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Original Research Article

PREPARATION AND EVALUATION OF ETHOSOMES OF MEDICINAL PLANT *TRIDAX PROCUMBENS* EXTRACT

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ABSTRACT

Since the beginning of time, medicinal herbs have been utilized in India to promote the health of both people and animals. However, there are drawbacks to their use, including decreased bioavailability, activity loss upon exposure to light and air, and worse patient compliance due to bad taste. The majority of issues related to the use of medicinal plants are addressed by the innovative drug delivery technology like ethosome. Thus this study deals with preparation and evaluation of ethosomes of medicinal plant *Tridax procumbens*. The plant material was collected & subjected to extraction by maceration method using aqueous solvent. Further the qualitative test & formulation, evaluation of herbal ethosomes was performed by standard methods. Results showed that plant contain alkaloids, glycosides, flavonoids, saponins, tannins and anthracyanin. Further, the prepared ethosomes noticed to be spherical in shape, uniform in size with smooth surface. The particle size of all four formulations was found within the range 1025.6 – 1916.7 nm. . Small vesicle size is formed with the F2 formulation having minimum concentration of lecithin i.e., 0.5%. In comparison with other formulations, TPF2 showed the less polydispersity index which indicated the homogenous population of ethosomes. So, among all four formulations TPF2 has better homogeneity. Zeta potential of the TPF formulations was found in the range -36.0 to -11.0 mV. The formulation TPF2 is more stable than others as it has the highest homogeneity and zeta potential. The λmax was found to be 243nm. Formulation TPF2 was found best one as it has the highest entrapment efficiency i.e. 94.92%. The entire study makes it abundantly evident that ethosomes made with Tridax procumbent extract exhibits a synergistic impact, & can produce outstanding results in the treatment skin infections

Keywords: Ethosomes, Transdemal drug delivery, Phytochemicals, Herbal medicines, Medicinal plants

INTRODUCTION

In the primary healthcare system, traditional medicine continues to be the most accessible and reasonably priced form of therapy for populations without access to modern drugs. Indigenous people have a long history of using traditional plants to treat illnesses. Phytochemicals are naturally occurring chemical substances that are physiologically active and are present in plants. They shield plant cells from environmental dangers such pollution, stress, dehydration, UV exposure, and pathogenic attack. These substances, also referred to as secondary plant metabolites, are

advantageous for human health. They are believed to work as synergistic agents, enabling the body to utilize nutrients more effectively. Low toxicity, low cost, easy accessibility, and biological properties like antioxidant activities, antimicrobial effects, modulation of detoxification enzymes, stimulation of the immune system, reduction of platelet aggregation, modulation of hormone metabolism, and antineoplastic properties are some of the advantages of phytochemicals (Chanda, 2014; Hussain *et al*., 2011).

There exist various methods for delivery of phytochemical inside the body but, In comparison to traditional medication delivery methods, such as oral and parenteral drug delivery, transdermal drug delivery has many advantages. One of the better options for maintaining stable plasma levels for extended periods of time is the transdermal route, which may also be favourable due to fewer frequent dosing schedules. The benefits listed include improved pharmacological and physiological response, decreased side effects, reduced utility of short half-life drugs, increased patient acceptability, avoidance of first pass metabolism, predictable and extended duration of activity, and prevention of drug level fluctuations. One such drug delivery system used for delivery of phytochemical through skin is ethosomes (Rastogi and Yadav, 2012; Mahale *et al*., 2011).

