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Evaluation of phytosomes containing Ethanolic extract of Aerial parts of *Mukia maderaspatana*

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Abstract : The aim of the present investigation was to formulate *Mukia maderspatana* loaded Phytosome for improved delivery. Phytosomal formulations were developed using different concentration of Cholesterol (1-3%) then optimized and characterized. Particle size, entrapment efficiency and vesicular shape were determined by Malvern Zetasizer, and Scanning Electron Microscopy, respectively. Particle size varied from 175 to 510 nm depending on the concentrations of Cholesterol. Entrapment efficiencies were exhibited of 38.42-84.26%, where it increased with concentration of cholesterol increased. Photomicrographs revealed that optimized Phytosomes were spherical in shape and uniform in size. Based on minimum particle size and maximum entrapment efficiency F9 (3% of Cholesterol concentration and 40% of ethanol concentration) was selected as optimization Phytosomal formulation.

Key Words : *Mukia maderspatana*, Optimization, Characterization, Phytosome.

Introduction:

Mukia maderaspatana, *Cucumis maderaspatana* or *Mukia scabrella* (family: Cucurbitaceae) is an annual monoecious herb, densely covered with white hairs. It is found throughout India ascending up to 1800 m in the hills. Mukia is rich in sugars, namely, arabinose, fructose, glucose, mannose, sucrose, xylose, galactose and ribose, together with uncharacterized steroids, triterpenes, alkaloids, phenols, glycoflavones, catechins and saponins[1]. Folklore medicine claims that it is a good diuretic, stomachic, antipyretic, and antiflatulent, antiasthmatic, and antibronchitis, hepatoprotective[2] and immunomodulatory effects[3] and antiarthritic activity properties[4]

Phytosome are more bioavailable as compared to simple herbal extracts owing to their enhanced capacity to cross the lipid rich biomembranes and finally reaching the blood. The lipid-phase substances employed to phytoconstituents, lipid compatible are phospholipids from so, mainly phosphatidylcholine (PC). Phospholipids are complex molecules that are used in all known life forms to make cell membranes. They are cell membrane building blocks, making up the matrix into which fit a large variety of proteins that are enzymes, transport proteins, receptors and other biological energy converters. In humans and other higher animals the

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phospholipids are also employed. They are miscible both in water and in oil/ lipid environments, and are well absorbed orally. Phospholipids are small lipid molecules in which the glycerol is bonded only to two fatty acids, instead of three as in triglycerides, with the remaining site occupied by a phosphate group. Most of the bioactive constituents of phytomedicines are flavonoids. However many flavonoids are poorly absorbed. It is a complex between a natural product and natural phospholipids, like soy phospholipids. Such a complex is obtained by reaction of stoichiometric amounts of phospholipids and the substrate in an appropriate solvent. In present status various dosage formed of herbal formulation are available in the market but the main problem regarding herbal formulation is it bio-availability, herbal formulation has less bio availability, without affection there safety parameter[5].

Phytosome protect valuable component like flavanoids (water soluble) of herbal extract from destruction by digestive secretion because of which they shows better absorption which produces better bioavailability. To improve and pharmacological and pharmacokinetic parameters of *Mukia maderaspatana* in the present study the water soluble phytoconstituents are loaded as phytosomes. Plant flavanoids have effect on immune and inflammatory cell function.[6,7]

Solubility studies

Solubility of plant extract was carried out in different solvents like 0.1N HCL, Water and 6.8 and 7.4 pH buffer, Saturated solutions were prepared by adding excess extract to the vehicles and shaking on the shaker for 24 hrs at 25°C under constant vibration. Filtered samples (1ml) were diluted appropriately with suitable buffer and was determined spectrophotometrically.

Determination of Absorption maxima by UV spectrophotometer:

Solution of plant extract was prepared in Methanol and scanned in the range of 200 to 400 nm UV spectrophotometer **Preparation of calibration curve of Metronidazole:**

1 ml of plant extract was dissolved in 10 ml of methanol by slight shaking (1000 mcg/ml). 1 ml of this solution was taken and made up to 10 ml with methanol, which gives 100 mcg/ ml concentration (stock solution). From the stock solution, concentrations of 2, 4, 6, 8, 10 and 12 µg/ml in methanol were prepared. The absorbance of diluted solutions was measured at 238 nm and a standard plot was drawn using the data obtained. The correlation coefficient was calculated.

Method of Preparation of Phytosomes[8,9]:

Selection of lipid and method for preparation of Phytosome:

For the present study cholesterol used as a lipid for the preparation of phytosome of *Mukia maderspatana* Ethanol in various concentrations (20% to 30%) was taken in 25ml beaker. cholesterol in various concentrations (1% to 3%) was dissolved in ethanol by mixing using magnetic stirrer (REMI stirrer) at 300C temperature. 2% of propylene glycol and 2% complex were dissolved in this ethanolic mixer. While stirring at 700rpm, Ethanolic mixer was added in aqueous solution by using 24 gauge hypodermic syringe at constant rate. After addition of ethanolic mixer stirring was continued for additional 30min. Finally, the formulation was stored under refrigeration at 4^0C.

