical Characterization of Microemulsions: Zeta potential of the optimized microemulsions was measured by Zetasizer ZS90 (M/s Malvern Instruments, UK). The percentage transmission was measured by a UV spectrophotometer at 287 nm. The refractive index of microemulsions was measured by the Abbe refractometer (Bausch and Lomb, New York, USA). The isotropic nature of microemulsions was verified by a polarizing microscope by placing a drop of formulation on a glass slide with a coverslip and observed under cross-polarized light. The electrical conductivity of microemulsions was measured by a conductivity meter (CM-180 ELICO, India). The viscosity of microemulsions was determined as such without dilution using Brookfield DV III Rheometer (Brookfield Engineering Labs, USA) with spindle LV III in 30 g samples using a small sample holder. The pH of the optimized formulation of microemulsion was measured by pH meter at 25 ºC (Electroquip, Delhi, India).

***Ex-vivo* Permeation Study:** A goat stomach mucosa was carefully excised and prepared for the permeation study. A Franz diffusion cell with an effective diffusion area of 2.54 cm^2 was used for the study. The nasal mucosa was placed between donor and receptor compartments of the Franz diffusion cell. The microemulsion was placed on the mounting area carefully, and the release profile was taken. The receptor compartment was filled with 0.1N HCl.

The receptor medium was maintained at 37±1 ºC and was magnetically stirred at 50 rpm. Samples were withdrawn at predetermined time intervals, filtered through 0.45 µm pore size cellulose membrane filter, and were analyzed by HPLC. The fresh buffer solution was immediately replaced into the receptor chamber after each sampling. The cumulative amount of drug in the receptor compartment was plotted as a function of time (min). The cumulative amount of DM permeated through the skin was determined as per the following equation:

$$Q_n = \frac{Cn \times V0 + \sum_{i=1}^{n-1} Ci \times Vi}{S}$$

Where C_n is the drug concentration of receptor medium after each sampling time, C_i is the drug concentration after i^{th} sample, V_0, and V_i are the volumes of the receiver solution and sample, respectively, and S is the effective diffusion area.

***In-vitro* Cytotoxicity Study on MCF7 Cell Line by MTT Assay:** [19, 20] The MCF7 cell line was procured from National Centre for Cell Sciences (NCCS), Pune, Maharashtra, India. The cells were cultured in Minimum essential medium (MEM) (Eagle) with non-essential amino acids, supplemented with 10% Foetal Bovine Serum

(FBS), 1% antibiotics solution (penicillin and streptomycin) in a humidified atmosphere of 5% CO_2 at 37 ºC. Cells were seeded with a density of 1 × 10^5/well in a 96-well flat-bottomed plate and incubated for 24 h at 37 °C and in 5% CO_2 atmosphere in an incubator. The MCF7 cells were exposed to the alcoholic extract of *E. nuda* and microemulsion loaded with alcoholic extract of *E. nuda* at four different concentrations of 100, 250, 500, and 1000 µg/ml for 48 h. Cells were then treated with MTT reagent (0.5 mg/ml as a final concentration in phosphate buffer saline) for 4 h at 37 °C in the dark. Then all the media and MTT reagent was removed from the wells, and 200µl DMSO solvent was added to each well to dissolve the formazan crystals. The optical density (OD) was recorded at 570 nm using a Microplate (ELISA) reader. The percentage cytotoxicity for MCF7 was calculated.

Stability Study: The microemulsions were subjected to accelerated stability studies as per ICH guidelines (40±2 °C and 75±5% RH) for a period of 3 months in stability chambers (Model-TH 90 S, Thermolab, India). The samples were taken out at 30, 60, and 90 days and were evaluated for drug content, % transmittance, globule size, and zeta potential to ensure physicochemical stability.

