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FORMULATION AND EVALUATION OF *ARGEMONE MEXICANA* LEAF EXTRACT LOADED TRANSFEROSOMAL GEL FOR ANTIMICROBIAL ACTIVITY

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ABSTRACT

This study was designed to evaluate the transferosomal gel of leaf extract of Argemone mexicana. Because of smaller size transferosomes can penetrate across the pores of stratum corneum and enters the underlying viable skin in intact form. Different formulations of transferosomal gel were prepared and assessed for their antibacterial activity. The extract has been standardized for its alkaloid content using berberine as biomarker. Transferosomes were prepared using phosphatidylcholine, cholesterol and edge activator like sodium cholate by thin film hydration method using rotary evaporator and evaluated for various parameters. Later transferosomes were added to Carbopol934 gel and consequently evaluated for their physicochemical properties. Gels were found to be smooth, homogenous and pH lying in the normal skin pH range and easily spreadable. The size of transferosomes was in the range of 236.86to397.78 nm whereas entrapment efficiency was found between 49.84 to 68.23%. Amongst the prepared formulations of Argemone mexicana, B4 (20% sodium cholate) was found to possess significant drug content and encapsulation efficiency. From the study, it can be concluded that transferosomal gel served as an efficient drug delivery system for herbal extract with potential antibacterial activity.

Keywords *– Argemone mexicana, antibacterial activity, transferosome, gel*

1. INTRODUCTION

Argemone mexicana, recognized as Ghamoya (class: Magnoliopsida Dicotyledons; order: Papaverales; family: Papaveraceae). It is an exotic weed native in South America but has widespread distribution across the tropical and sub-tropical countries. This plant is very common and found everywhere by roadsides and fields in India as well. The plant raises upright of about 1 m height, leaves

are spiny and typically 5 to 11 cm long, more or less blotched with green and white, half-clasping the stem prominently sinuate-lobed, glaucous broad at the base. The flowers turn out 4 to 5 cm in diameter, and are terminal, yellow, and scentless. The capsules are spiny, obovate or elliptic-oblong and are around 3 cm in length. The seeds are spherical, shining, black and pitted [1,2]. The plant is self-fertile, self-pollinated and usually prefers light (sandy) and well-drained soils, and can grow in nutritionally poor soil. It cannot grow in the shade. It needs dry or moist soil and can bear drought [3].

Traditionally, the leaves are useful in cough, warts, cold sores, wounds, ulcer, skin diseases, itches, cutaneous infections, etc. The continuous evolution of bacteria resistance to currently available antibiotics has necessitated the search for novel and more effective antimicrobial agents [4,5]. Skin infections are a common occurring during consultation in primary and in dermatological practice.15 per cent of the total out-patients in a general hospital Patients and mostly children will have a skin infection at some time [6,7]. Observing ethnobotanical studies, a number of plants are analysed for antibacterial activities and they exhibited positive results. Pharmaceutical companies are interested to discover new medicines from plant origin to cure microbial disorders, as they are safe to use, cost is low and will be in approach to population [8]. Comparatively, Synthetic medicines against skin infections are expensive. In this research work antibacterial and antifungal activity was performed on the *Argemone mexicana* plants that are in easy approach to everyone, to investigate the antibiotic agents, which may be helpful for pharmaceutical companies to design a less expensive medicine [9]. Fungi that originate skin problems mostly occurring in the upper top layer of skin, dead parts of skin and moist areas of body like under arms and under breasts. Usually, fungi living in such places can cause minor infections that can be easily curable. Sometime, fungi can cause serious skin infectious diseases by penetrating deep into the skin and that can be more hazardous. Fungal infection at one place of the body can imitate its reaction to somewhere else, for example a person suffering from infection between toes may spread a reaction on the fingers or hands on contacting to that part. *Microsporum canis, Epidermophyton, and Trichophyton* are mostly responsible for such types of infectious problems. For treatment of such diseases a special clinical care is required by a physician [10]. The objective of this study is to screen the extract of *Argemone mexicana leaf* for antibacterial and antifungal activity; and formulate the extract into transferosomal gel using Carbopol 934 as gelling agent.