Different batches of Phytosomal formulation

Formulation Code	Drug+polymer complex	Propylene glycol	Ethanol	Water
F1	1:1	2%	20	Q.S
F2	1:2	2%	20	Q.S
F3	1:3	2%	20	Q.S
F4	1:1	2%	30	Q.S
F5	1:2	2%	30	Q.S
F6	1:3	2%	30	Q.S
F7	1:1	2%	40	Q.S
F8	1:2	2%	40	Q.S
F9	1:3	2%	40	Q.S

Evaluation Parameters of Phytosomes:[10,11]

Visual Appearance and Clarity Visual appearance and Clarity was done under fluorescent light against a white and black back ground for presence of any particulate matter.

pH Measurement The pH measured using pH meter.

Particle Size and Size Distribution: The particle size and size distribution of Phytosome were determined by Malvern Zetasizer based on laser light scattering principle.

Determination of drug content Accurately 10 ml of formulation from different batches was measured and transferred to 100 ml volumetric flask. To this 50-70mL of 0.1 N HCL was added and sonicated for 30 min. Complete dispersion at maximum absorbance at 238 nm using UV-Visible Spectrophotometer

Entrapment efficacy:

Entrapment efficiency of phytosomal vesicles were centrifuge in (REMI ultra-centrifugation) at 10,000 rpm for 90 minutes at a temperature maintained at 4ºC. The absorbance of the drug was noted at 238 nm by UV analysis.

%Entrapment efficiency= Drug content *100/Drug added in each formulation

In-Vitro Release Studies

In vitro drug release studies were carried out by putting the formulation on Millipore membrane filter (0.15 mm) between the donor and receptor compartments of an all-glass modified Franz diffusion cell The receptor compartment of an all-glass modified Franz diffusion cell was filled with 10 mL freshly prepared 7.4 pH, and all air bubbles were expelled from the compartment. An aliquot (1 mL) of test solution was placed on the Millipore membrane filter, and the opening of the donor cell was sealed with a glass cover slip. The receptor fluid was kept at 37 ± 0.5°C with constant stirring using a Teflon-coated magnetic stir bead. Permeation study was continued for 10 hr, and samples were withdrawn from receptor and analyzed for drug content by measuring absorbance at 238 nm in a spectrophotometer.

Kinetics of Drug Release

The mechanism of drug release for the formulated phytosomes was determined using zero order and first order.The results of in vitro release profile obtained for all the formulations were plotted in modes of data treatment as follows:-

1. Zero – order kinetic model – Cumulative % drug released versus time.
2. First – order kinetic model – Log cumulative percent drug remaining versus time.

Zero Order Kinetic

It describes the system in which the drug release rate is independent of its concentration.

$Qt = Qo + Ko\ t$
Qt= Amount of drug dissolved in time t
Qo = Initial amount of drug in the solution, which is often zero
Ko = zero order release constant.

If the zero order drug release kinetic is obeyed, then a plot of Qt versus t will give a straight line with a slope of Ko and an intercept at zero.

First Order Kinetic

It describes the drug release from the systems in which the release rate is concentration dependent.

$Log\ Qt = log\ Qo + kt/\ 2.303$

Where,

Qt = amount of drug released in time t.

Qo = initial amount of drug in the solution k = first order release constant If the first order drug release kinetic is obeyed, then a plot of log (Qo- Qt) versus t will be straight line with a slope of kt/ 2.303 and an intercept at t=0 of log Qo.

Results and Discussion

Saturation Solubility of Phytosomes: Solubility of Phytosomes was determined in water, 0.1 N HCL, & 6.8 and 7.4 pH phosphate buffer

Table no.1

S.No	Buffer	Solubility
01	WATER	0.097
02	0.1 N HCL	0.124
03	6.8 PH Buffer	0.147
04	7.4 ph Buffer	0.285

Solubility studies

Discussion:

Phytosomes has more solubility in 7.4pH buffer.

Determination of absorption maximum (λmax) of Phytosomes:.