RESULTS:

Selection of Components for Microemulsions: The solubility of alcoholic extract of *E.nuda* was determined in different oils and was found highest in oleic acid (131.21±2.42 mg/ml, **Fig. 1**). Also, oleic acid is a lipophilic permeation enhancer and can be useful to improve the membrane permeability [21]. Hence, oleic acid was selected as an oil phase for the microemulsion formulation. The type of microemulsion formed depends on the properties of the oil, surfactant, and co-surfactant. An important criterion for the selection of the surfactant is that the required hydrophilic-lipophilic balance (RHLB) value to form oil in water microemulsion be greater than 10. Both Labrasol® and Tween 80 are non-ionic, GRAS listed excipients, and widely used in pharmaceutical preparations. Alcoholic extract of *E. nuda* showed higher solubility in Tween 80 (14.70±1.22 mg/ml) among the screened surfactants. PEG 400 showed the highest solubility of DM (18.23±3.12 mg/ml) among the tested co-surfactants; hence it was chosen for further studies.

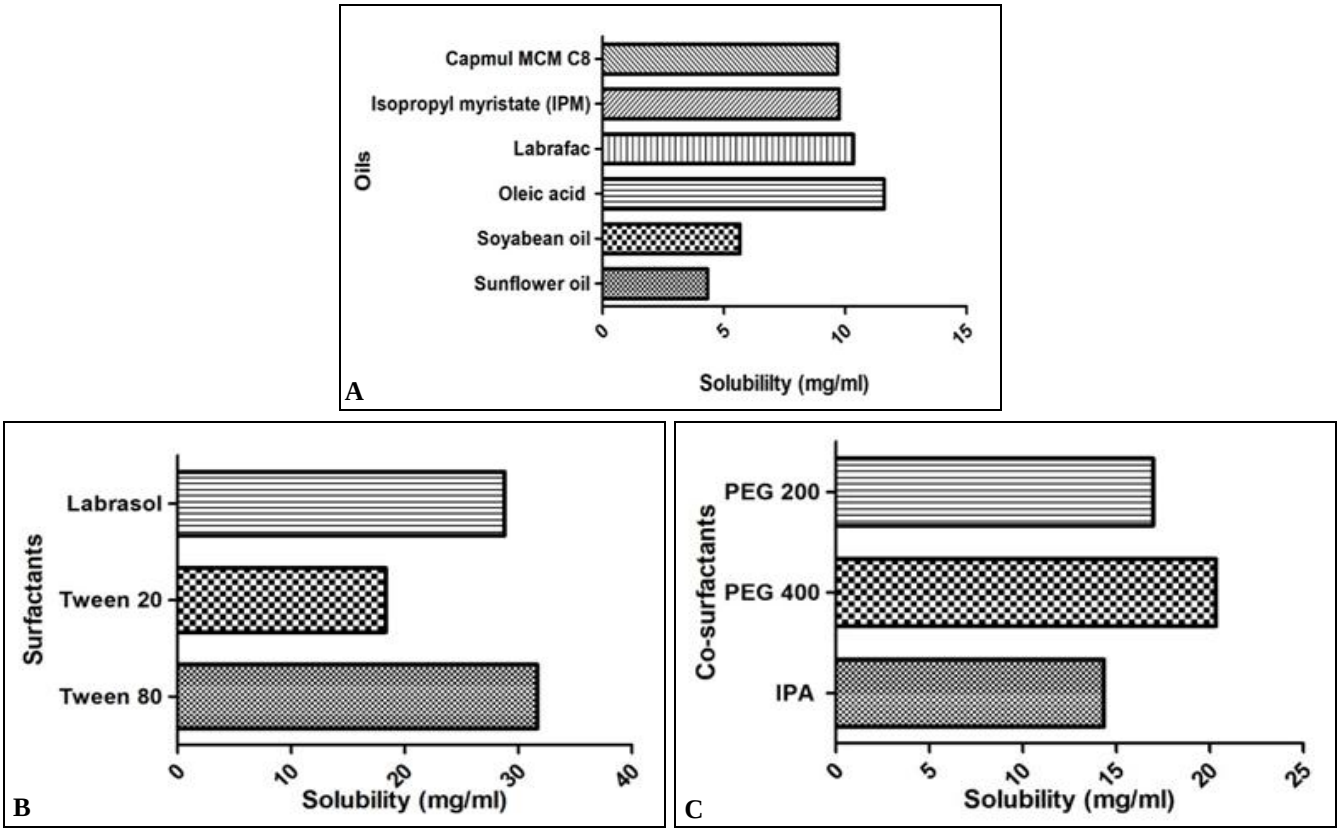

FIG. 1: SOLUBILITY STUDY OF ALCOHOLIC EXTRACT OF *E.NUDA* IN (A) OIL, (B) SURFACTANT AND (C) CO-SURFACTANT

Compatibility Studies: The blends of oil and surfactant and the blends of oil, surfactant, and co-surfactant showed miscibility and transparency, which are an indication of physical compatibility. Also, no sign of phase separation was observed. To evaluate the compatibility of an extract with microemulsion excipients, an FT-IR study by diffuse reflectance was carried out. **Fig. 2** shows the spectra of alcoholic extract of *E. nuda*, a mixture of oil, surfactant and co-surfactant and microemulsion, respectively in the region of 400-4000 cm^{-1}. The characteristic absorption peaks of alcoholic extract of *E. nuda* were retained in microemulsion without any additional peaks, showing no significant interaction between alcoholic extract of *E. nuda* and microemulsion components.