2. MATERIALS AND METHODS

Plant Material: *Argemone mexicana* leaves were collected in June 2019 from the waste lands of the village Karjat, Raigad district of Maharashtra, India. The taxonomic identities of this plant were determined by Professor Mr. Pritam Juvatkar (HOD of Pharmacognosy Department, Konkan Gyanpeeth Rahul Dharkar College of Pharmacy & Research Institute, Karjat), University of Mumbai, India. Lecithin, Cholesterol, Sodium Cholate, Ethanol, chloroform and other chemicals were of analytical grade.

2.1 Preparation of the extracts

After collection, plant parts were first cleaned with running tap water, cut into small pieces and then kept under shade until drying. After appropriate drying, dried leaves were pulverized into powdered form and subsequentlyextraction of the powder carried out using Soxhlet apparatus and suitable solvents such as water, ethanol and petroleum ether for separation of polar and non-polar components. All the extracts were concentrated to dryness under reduced pressure and controlled temperature (40°C – 50 °C). Chemical test of both polar and nonpolar extract was carried out and test for steroids was found to be positive (Liebermann Burchard Test) [11].

2.2 Thin layer chromatography for crude extracts and berberine

The leaf extracts of *Argemone mexicana* were characterized for the presence of berberein which is responsible for the antimicrobial activity. The ethanolic extracts of *Argemone mexicana*leaf's and berberine were carried out by dissolving in ethanol (mg/ml) and collected in two test tubes. Silica gel plate were used as stationary phase and an aliquot of 20 µl sample was loaded onto the thin layer chromatography plate and put in a buffer tank containing Hexane: Diethylacetate (9:1, V/V) as a mobile phase; the resulting chromatogram bands were visualized by iodine vaporization.5 bands of different steroids were found, Rf of one of the steroids was matching with berberine which can be responsible for the activity [12].

2.3 Antibacterial Sensitivity Test

Four bacterial strains were used during the study. Gram-positive bacteria include *S. aureus* and *B. subtilis* and Gram-negative bacteria include *E. coli* and *P. aeruginosa*. Cup plate method was used for antibacterial sensitivity test, where agar plats were allowed to solidify and then agar was punched with sterile cork borer. Thereafter introduced the bacterial strains and a dose of 80µl test solution into wells and incubated the Petri plates at 37°C for 24 hours. Plates were then examined and the diameters of the inhibition zones were measured in mm [9].

2.4 Formulation development of *Argemone Mexicana* extract loaded transferosomes

The transferosome were prepared by thin film hydration method using rotary evaporator. The excipients phosphatidyl choline, sodium cholate & cholesterol were solubilized in 5 ml chloroform-methanol (4:1) mixture and place in a clean dry bottom flask. These were then dissolved by shaking. Thin film was then formed by keeping it in the rotary vacuum evaporator at 40°C. Final traces of solvent are removed under vacuum. The lipid film on the wall of the flask were hydrated with the suitable phosphate buffer by rotation at 60 rpm for 1 hour at room temperature. The resultant vesicles were swollen for 2 hours at room temperature. The multilamellar lipid vesicles (MLV) are then sonicated using probe sonicator for 30 minutes at 40°C. Formulation batches and quantities of respective ingredients are given in Table I.

2.5 Evaluation of the prepared transferosomes

The evaluation was done on the basis of microscopic studies, vesicular size and % entrapment efficiency.

2.5.1 Microscopic Study

The transferosomes dispersion was spread on the glass slide using a glass rod. Multilamellar vesicles (MLV) was confirmed by observing the transferosomes suspension under an optical microscope with magnification power of 100X. Photographs of vesicles were taken using Olympus camera.

2.5.2 Vesicular size

Average vesicular size of prepared transferosome performed using the particle size analyser (Malvern Master Sizer, Malvern Instruments Ltd., Malvern, UK). For analysis small amount of formulation suspended in aqueous dispersion phase and stirring for 5min on vortex mixture at room temperature.