Ethosomes are lipid vesicles that contain phospholipids, alcohol (ethanol or isopropyl alcohol) in a relatively high concentration, and water. This lipidic vesicular system that contains ethanol. It has been claimed that ethosomes enhance the skin absorption of different medications. Propylene glycol has been used to prepare ethosomes, and these ethosomes demonstrated improved penetration efficacy. Alcohol and sodium cholate, two age-activator chemicals, significantly increase carrier penetration through the stratum corneum, enabling effective local and systemic distribution of both hydrophobic and hydrophilic substances. Ethosomes have been shown to be an effective transdermal delivery vehicle, and their enhancing impact is well known. Among the additives employed in ethosomal composition are phospholipids, polyglycol, alcohol, cholesterol, and colour (Kalra *et al*., 2020). Thus this study deals with preparation and evaluation of ethosomes of medicinal plant *Tridax procumbens* which is a particularly promising species, *Tridax procumbens,* is known to produce secondary metabolites with a range of purported medical benefits, including anaesthetic, anti-inflammatory, anti-diabetic, and anti-anemic activities. Various communities have traditionally used this plant in traditional ways (Andleeb *et al*., 2021).

MATERIALS AND METHODS

Collection of Plant

For the purpose of formulation of the *Tridax procumbens* ethosomes, plant leaves were collected from farms and besides the roads in Barshi region and the plant was authenticated in flora of Solapur district (Salahdeen *et al*., 2004).

Preparation of extract

Leaves of the collected plant were taken and washed with water, and then dried in shade for nearly 7 days. The leaves were powdered into coarse powder in blender.

The powder was dissolved in distilled water in proportion of 1:10 for the maceration. It was kept for 48hours with occasional shaking with mechanical shaker. It was then filtered by muslin cloth and kept for evaporation using water bath. The solid extract was obtained (Bhagwat *et al*., 2008).

Phytochemical screening of the extract

The extract was subjected for the qualitative phytochemical screening for the presence of the phytochemical constituents.

Preparations of Ethosomal Formulation

Four different formulations of *Tridax Procumbens* Ethosomes were prepared by cold method of preparation. The composition of ethosomes was as shown in the table. Measured quantities of phospholipid, cholesterol and drug were dissolved in ethanol in the 100 ml covered round bottom flask. It was vigorously stirred at 30°C. After 5 min, propylene glycol was added drop wise during the continuous stirring on magnetic stirrer. Water which was separately heated in 100 ml beaker at 30°C was added drop wise to the organic phase under constant mixing. The stirring was continued for another 30 min. The prepared suspensions were sonicated for 5 min at 30°C to decrease the vesicle size and stored in the refrigerator in the amber-colored glass bottle (Patrekar *et al*., 2015; Nandure *et al*., 2013).

Evaluation of ethosomal formulations

Microscopic examination: The surface morphology of the prepared ethosomal formulation was observed through the microscope to estimate the shape of the ethosomal vesicles.

Particle size and polydispersityindex: The vesicle size, polydispersity of prepared formulations of the ethosomes were measured by using the Horiba scientific sz-100 zeta potential analyzer windows [ztype] ver 2.40. The particle size of ethosomal formulations was determined by dynamic light scattering method. The particle size is determined by measuring the diffusion coefficient of nanoparticles. The sample was diluted in the proportion such that 10 mg sample is dissolved in 10ml of the distilled water. Then sonicated for 10min. and filtered through whatsman filter paper (Andlee *et al*., 2021). Filtrate is taken for analysis. The sample is kept in glass cuvette and run for 120 sec. The light from the laser light illuminates the sample and the scattered light is collected with the detectors at 90°C.

Zeta potential:

Zeta potential measures of the drug loaded ethosomes were done by using the Horiba scientific sz-100 zeta potential analyzer for windows [z type] ver 2.40. All formulations were analyzed at 25.0 ± 1°C. The filtrate for analysis was prepared as mentioned above and kept in cuvette (carbon 5mm electrode cell) run for 80 sec. View the reports in terms of positive or negative numerical values.

Determination of λ_{max}

Accurately weighed 10 mg of extract was dissolved in 10 ml of 0.1 N HCl in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipetted out and transfered into 10 ml volumetric flask and volume was made up with 0.1 N HCI. Suitable dilution was

made to a concentration range of 5-25 ug/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia).