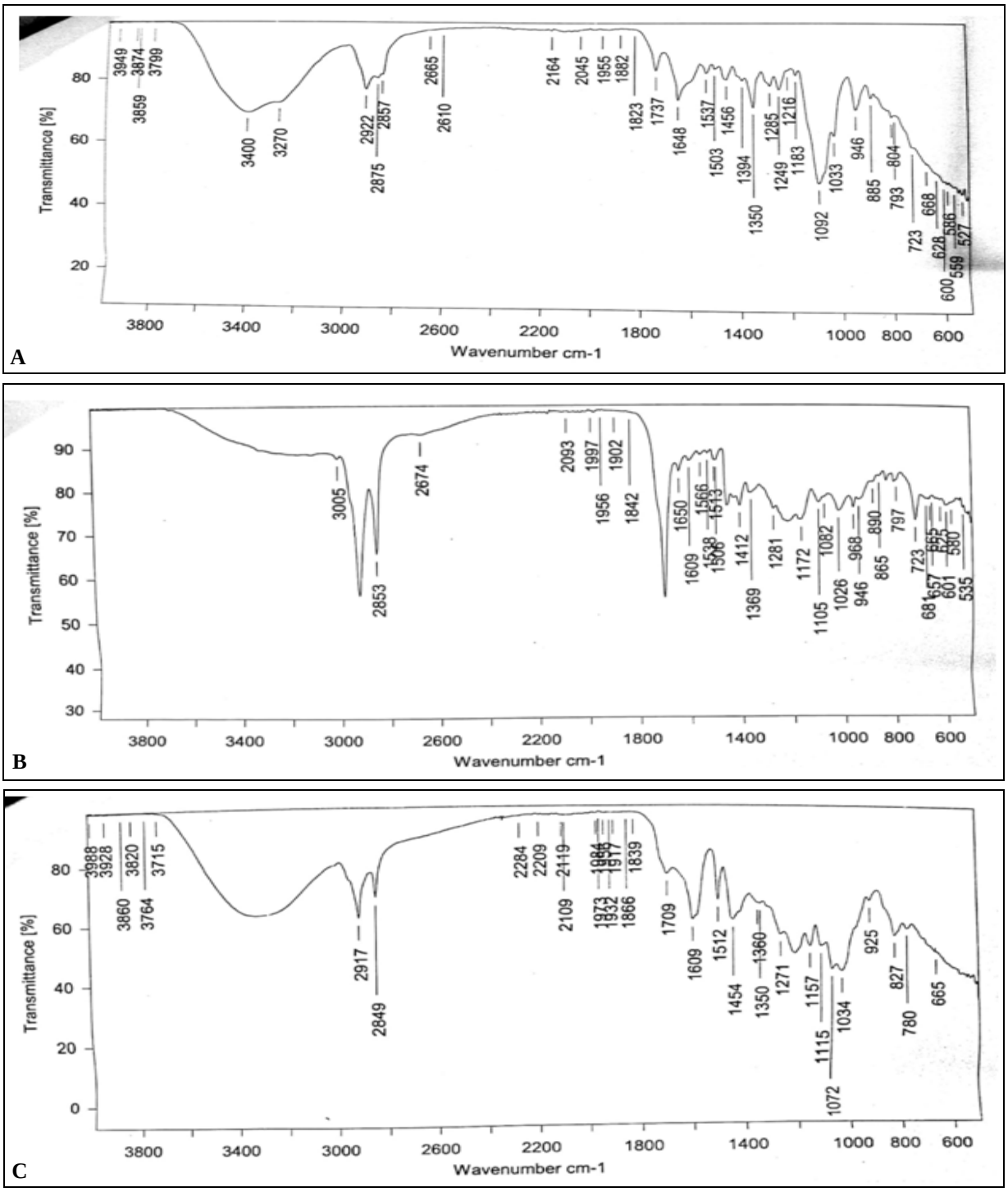

FIG. 2: FT-IR SPECTRA OF (A) PURE ALCOHOLIC EXTRACT OF *E. NUDA*, (B) PHYSICAL MIXTURE OF OIL, SURFACTANT, AND CO-SURFACTANT, (C) MICROEMULSION

Construction of Pseudo Ternary Phase Diagrams: In order to obtain an appropriate concentration range of the microemulsion components, the pseudo ternary phase diagrams were constructed using different S_{mix} (Tween 80: PEG 400) ratios viz. 1:1, 2:1, 3:1 and 4:1. Apexes of the ternary phase diagrams represent 100% of the respective component and shaded area, as shown in **Fig. 3** represents the microemulsion region. The largest microemulsion region was obtained in 1:1. Thus, S_{mix} ratio 1:1 was selected for the formulation of the microemulsion.