2.5.3 Entrapment efficiency

Entrapment efficiency of prepared transferosome was determined by measuring the concentration of unentrapped free drug in aqueous medium. About 1 ml of the extract loaded transferosomes dispersion was centrifuged at 10,000 rpm for 30 min. The *Argemone Mexicana* extract loaded transferosome along were separated at the bottom of the tubes. Plain transferosome without

extract was used as blank sample and centrifuged in the same manner and measure the absorbance using UV spectrophotometer [13].

2.6 Preparation of transferosomal gel

The gels were prepared by dispersion method using 0.8% carbopol 940 (optimized by preparing different concentrations) as gelling agent in distilled water. Then the mixture was allowed to swell overnight and added few drops of glycerol to balance its viscosity. To this gel solution, 1gm of optimized transferosomal dispersion was added and mixed properly. Mixing was continued until a transparent gel appeared. The prepared gels were filled in glass vials and stored at 4-8 °C[13].

2.7 Evaluation of transferosomal gel

2.7.1 Determination of pH: 50 gm of gel formulation were weighed and transferred in 10 ml of beaker and measured the pH by using the digital pH meter. pH of the topical gel formulation should be around the pH of skin to treat the skin infections [13].

2.7.2 Spreadability: 1 g of gel between placed in between two horizontal plates (20 cm x 20 cm) and standard weight of 125gm was applied on upper plate. The spreadability of gel formulation was confirmed by determining the spreading diameter of after 1 min [14].

2.7.3 Drug content: Mixed 1 gm. of the prepared gel with 100 ml of ethyl alcohol and prepared the aliquots of different concentrations by suitable dilutions after filtering the stock solution and measured the absorbance on UV-Spectrophotometer. Drug content was calculated by linear regression analysis of the calibration curve [13].

***2.7.4 In-vitro* diffusion study:** Modified Franz diffusion cell was used to study *in-vitro* drug release study. Dialysis membrane was obtained from Hi Media (Molecular weight 5000 Daltons) and placed between receptor and donor compartments. Transferosomal gel of *Argemone Mexicana* extract was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (24 ml). The diffusion cell was maintained at 37±0.5°Cthroughout the experiment with stirring speed of 50 rpm. 5 ml of aliquots were withdrawn at different time intervals from side tube of receiver compartment and analyzed for drug content by UV Visible spectrophotometer [15].

3. RESULTS AND DISCUSSION

3.1 Anti-bacterial activities

Results of anti-bacterial activities are provided in Table 2, which provides clear indication and understanding of inhibition zones. Zones of inhibitions were measured in mm (millimetres) and compared with ampicillin for interpretation of results. It was found that crude ethanol extract was more potent against bacteria, followed by thewater and petroleum ether extract of *A. mexicana* which revealed that all three crude extracts of *A. mexicana* exhibited considerable antibacterial activity. *S. aureus* was found to be least resistant to crude plant extracts followed by *Bacillus subtilis, P. aeruginosa* and *Escherichia coli.*

It is observed that antibacterial activity depends upon choice of solvent used for extraction of active ingredients. Method used for Antibacterial activity was easy to handle and cheap technique to identify and screen a potential of antibiotic drug discovery from any plant or natural compound. Solvent selection found to play a significant role in extraction of active ingredients, which has a great influence towards development of new drug discovery. Our results stated that ethanol is the best choice of solvent for extraction of active compounds from plant and well explained by bar graph as shown in Figure 1.

3.2 Microscopic Study

Microscopic study of formulated transferosome showed that the transferosome prepared by thin film hydration method were found small in size, unilamellar and spherical in shape with smooth surface as given in Figure 2.

