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FORMULATION AND *IN-VITRO* ANTIMICROBIAL EVALUATION OF HERBOSOMAL GELS OF EXTRACTS OF *QUERCUS INFECTORIA* AND *ACORUS CALAMUS*

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ABSTRACT

The emerging technology of drug delivery is being applied to phyto-pharmaceuticals to improve the bioavailability of herbal extracts for medicinal applications. Plant extracts can be standardized accordingly and may be formulated as phytosomes for systematic investigation for any improved potential use. Phytosomal herbal formulations are better absorbed, and as a result produce better bioavailability and actions than the conventional phytomolecules or botanical extracts. The term “Phyto” means plant while “some” means cell-like. It is also often known as herbosomes. Phytosomes are produced by a process whereby the standardized plant extract or its constituents are bound to phospholipids, mainly phosphatidylcholine producing a lipid compatible molecular complex. This phyto-phospholipid complex i.e. phytosome resembles a little cell. Phytosomes exhibit better pharmacokinetic and pharmacodynamic profile than conventional herbal extracts. These drug-phospholipid complexes can be formulated in the form of solution, suspension, emulsion, syrup, lotion, gel, cream, aqueous micro dispersion, pill, capsule, powder, granules and chewable tablet.

KEY WORDS

Herbosomal gel, Bioavailability and Herbal extracts.

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INTRODUCTION

It is widely accepted that the use of herbal medicine is well established and safe¹. Herbal medicine, now-a-days are gaining importance for treating many diseases due to their significant effect and lesser side effects as compared to allopathic medicines². The goal of topical antimicrobial therapy in skin infections is to control microbial colonization and subsequent proliferation thus promoting the healing of the wounds³. In this research, *Quercus infectoria* is a small tree or a shrub belonging to the Fagaceae

(Quercaceae) family and here it was studied in order to investigate its antimicrobial properties. Gall of *Quercus infectoria* (QI), or better known as Manjakani, is originated from Western Asia and Southern Europe. Galls are irregular plant growth, which is stimulated by the reaction between plant hormones and powerful growth regulating chemicals produced by insects or mites⁴. The QI galls are produced by the insect, *Cynipsquercuifolii*, for depositing its eggs⁵. The chemical constituents of the galls have been reported to comprise a large amount of tannins and small amounts of free gallic acids, ellagic acid and synergic acid⁶. *Acorus calamus* Linn. belongs to the family Acoraceae, commonly known as “sweet flag” or “calamus”, is a semiaquatic, perennial, aromatic herb with creeping rhizomes. The plant is found in the northern temperate and subtropical regions of Asia. *Acorus Calamus* (AC) has been used in traditional Indian prescriptions for its beneficial effects on antiproliferative^{7, 13}, antidiarrhoeal, antioxidant⁸ and hypolipidemic activity⁹. The aim of the present study was to screen for the aqueous and ethanolic extracts of AC that could be useful for the development of new tools as antibacterial agents for the control of infectious diseases. The herbosome process has been applied to many popular herbal extracts, including *Milk thistle*, *Ginkgo biloba*, *Grape seed*, *Green tea*, *Hawthorn*, *Ginseng* etc. The herbosome technology, markedly enhances the bioavailability of select phytomedicines, by incorporating phospholipids in to standardized extracts and so vastly improve their absorption and utilization¹⁰.

MATERIALS AND METHOD

Preparation of extracts

The galls of *Quercus infectoria* and rhizomes of *Acorus calamus* used in this study were collected and washed with distilled water, and dried in air. The galls were crushed in mechanical mortar. Ethanol extracts were prepared by the following method. 50 gm of gall and rhizome powders were used with 300 ml of solvents, separately with an extraction period 24-72 hours. The extracts were filtered using filter paper and the solvents were evaporated using rotary

distillation apparatus. In order to obtain a completely dry extract, the resultant extracts were transferred to glass dishes, and were left in 50 C° ovens for 24 hours. Then, they were left¹⁴ at 4 C°.