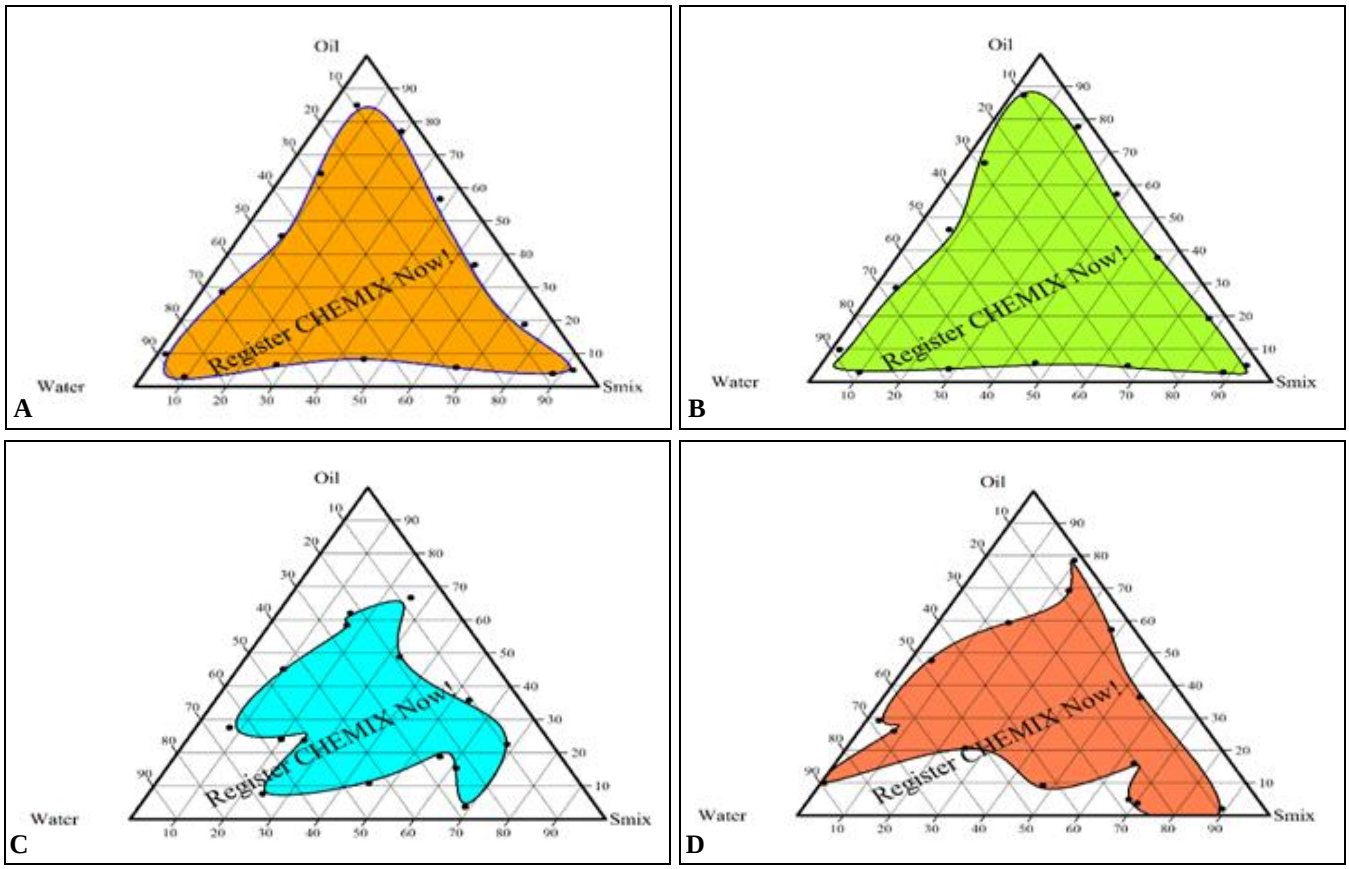

FIG. 3: PSEUDOTERNARY DIAGRAM OF Smix IN RATIO (A) 1:1, (B) 2:1, (C) 3:1 AND (D) 4:1

Optimization of Microemulsion: The results of the evaluation parameters of microemulsion are depicted in **Table 1**. Upon analyzing the results, it is clear that drug loading is maximum in F2 batch due to a higher concentration of S_{mix}. Further, it shows no significant difference in the case of other parameters like % transmittance, refractive index, conductivity, viscosity, pH, and drug loading. Globule size was found to be minimum in all formulations where the amount of S_{mix} was high, drug permeation was found to be similar in each batch, and no significant difference was observed. Based on all the results, the F2 batch was considered as the optimized formulation and was evaluated for *in vitro* cytotoxicity study.

TABLE 1: FORMULATION COMPONENTS OF MICROEMULSION OF ALCOHOLIC EXTRACT OF *E. NUDA* WITH RESULTS