3.3 Evaluations of transferosomes for Vesicle size and Entrapment efficiency (EE)

Table 3 summarizes the value of vesicle size and entrapment efficiency. The vesicle size of all transferosomes varied between 236.86 and 397.78 nm whereas entrapment efficiency was found between 49.84 to 68.23%. Results showed that ingredients used in formulation of batchB4 although not gives smallest vesicle size but showed increase in entrapment efficiency. Hence batch B4 (which contains 40% phosphatidylcholine, 40% cholesterol and 20% of sodium cholate) selected as optimized formulation for further evaluation. Further It was also found that vesicle size of *A. Mexicana* extract loaded transferosomes decreased with increase in sodium cholate concentration and increased with increase in phospholipid concentration.

The EE in transferosomes depends on the sodium cholate concentration in the lipid bilayer. Transferosomal formulations containing varying percent of sodium cholate (5-30%). Initially, with increase in quantity of sodium cholate (5%–20%), there was an increase in EE, but it was observed that above 20% concentration of sodium cholate EE decreased. This may be owing to micelles formation in the vesicle membranes at higher sodium cholate concentration. This confirms with the report which states that micelles having a lower drug carrying capacity and deprived skin permeation owing to their internal structural feature.

3.4 Evaluations of transferosomal gel for pH, Spreadability, Drug content and In-vitro diffusion studies

The prepared gels were evaluated for pH, spreadability, drug content and invitro diffusion studies as depicted in Table 4. Gels were found to be smooth, homogenous and pH lying in the normal skin pH range, easily spreadable. The spreadability was performed on the basis of slip and drag characteristics of the gels and was in the range of 10.95 – 13.55 gms.cm/sec. The gels should have optimal spreadability because very high and very low spreadability values indicate that the application of the gel to the site is difficult. The spreadability of optimized formulation B4 was found to be 12.77.

Drug content is most significant in transferosomes formulation and the appropriate data was found. It was found in range of75.65 to 87.18% which illustrates the good capacity of formulation to hold the drug. The maximum drug content was found in formulation B4 (87.18%). The cumulative amount of drug release was calculated for each formulation, and it was found that percent cumulative amount of drug release in 24 hr ranges from 76% to 93%. Among the six transferosomal batches, batch B4 (20% sodium cholate) showed maximum drug release up to 24 h, may be due to highest EE. This may be due to optimum sodium cholate concentration, (20%) which linked with phosphatidylcholine bilayer, thus enabling partitioning of drug across the vesicle easily and higher percentage drug release.

Table 1: Formulation batches of *Argemone mexicana* extract loaded transferosomes

Batch	Drug (mg)	Quantity of phosphatidyl-choline (mg)	Quantity of Cholesterol (mg)	Quantity of sodium cholate(mg)
B1	100	47.5	47.5	5
B2	100	45	45	10
B3	100	42.5	42.5	15
B4	100	40	40	20
B5	100	37.5	37.5	25
B6	100	35	35	30

Table 2: Antimicrobial activity of Aqueous, ethanol and petroleum ether extracts of leaves

Different extracts of *A. Mexicana* Leaves	Diameter of the inhibitory zones (mm)			
	S. aureus	*Bacillus subtilis*	*E. coli*	*P. aeruginosa*
Aqueous extract	8.4±0.21	13.6±0.49	12±0.48	10.9±0.3
Ethanolic extract	18.3±0.66	14.8±0.52	12.3±0.46	13.7±0.46
Pet. ether extract	10.1±0.21	8.4±0.18	6.6±0.22	7.9±0.22

Table 3: Evaluations of transferosomes for Vesicle size and Entrapment efficiency

Batch	Vesicle size	Entrapment efficiency
B1	346.37±3.21	54.28±2.32
B2	301.83±2.68	59.84±3.41
B3	287.19±1.97	62.93±2.18
B4	236.86±3.21	68.23±1.22
B5	212.78±3.89	65.29±2.27
B6	197.28±2.19	63.79±2.22

