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Development of phytosomes of *Agele marmelos* and *Pterocarpus marsupium*

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Abstract
Plant extract have excellent bioactivity but less *in-vivo* action due to poor lipid solubility, variating molecular size and destruction in gut phytosome is a non-vesicle compound in which plant extract and phospholipid are combine to form a more soluble complex provide better absorption bio availablity is increase due to capacity of extract to cross lipid rich bio membrane and protect destruction of valuable component of extract by digestive secretion phytosome phytosome have capacity to delivery the standard dose of extract through different route of administration the purpose of this study is to formulate phytosome of bael fruit extract with good physiochemical properties so can improove the stability and properties of plant extract during present investigation physiochemical properties of selected plant were studied phytosome was prepared by solvent precipitation method and evaluated by X-ray, FTIR, Opttical microscopy, particle size and zeta potential the study also suggest that phytosome is act as promising drug delvery system in delivering plant constituent of medical value.

Keywords: phytosome, phospholipid, bioavailability, cholesterol

Introduction
Herbal medicine are popular health care choice not only for health maintenance but also for enhancement of function or processes of human body medicinal plant constituent are mostly water soluble and having a size greater than lipid soluble constituent [1]. Water soluble constituent are poorly absorbed because of their size limitation and do not cross the lipid rich cell membrane causes low bioavailability [2].
Phytosome a technology developed for drug and nutraceutical to integrate water soluble phytoconstituent into phospholipids to produce lipid compatible molecular complex known as phytosome with improove bioavailability and absorption behavior [3].
Bael (*Agele marmelos*) is one traditional and imperative plant of family Rutacae the various phytoconstituent present in fruit are carbohydrates, protein, fiber, fat, calcium, phosphorous, potassium, minerals and vitamins, steroids, terpenoids, flavanoids, phenolic compound, lignin, alkaloids, and cardiac glycosides [4] the plant have many medicinal activity like anti-diabetic, hepatoprotective, anti-microbial, analgesic, anti-inflammatory, antipyretic, and anticancer activity [5].

Material and Methods
Plant *Agele marmelos* fruits were collected from malwa region of madhya pradesh the plant were selected on the basis of chemical composition and traditional usage. Soya lecithin and cholesterol purchased from chemdyes corporation Rajkot.

Extraction
The fruit of bael for the study were collected fresh. These fruit were kept in sunlight and the dried for 10 days. The dry sample was ground into powder.
The powdered plant sample (50gm) was packed into thimble of soxhlet apparatus and extracted firstly with petroleum ether to remove fatty material and chlorophyll for 12hrs. Then after that marc was collected and placed it for complete removal of petroleum ether and again marc was extracted with methanol (250ml) the process of extraction is continued till the solvent in siphon tube become colorless than extract was taken in beaker and kept on hot plate and heated at 30-40 °C till solvent get evoparated. The dried extract was kept at 4 °C in refrigerator for further use [6]

Phytochemical Screening
Qualitative photochemical screening was with freshly prepared reagent for chemical identification the chemical test for both of extract were performed to show presense of alkaloid, carbohydrate, protein and amino acid, glycosides, flavanoid, tannis and phenolics, steroid, volatile oil and fat The extract were tested for presence of bioactive compound by using standard method [7-12].

Preparation of Phytosome
Phytosomes was prepared by anti solvent precipitation technique different phytosome complex of agele marmelos F1, F2, F3,F4 containing molar ratio of 1:1, 1;2, 2;1, 2;2 of soya lecithin and phosphatidyl choline were prepared. Specific amount of extract phospholipid and cholesterol was taken in 100ml round bottom flask and refluxed with 20ml dichloromethane at a temperature not more than 60°C for 2hrs. when the mixture is concentrated to 5-10ml hexane(20ml) was added with continous stirring, preciptate formed was filtered and collected then stored in vaccum dessicator overnight the dried precipitate obtained was crushed in mortar sieved and placed in amber colour glass bottle stored at room temperature [12-14].

Table 1: Preparation of Phytosome

S.No	Phytosome	Phytosome (Molar Ratio of Soya Lecithin: Phophatidyl Choline)	Extract 1	Extract 2	Solvent
1.	F1	1:1	Aegle Marmelos	Pterocarpus marsupium	dichloromethane+n-hexane
2.	F2	1:2	Aegle Marmelos	Pterocarpus marsupium	dichloromethane+n-hexane
3.	F3	2:1	Aegle Marmelos	pterocarpus marsupium	dichloromethane+n-hexane
4.	F4	2:2	Aegle Marmelos	pterocarpus marsupium	dichloromethane+n-hexane

Evaluation of Phytosomes

Microscopy

Phytosome was obseved under microscope (cippon Japan) one drop of diluted extracted loaded phytosome suspension was deposited on glass slide excess of solution was allow to drained off then the sample was examined by optical microscopy

Percentage yield

The prepared phytosomes were dried properly and weighed accurately. This total weight of phytosomes was divided by total weight of extract [14].

$$\% \text{ yield} = \frac{\text{Practical yied} \times 100}{\text{Theoratica yield}}$$

Entrapement Efficiency

Entrapement efficiency was determined by measuring the entrapped plant constituent. Specific amount of phytosome dispersion was transferred to centrifuge tube and centrifuged for 5min at 5000 rpm then supernant was collected and amount of free extract was determine by UV spectroscopy [15].

$$\% \text{ E.E} = \text{W} \frac{\text{(Added Drug)} - \text{W(Free Drug)}}{\text{W (Added Drug)}} \times 100$$

Total Flavanoid Content of Phytosome

Total flavanoid content was determine by aluminium chloride method standard solution of quercetin was prepared by 10 mg of quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol and used as standard. Phytosome solution was prepared by dissolving 10 mg of dried phytosomes in 10 ml methanol and filter. Three ml (1mg/ml) of this was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of each extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm [16-18].