Code	Oil (%)	S_{mix} (%)	Water (%)	Globule size (nm)	% transmittance	Refractive index	Conductivity	Viscosity	pH	Drug loading	% drug permeated
F1	5	55	40	25.5±0.27			78.3	10.72	7.12	69.97±1.32	72.15
F2	7	55	38	20±0.54			75.9	8.21	7.38	90.18±1.54	73.65
F3	10	55	35	23.5±0.75			73.6	11.59	7.05	80.01±1.43	71.12
F4	7	40	53	24±0.89	>99.0%	1.45	86.8	9.59	7.28	84.99±0.98	70.12
F5	7	50	43	27±0.67			81.2	8.97	7.18	75.78±1.67	73.56
F6	7	60	33	26±0.43			70.2	7.98	7.56	80.89±1.36	76.23

***Ex-vivo* Permeation Study:** *Ex-vivo* permeation study of microemulsion revealed depicted successful permeation profile through goat stomach mucosa, and the results are presented in **Fig. 4**.

Prepared microemulsion could act as a drug reservoir from which the drug will be released from the internal phase to the external phase and finally onto mucosa. Microemulsion showed better permeation (73.65%) than the alcoholic extract of *E. nuda* (67.23%). This can be attributed to components of microemulsion (oil and S_{mix}), which helps in higher permeation.