Table 4: Evaluation of Transferosomal Gel

Batches	pH	Spreadability	Drug Content	In-vitro Drug Release
B1	6.32±0.21	11.23±1.21	75.65±1.12	78.65±1.21
B2	6.21±0.22	10.95±0.98	78.12±0.96	86.96±1.28
B3	6.29±0.12	12.43±0.25	86.01±1.23	91.20±1.65
B4	6.92±0.29	13.39±1.11	87.18±1.09	93.01±1.88
B5	6.49±0.22	13.55±0.87	77.93±1.28	83.32±1.39
B6	6.59±0.27	12.98±0.94	70.19±0.96	76.12±2.01

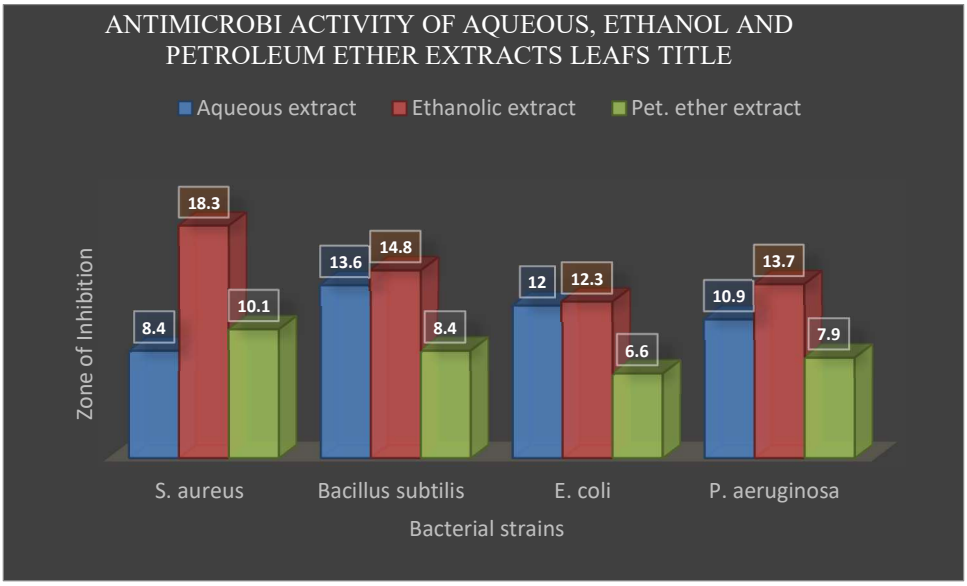

Figure 1: Bar diagram showing Antimicrobial activity of different extract

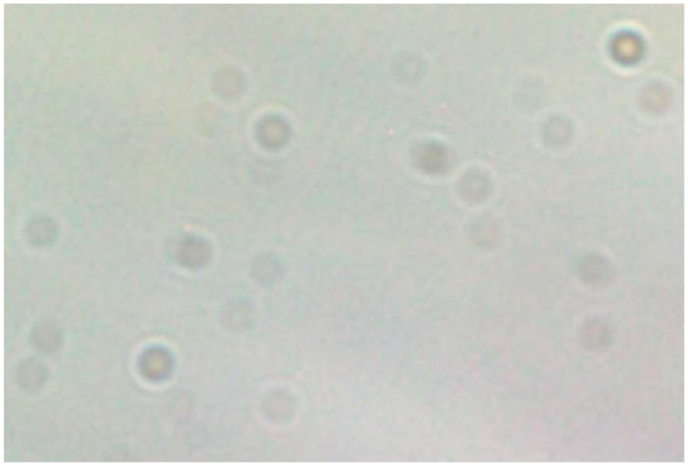

Figure 2: Microscopy image of transferosome

4. CONCLUSION

Current research work emphasizes on novel drug delivery system bearing herbal extracts of *Argemone mexicana* and we reached to the conclusion that novel herbal delivery systems increase the therapeutic efficacy by reducing toxicity but also increases the bioavailability. Remarkable antimicrobial activities observed against all the tested microorganisms. Solvent selection has been proved to be significant in extraction of herbal drugs and also, we reached to the conclusions that ethanol can act as potential solvent to extract antibiotic agents. These positive results are the basic outline for pharmaceutical industries to pay special attention on this plant for further studies.

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