Formulation of Phytosomal Gel

Herbosomes are prepared by reacting the herbal extract and soy lecithin and cholesterol in a ratio of 1:1 and dissolving them in ethyl acetate. After solubilization has completed, the complex compounds are removed by solvent evaporation technique. Gel was prepared by using Carbopol 940 as the gelling agent. Then the prepared herbosomes were incorporated into the gel and thus the herbosomal gel was obtained.

Physicochemical Evaluation of Phytosomal Gel

Physical Evaluation

Preliminary evaluation was carried out as follows:-

pH

The pH meter was calibrated using standard buffer solution such as pH 4.0 and 7.0. About 0.5g of the gel was weighed and dissolved in 50 ml of distilled water and its pH was measured.

Viscosity

Viscosity of the formulation was determined by Brookfield Viscometer at 100 rpm, using spindle no 7.

Homogeneity

The formulations were tested for the homogeneity by visual appearance and by touch.

Spread ability

Two glass slides of 20 x 20 cm were selected. A small amount of sample was sandwiched between the two glass slides. A 100g weight was placed upon the upper slide so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and then fixed to a stand without slightest disturbance in such a way that only the upper slide, slides off freely, to the force of weight tied to it. The time taken for the upper slide to separate away from the lower one was noted using a stop clock. This parallel plate method is the most widely used method for determining and quantifying the Spread ability of semisolid preparations.

$$S = M \times L/T$$

Where, S – Spread ability; M - Weight tied to the upper slide; L - Length of the glass; T - Time taken in seconds.

Extrudability

It is an empirical test to measure the force required for the gel to extrude out from the tube. The prepared cream was filled into a collapsible tube and it was sealed and the weight of the tube was recorded. Placed a 500g weight on the tube and the amount of gel that extruded out was collected and weighed. Then the percent of gel extruded was calculated. The packing of gel have gained a considerable importance in the delivery of desired quantity of cream, therefore measurement of Extrudability have become an important criteria for creams.

Characterization of Herbosomal Gel

The behavior of Herbosomes in both physical and biological system is governed by the factors such as physical size, shape, stability and its distribution.

Visualization

Visualization of Herbosomes can be achieved by using scanning electron microscopy (SEM).

Vesicle size and Zeta potential

The particle size and zeta potential can be determined by Dynamic Light Scattering (Nicomp 380 DLS) using a computerized inspection system and photon correlation spectroscopy.

Vesicle stability

The stability of vesicles can be determined by assessing the size of the vesicles overtime. Mean size is measured by DLS (Nicomp 380 DLS).

Stability studies

Stability studies were performed according to the ICH (International Conference on Harmonization) guidelines. The optimized formulation was kept at two different temperatures, i.e. $30 \pm 2^{\circ}\text{C}$ and $4 \pm 2^{\circ}\text{C}$ for 90 days.

Comparison of the Optimized Herbosomal gel with marketed Anti-microbial Gel

The prepared antimicrobial Herbosomal gel was compared with a standard drug Gentamycin ($1\mu\text{g/ml}$) and Nystatin ($1\mu\text{g/ml}$) as antibacterial and antifungal standard drug.

RESULTS AND DISCUSSION

Herbal medicines are the important tool for many diseases because of their less side effects, compared to allopathic medicines.

So medicinal plants extracts with antibacterial and antioxidant potential were utilized for the development of herbosomal gel formulation and assessment of *in-vivo* skin permeation was the main approach.

Formulation and Evaluation of Herbosomal Gel

Herbosomal gels were prepared by using carbopol 940as gelling agent. Physical evaluation like pH, Viscosity, Homogeneity, Spreadability, Extrudability, were determined as shown in Table No.1.