Entrapment efficiency

The entrapment efficiency of the prepared ethosomes was determined by the centrifugation technique. The vesicles were separated in cooling centrifuge at 20000 rpm for an hour. The sediment and supernatant were separated. The absorbance of supernatant liquid for non entrapped extract was recorded at lambda max using UV visible spectrophotometer.

Table No 1: Different formulations of ethosomes

Formulation	Extract conc (%)	Phospholipid Conc (%)	Cholesterol Conc (%)	Propylene Glycol(ml)	Ethanol (ml)	Water (ml)
TPF1	2	0.5	2	10	20	q.s
TPF2	2	1	2	10	20	q.s
TPF3	2	1.5	2	10	20	q.s
TPF4	2	2	2	10	20	q.s

Table No. 2: Phytochemical screening of the extract

Sr. No.	Chemical Tests	Observations	Results
1	Alkaloids	Brownish red colour	Positive
2	Glycosides	Reddish brown colour	Positive
3	Flavonoids	Intense yellow colour	Positive
4	Saponins	Formation of foam	Positive
5	Anthracyanin	Pink red colour	Positive
6.	Tannin	Brownish green colour	Positive

Table No. 3: Results of particle size analysis of prepared formulations

Sample	Mean	S.D	Mode
TPF1	1025.6 nm	233.3 nm	1000.2 nm
TPF2	1078.5 nm	195.2 nm	1014.5 nm
TPF3	1281.3 nm	332.5 nm	1141.1 nm
TPF4	1916.7 nm	570.8 nm	1646.9 nm

Table No. 4: Particle size, polydispersity index and zeta potential of prepared formulations

Formulation code	Particle size	Polydispersity index	Zeta potential
TPF1	1025.6 nm	0.731	-3.3 mV
TPF2	1078.5 nm	0.626	-36.0 mV
TPF3	1281.3 nm	0.721	-16.0 mV
TPF4	1743.5nm	0.995	-11.0mV