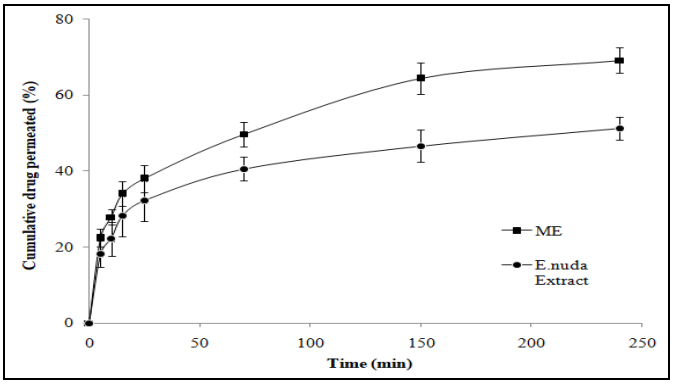

FIG. 4: *EX-VIVO* PERMEATION STUDY OF MICROEMULSION (ME) F2 BATCH COMPARED WITH ALCOHOLIC EXTRACT OF *E. NUDA*

***In-vitro* Cytotoxicity Study on MCF 7 Cell Line by MTT Assay:** The result of the *in-vitro* cytotoxicity study is depicted in **Fig. 5**. The results clearly indicated higher cytotoxicity in the case of micro-emulsion compared to the alcoholic extract of *E. nuda*. This higher cytotoxicity may be attributed to microemulsion as it helps in better permeation, due to its smaller globule size it may permeate to the cells between the cell walls.

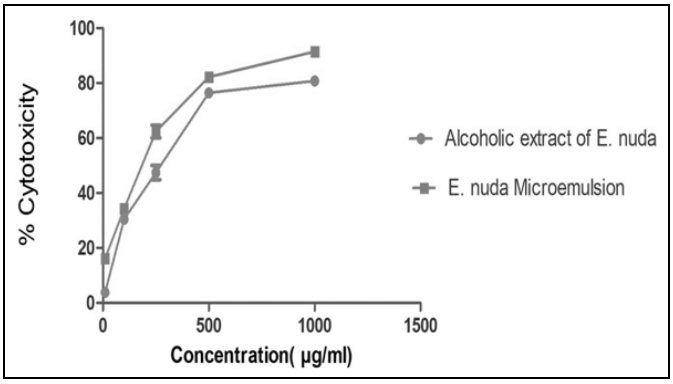

FIG. 5: *IN-VITRO* CYTOTOXICITY STUDY OF MICROEMULSION (ME) F2 BATCH COMPARED WITH ALCOHOLIC EXTRACT OF *E. NUDA*

Stability Studies: In stability studies, the microemulsion exhibited no precipitation of drug, creaming, phase separation, and flocculation on visual observation. The results of stability studies showed that there are negligible changes (P>0.05) in the parameters such as drug assay, % transmittance, globule size, PDI, and zeta potential of microemulsion after 3 months of storage.

CONCLUSION: The tubers of *E. nuda* has been reported for its anti-cancer use in the literature. The efficacy of herbal drugs could be improved by formulating them into novel drug delivery systems. Microemulsions have several advantages for pharmaceutical use, such as ease of preparation, long-term stability, and high drug solubilization capacity. They are suitable for the incorporation of poorly water-soluble drugs to improve oral absorption, and hence, in our study, formulation and characterization of microemulsion loaded with alcoholic extract of *E. nuda* were done. The o/w microemulsion was prepared using the water titration method. The results of the *ex-vivo* permeation study of the prepared microemulsion depicted that the microemulsion showed enhanced permeability as compared to the alcoholic extract of *E. nuda*. Further, microemulsion loaded with alcoholic extract of *E. nuda* was also studied for their *in-vitro* cytotoxicity on MCF7 cell line by using MTT assay. The microemulsion loaded with alcoholic extract of *E. nuda* showed more cytotoxicity as compared to that of the alcoholic extract *of E. nuda*, which is suggestive of the enhanced drug solubility and permeability.

ACKNOWLEDGEMENT: The authors are thankful to DST- INSPIRE, New Delhi, India, for providing financial assistance for the project.

CONFLICTS OF INTEREST: There is no conflict of interest.