Physical Evaluation of Phytosomal Gel

The ethanolic extracts of were formulated into a herbosomal gel and all studied formulations did not show a considerable change in characters like color, odor and consistency with the viscosity of 78, 000-98, 000cPs and homogenous. The values are represented in the Table No.1. This showed that in the case of a gel, the consistency depended on the ratio of the solid fraction, which produced the structure of the liquid fraction. The spreadability of the formulation is recorded in Table No.1. The spreadability of the formulations was acceptable. The extrusion of the topical formulation from the tube is important during the application, as also patient acceptance. The study showed that the extrudability of the gel was comparatively better than the ointment.

Characterization of Herbosomal Gel

Visual Inspection

The prepared Herbosomal gel was visually inspected and it showed that the formulation was homogeneous without any gritty particles and was of optimum consistency.

Globule Size Determination

Microscopic Evaluation

The prepared antimicrobial Herbosomal gel was observed under optical microscope at 100x and observed that the formed vesicles were of uniform size.

Size and Size distribution

The vesicular size and size distribution was evaluated by using dynamic light scattering, the results showed that increase in extract phospholipid concentration increases the mean vesicular size. The vesicular size was between 52nm-115nm. The polydispersity index was found to be low, shows that the particles were of low value shows that Herbosomes formed by hydration was of uniform size.

Zeta Potential Determination

The magnitude of zeta potential gives a potential stability of the colloidal dispersion. If the particles have, large positive or negative charge reveals that they repel each other and there is dispersion stability. The zeta potential of the optimized formulation showed that the sample is sample is highly stable. It was found as -22.21, and hence this indicates that the prepared formulation is stable.

Scanning Electron Microscopy

From the Figure 6, it is clear that the particle size of the optimized formulation was confirmed to be 52-115nm. This was in accordance with the particle size of Herbosomes in the literature.

Stability Studies

The best formulation of the Herbosomal gel was kept at varying conditions of temperature. The system was stable at 25⁰C. There were no significant changes in the formulation when kept at room temperature (30 ±2⁰C) and also at refrigerated temperature (4 ±2⁰C). Not much change of pH, viscosity, homogeneity, Spreadability, Extrudability and degradation of the samples were observed during 45 days period. Table 2 shows the datas for stability studies for antimicrobial Herbosomal gel F2. There was no much change in the zeta potential of the sample and this proves that the Herbosomal gel system remains stable.

Table No.1: Physical parameters of the formulation

S.No	Formulation Code	pH	Viscosity (cp)	Homogeneity	Spreadability (gm/sec)	Extrudability
1	F1	6.0	26155	Homogeneous	38.1	94.85
2	F2	60.4	39481	Homogeneous	36.9	95.21
3	F3	60.6	37182	Homogeneous	21.3	93.29
4	F4	60.4	31094	Homogeneous	23.8	96.77
5	F5	60.4	27165	Homogeneous	27.7	94.28
6	F6	60.4	28920	Homogeneous	33.9	98.65
7	F7	60.6	22371	Homogeneous	27.5	93.43
8	F8	60.7	59172	Homogeneous	26.4	92.48
9	F9	60.5	56193	Homogeneous	24.3	91.22

Table No.2: Size Distribution and Polydispersity of Formulations

S.No	Formulation Code	Vesicular size(nm)*	Polydispersity**
1	F1	52.44±31.45nm	0.145
2	F2	53.23±36.79nm	0.1453
3	F3	98.28±48.36nm	0.1621
4	F4	65.45±25.46nm	0.08335
5	F5	76.56±46.79nm	0.1478
6	F6	107.89±36.57nm	0.1759
7	F7	103.28±21.45nm	0.1109
8	F8	115.14±26.79nm	0.1982
9	F9	110.23±21.34nm	0.1050