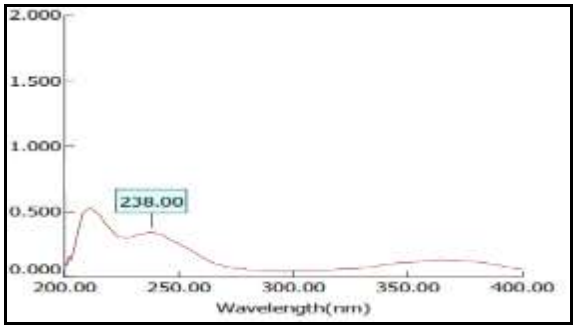


Figure no:1 Absorption maximum (λmax) of phytosomes

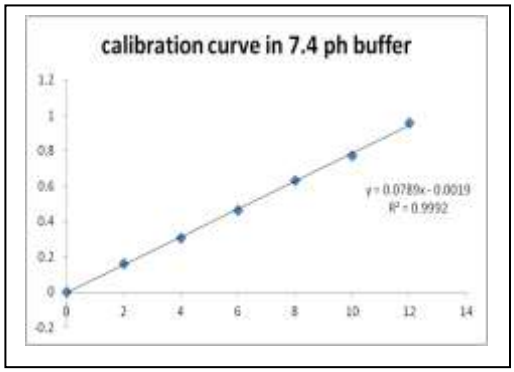


Figure no.2

Standard calibration curve of Phytosomes:

Table no.2

Concentration	Absorbance
0	0
2	0.164
4	0.309
6	0.463
8	0.632
10	0.773
12	0.959

Discussion:

The regression value was closer to 1 indicating the method obeyed Beer-lamberts' law as it was linear.

Table no.3

Formulation	Color	Homogeneity	Consistency
F1	Pale yellow	Excellent	Excellent
F2	Pale yellow	Excellent	Excellent
F3	Pale yellow	Excellent	Excellent
F4	Pale yellow	Excellent	Excellent
F5	Pale yellow	Excellent	Excellent
F6	Pale yellow	Excellent	Excellent
F7	Pale yellow	Excellent	Excellent
F8	Pale yellow	Excellent	Excellent
F9	Pale yellow	Excellent	Excellent

Particle size :

The Particle Size Of The Formulated Batches Were Given In The

Table no.4

Formulation code	Particle size(nm)
F1	459
F2	310
F3	175
F4	486
F5	342
F6	209
F7	510
F8	418
F9	312

Entrapment Efficiency:

The Entrapment Efficiency was found to be in the range of 38.42-84.26%

Table no.5

Formulation code	Entrapment Efficiency
F1	38.42
F2	47.21
F3	56.32
F4	52.12
F5	62.63
F6	78.28
F7	56.63
F8	72.81
F9	84.26

Entrapment Efficiency values

Discussion:

The % entrapment for prepared formulations were found to be in the range of 38.42-84.26%

pH Measurement The pH of formulations was found in the range of 6.0-6.8.

Table no.6

Formulation code	pH
F1	5.8
F2	6.2
F3	6.0
F4	6.6
F5	6.4
F6	6.2
F7	5.9
F8	6.2
F9	6.0

pH values

Discussion : The pH of formulation F1-F9 was found to 5.8-6.6

Morphology determination by scanning electron microscopy (SEM):

It was observed that the phytosomes were spherical, and uniform with no drug crystals on the surface.

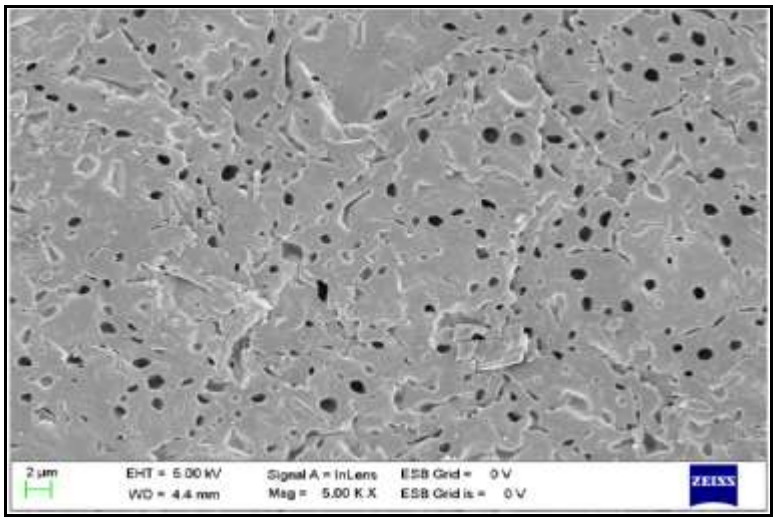


Fig no.3 Phytosome structure optimized formulation (F9)

Invitro drug release study:

The *in vitro* release study conducted at 7.4pH for period of 12 hours. The highest drug release of 96.19 % was observed with formulation F9.