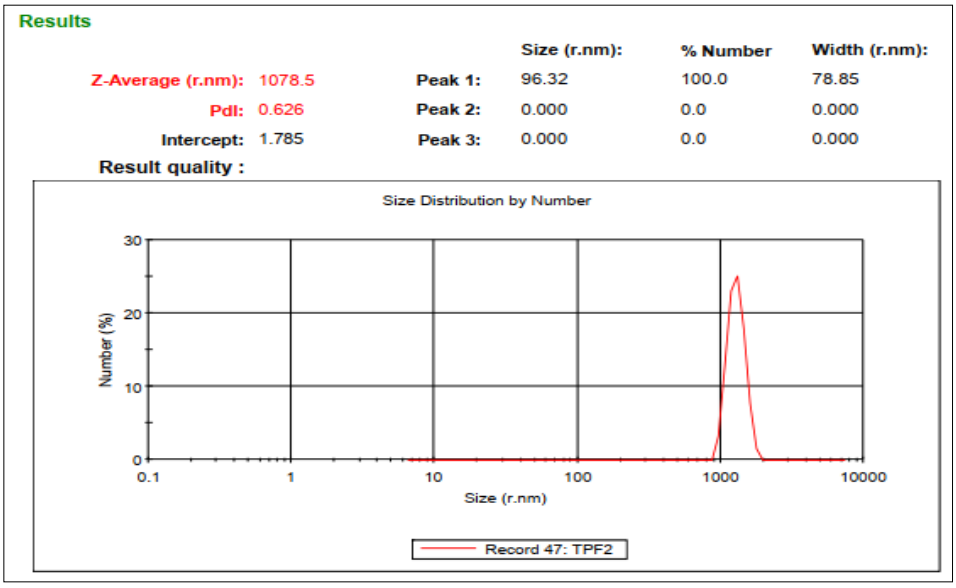

Figure 1: Particle size, polydispersity index of formulation TPF2

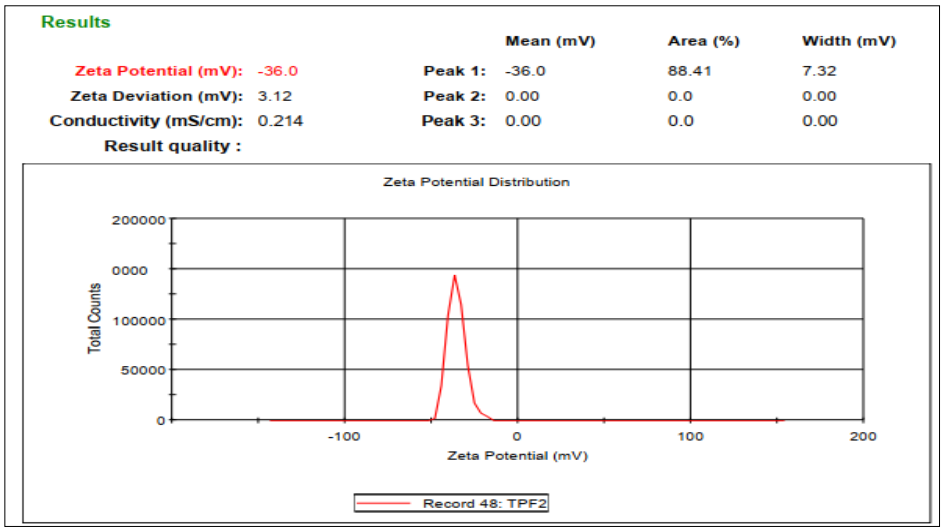

Figure 2: Zeta potential of formulation TPF2

Table No. 5: Result of Lamda max determination

Name of sample	Lambda max
Tridax procumben extract	243nm

Table No. 6: Entrapment efficiency of drug loaded ethosomes

Formulation Code	Entrapment Efficiency (%)
TPF1	81.24
TPF2	94.92
TPF3	91.65
TPF4	84.01

RESULTS AND DISCUSSION

The phytochemical analysis showed the presence of the Alkaloids, glycosides, flavonoids, saponins, tannins and Anthracyanin in aqueous extract of *Tridax procumbens*. The morphological analysis by microscope revealed that Ethosomal vesicles to be spherical in shape, uniform in size with smooth surface. The particle size of all four formulations was found within the range 1025.6 – 1916.7 nm. Small vesicle size is formed with the F2 formulation having minimum concentration of lecithin i.e., 0.5%.

Polydispersity is the size distribution of the vesicles within the formulation. In comparison with other formulations, TPF2 showed the less polydispersity index which indicated the homogenous population of ethosomes. So, among all four formulations TPF2 has better homogeneity. Zeta potential of the TPF formulations was found in the range -36.0 to -11.0 mV. The formulation TPF2 is more stable than others as it has the highest homogeneity and zeta potential. The λ_{max} was found to be 243nm. Formulation TPF2 was found best one as it has the highest entrapment efficiency i.e. 94.92%.

From the aforementioned findings, it can be concluded that when the other ingredients are held constant, variations in the concentration of lipid have a substantial impact on entrapment efficiency as well as particle size. The results of all the parameter show that the formulations have the ideal pH and viscosity, making them ideally suited for cutaneous therapy.

CONCLUSION

Novel drug delivery system incorporated various plant constituents showed enhanced therapeutic effect also increase the bioavailability of the drug. Thus, development of novel drug delivery system

for valuable herbal drugs has great potential as they provide the efficient and economical drug delivery. Ethosomes loaded with the drug *Tridax procumbens* which has the great therapeutic value have been prepared and evaluated. Ethosomes can be used as novel drug delivery tool in various health disorders and can potentiate their antimicrobial effect.

Ethosomes having the herbal drug *Tridax procumbens* have been prepared. The characterization of the prepared ethosomes have been done for the particle size, polydispersity, zeta potential and drug entrapment which shows that the formulation TPF2 is best as it has better stability, lower particle size and most negative zeta potential.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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