*Data obtained from Nicomp 380 DLS, **PI=Standard deviation/mean vesicular size

Table No.3: Stability Studies of Herbosomal Gel, F2

S.No	Months	Temperature	Formulation	Parameters				
				pH	Viscosity	Homogeneity	Spreadability	Extrudability
1	0	RT 30 ⁰ c±2 ⁰ c	2	6.4±0.2	27634±	Homogeneous	35.7±0.2	90.9±0.9
		4 ⁰ c±2 ⁰ c	2	6.5±0.1	26889±	Homogeneous	25.4±1.1	93.1±1.0
2	1	RT 30 ⁰ c±2 ⁰ c	2	6.5±0.1	31279±	Homogeneous	36.1±1.1	90.3±1.2
		4 ⁰ c±2 ⁰ c	2	6.3±0.1	27736±	Homogeneous	24.3±1.1	92.5±1.0
3	2	RT 30 ⁰ c±2 ⁰ c	2	6.3±0.2	39127±	Homogeneous	37.1±1.1	93.5±0.9
		4 ⁰ c±2 ⁰ c	2	6.2±0.1	48193±	Homogeneous	26.7±1.1	92.5±0.9
4	3	RT 30 ⁰ c±2 ⁰ c	2	6.1±0.1	37162±	Homogeneous	34.2±1.3	91.5±1.2
		4 ⁰ c±2 ⁰ c	2	6.8±0.1	51092±	Homogeneous	31.5±0.8	93.2±0.8

Parameters are derived using mean ± SD

Table No.4: Comparison Antimicrobial Activity (Agar Well Diffusion Method)

S.No	Formulation		INHIBITION ZONE (Mean± SD,n=3)			
			<i>Staphylococcus aureus</i> (ATCC29737)	<i>Escherichia coli</i> (ATCC2068)	<i>Pseudomonas aeruginosa</i> (ATCC9027)	<i>Candida albicans</i> (ATCC10231)
1	Phytosomal Gel		14.0±0.	15.0±0.	15±0.	20.0±0.
2	Positive Control	Gentamycin (1µg/ml)	22±0.0	21±0.0	23±0.0	NT
		Nystatin (1 µg/ml)	NT	NT	NT	16±0.0
3	Negative control	AQ, ET, PT	-----	-----	-----	-----