Table no.7:

TIME	F1	F2	F3	F4	F5	F6	F7	F8	F9
0min	0	0	0	0	0	0	0	0	0
1hr	29.81	23.26	21.16	28.67	25.36	24.81	20.21	19.80	16.16
2hr	36.92	32.12	29.61	37.33	33.21	31.24	34.82	30.26	22.04
3hr	48.07	43.37	35.67	45.11	40.28	39.06	39.21	36.21	35.73
4hr	54.81	52.92	49.59	51.86	48.65	42.12	52.36	48.20	43.02
5hr	66.23	62.47	55.93	60.74	54.24	49.89	61.98	59.36	51.97
6hr	72.58	76.12	73.46	72.86	68.21	58.25	72.21	66.24	63.37
7hr	86.08	88.46	83.4	78.41	72.92	69.11	79.99	76.21	79.47
8hr	90.23	98.23	94.35	82.22	86.26	79.97	84.26	89.96	88.28
10hr	99.18		98.26	94.28	92.26	88.46	82.98	96.26	94.86
12hr				99.89	98.82	97.26	99.26	98.92	96.19

Table: invitro drug release

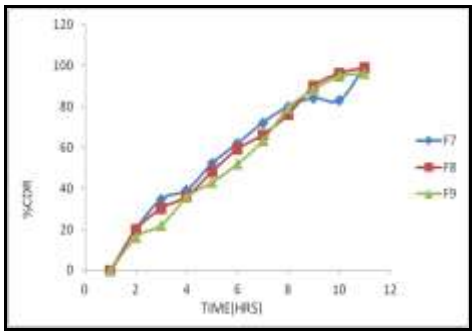


Figure no. 4 Invitro dissolution profile graphs:

Drug release kinetic studies:

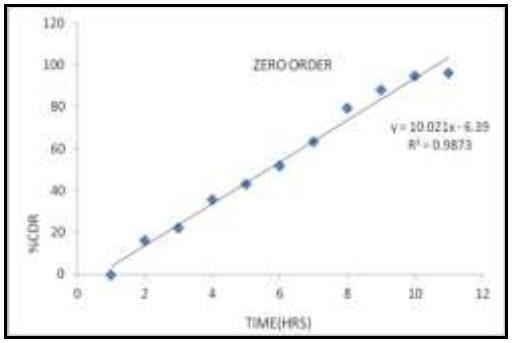


Figure no. 5 zero order release kinetics:

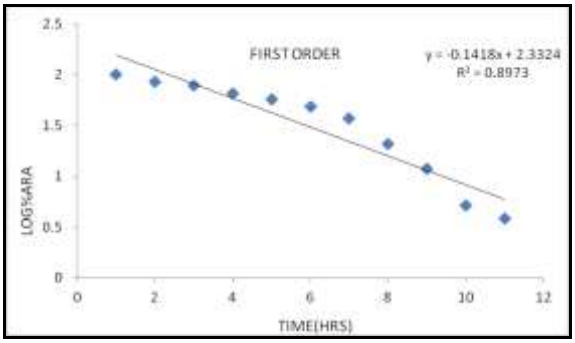
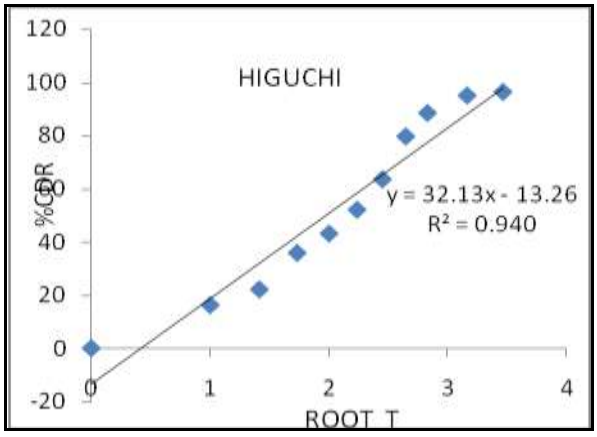


Figure no. 6 First order release kinetics:

Higuchi release plot:



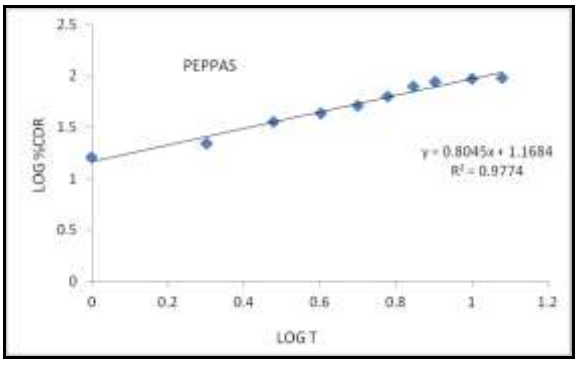


Fig no.7 higuchi release graph **Fig no.8 peppas release graph**

Table no: 8

R^2 values					**n values**
Formulation	**Zero order**	**First order**	**Higuchi**	**Korsmeyer – Peppas**	**Korsmeyer-Peppas (n)**
F9	**0.987**	**0.897**	**0.940**	**0.9774**	**0.804**

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