REFERENCES:

1. The Wealth of India: A dictionary of indian raw materials and industrial products. New Delhi: Council of Scientific and Industrial Research 2002; 75-78, 107.
2. Chopra RN, Nayar SL, Chopra IC, Asolkar LV, Kakkar KK and Chakre OJ: Glossary of Indian medicinal plants. New Delhi: Council of Scientific & Industrial Research 1956-92.
3. Nadkarni K: Indian Materia Medica. Bombay: Popular Prakashan Private Limited 1976. 411-12, 14-18, 80-84, 519, 1202-10, 92-94.
4. Cooke T: The flora of the presidency of Bombay Calcutta: Botanical Survey of India 1967: 649.
5. Mali PY and Bhadane VV: Some rare plants of ethnomedicinal properties from Jalgaon district of Maharashtra. International Journal of Green Pharmacy 2008; 2(2): 76-78.

6. Singh A and Duggal S: Medicinal orchids-an overview. Ethnobotanical Leaflets 2009; 3: 3.
7. Jagdale S, Shimpi S and Chachad D: Pharmacological studies of 'Salep'. Journal of Herbal Medicine and Toxicology 2009; 3(1): 153-56.
8. Mahekar PD and Yadav S: Medicinal Plant of South Western Maharashtra. Biodiversity of India 2006: 75-99.
9. Cragg GM and Newman DJ: Plants as a source of anti-cancer agents. Journal of Ethnopharmacology 2005; 100(1-2): 72-79.
10. Kumar K and Rai A: Miraculous therapeutic effects of herbal drugs using novel drug delivery systems. International Research Journal of Pharmacy 2012; 3(2): 27-30.
11. Devi VK, Jain N and Valli KS: Importance of novel drug delivery systems in herbal medicines. Pharmacognosy Reviews 2010; 4(7): 27.
12. Karasulu E, Karaca B, Alparslan L and Karasulu HY: Places of microemulsion and emulsion in cancer therapy: *In-vitro* and *in-vivo* evaluation. Microemulsions: CRC press 2008: 344-61.
13. Hu L, Jia Y, Niu F, Jia Z, Yang X and Jiao K: Preparation and enhancement of oral bioavailability of curcumin using microemulsions vehicle. Journal of Agricultural and Food Chemistry 2012; 60(29): 7137-41.
14. Barot BS, Parejiya PB, Patel HK, Gohel MC and Shelat PK: Microemulsion-based gel of terbinafine for the treatment of onychomycosis: optimization of formulation using D-optimal design. AAPS Pharm Sci Tech 2012; 13(1): 184-92.
15. Kawakami K, Yoshikawa T, Moroto Y, Kanaoka E, Takahashi K and Nishihara Y: Microemulsion formulation for enhanced absorption of poorly soluble drugs: I. Prescription design. Journal of Controlled Release 2002; 81(1-2): 65-74.
16. Wu Z, Guo D, Deng L, Zhang Y, Yang Q and Chen J: Preparation and evaluation of a self-emulsifying drug delivery system of etoposide-phospholipid complex. Drug Development and Industrial Pharmacy 2011; 37(1): 103-12.
17. Cho W, Kim MS, Kim JS, Park J, Park HJ and Cha KH: Optimized formulation of solid self-microemulsifying sirolimus delivery systems. International Journal of Nanomedicine 2013; 8: 1673.
18. Singh B, Singh R, Bandyopadhyay S, Kapil R and Garg B: Optimized nanoemulsifying systems with enhanced bioavailability of carvedilol. Colloids and Surfaces B: Biointerfaces 2013; 101: 465-74.
19. Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods 1983; 65(1-2): 55-63.
20. Marshall NJ, Goodwin CJ and Holt SJ: A critical assessment of the use of microculture tetrazolium assays to measure cell growth and function. Growth Regul 1995; 5(2): 69-84.
21. Pierre MBR, Ricci E, Tedesco AC and Bentley MVLB: Oleic acid as optimizer of the skin delivery of 5-amino-levulinic acid in photodynamic therapy. Pharmaceutical Research 2006; 23(2): 360-66.

How to cite this article:
Bhatt D, Suthar D, Jethva K, Deshpande S and Zaveri M: Formulation development and characterization of microemulsion of extract of *E. nuda*. Int J Pharm Sci & Res 2020; 11(8): 3709-16. doi: 10.13040/IJPSR.0975-8232.11(8).3709-16.