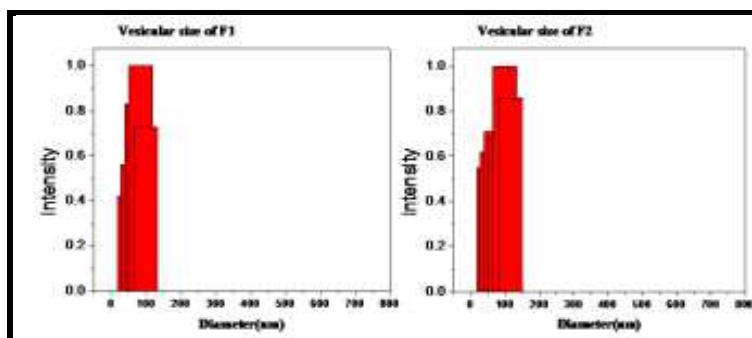


Figure No.1: Vesicular Size of Herbosome F1-F2

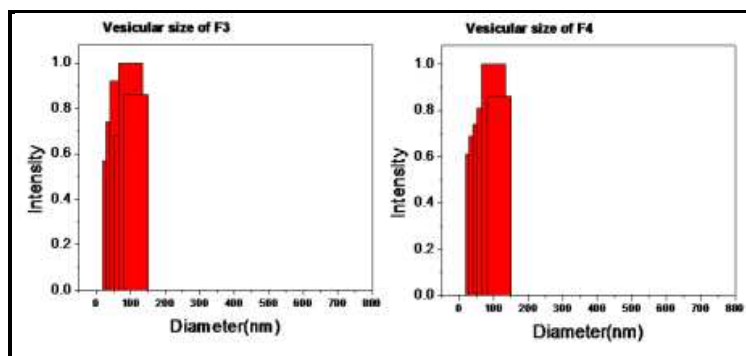


Figure No.2: Vesicular Size of Herbosome F3-F4

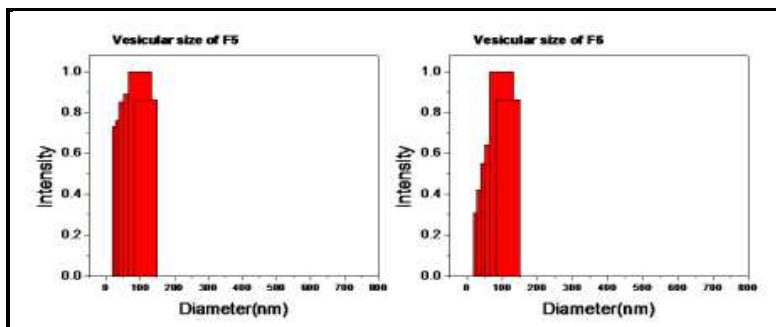


Figure No.3: Vesicular Size of Herbosome F5-F6

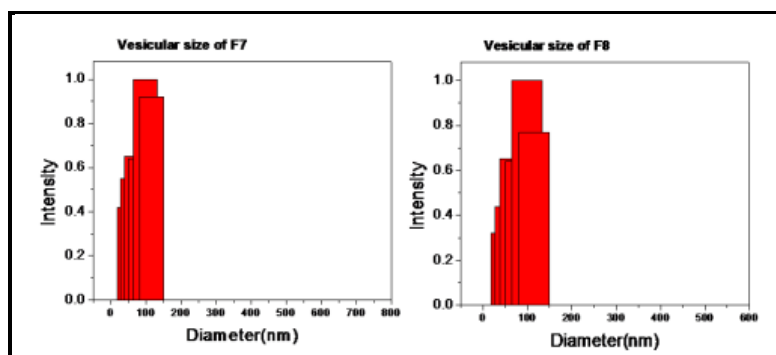


Figure No.4: Vesicular Size of Herbosome F7-F8

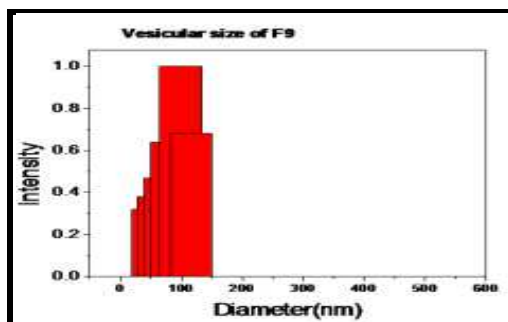


Figure No.5: Vesicular Size of Herbosome F9

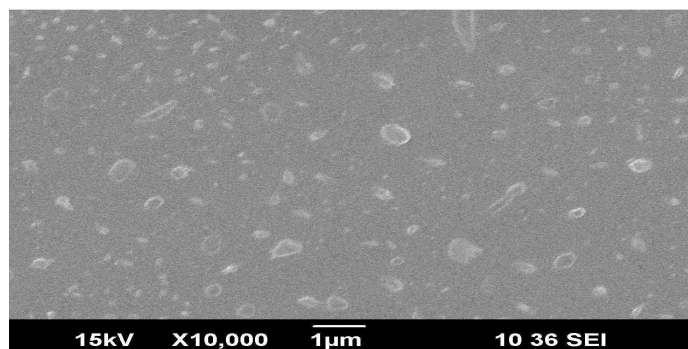


Figure No.6: Scanning Electron Microscopy of Optimized Formulation

CONCLUSION

The above article gives an outline about the preparation and comparison of Herbosomes. This technology of drug delivery aids to explore maximum therapeutic potential of plant constituents of polar nature exhibiting remarkable therapeutic efficacy. Herbosomal Gels containing *Quercus infectoria* and *Acorus calamus* were successfully prepared. Physical evaluation, Characterization and Stability studies of the prepared gels were done and compared with the marketed preparation.

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