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra were measured to investigate interaction of extracts and phospholipid in phytosomal complex the spectra were measured by using FTIR mocrocsope in a scanning range from 4000 and 500 cm^{-1} [19, 20].

X-ray diffraction

XRD was done on pure on pure extract and phytosome to see the crystallinity in substance.

Sample in was scanned in the angular range of 5-60° in X-ray diffractrometer [21].

Particle size and

The particle size of phytosomes is a very important property as it affects the stability and bioavailability of phytoconstituent systems. Smaller particles have a large surface area as well as faster release and higher stability The particle size of phytosomes were determined by particle size analyzer (Malvern Instruments Ltd). For that, 100 µl of the formulation was diluted with an appropriate volume of distilled water and the diameter of the vesicle was determined [22, 23].

Zeta potential

The zeta potential of a particle may be define as the overall charge that a particle carries in a particular medium. Evaluation of zeta potential help to determine the stability of phytosomal systems zeta potential of optimized phytosomes formulation were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster ZEM 5002, Malvern, UK) [5]. The electric potential of the phytosomes, including its Stern layer (zeta potential) was determined by injecting the diluted system into a zeta potential measurement cell [23, 24].

Result and Discussion

Preparation of phytosome

F1 formulation having 1:1 ratio of soyalechithin and phosphatidylcholine was selected for further evaluation as this ratio show better percentage yield and entrapement efficiency.

Phytochemical Screening

Observations in phytochemical investigation of alcoholic extract of Aegele Marmelos fruit and Pterocarpus Marsupium shown in table.

Table 2: phytochemical investigation of Aegle Marmelos fruit extract

S.No	Test	Result
1.	Alkaloid	+
2.	Carbohydrate	+
3.	Glycoside	+
4.	Flavanoid	+
5.	Polyphenol	+
6.	Saponin	+
7.	Steroid	-
8.	Triterpenoids	-

Table 3: phytochemical investigation of extract Pterocarpus Marsupium

S.No	Test	Result
1.	Carbohydrate	+
2.	Protein	+
3.	Phenol	+
4.	Flavanoid	+
5.	Alkaloid	+
6.	Steroid	+
7.	Saponin	-
8.	Tannis	+
9.	Glycoside	+

Preparation of Phytosome

Percentage yield

Table 4; percentage yield of Phytosome

S.No	Phytosome	% yield of Agele Marmelos phytosome	% yield of Pterocarpus Marsupium phytosome
1.	F1	80.40%	63.23%
2.	F2	76.47%	58.82%
3.	F3	72.02%	56.30%
4.	F4	67.04%	52.30%

Entrapment Efficiency

Table 5: entrapement efficiency of phytosome

S.No	Phytosome	% E.E. of Agele Marmelos phytosome	% E.E. of Pterocarpus Marsupium phytosome
1.	F1	70.05%	54.0%
2.	F2	68.80%	44%
3.	F3	64.04%	42%
4.	F4	60.03%	50%

Total Flavanoid Content (TFC) of Phytosome

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y=0.022X + 0.200$, $R^2=0.994$, where X is the quercetin equivalent (QE) and Y is the absorbance. And found to be

Table 6: Total flavanoid content of phytosome

S.no.	Phytosomes	TFC (mg/100mg)
1.	*Aegle marmelos*	3.08
2.	*Pterocarpus marsupium*	2.72

Table 7: Preparation of calibration curve of quercetin

S. No.	Concentration (µg/ml)	Absorbance
1.	5	0.324
2.	10	0.408
3.	15	0.540
4.	20	0.632
5.	25	0.767

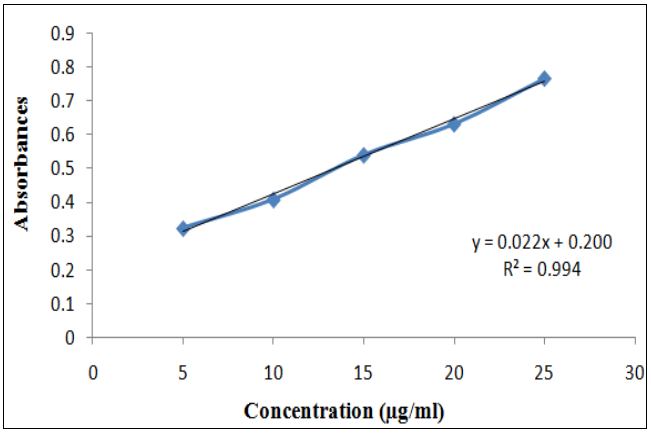

Fig 1: Graph of estimation of total flavonoids content

Ftir Spectra

The spectra of phytosomes show a decrease in OH peak as compared to extract thus proves complex was formed between extract and phospholipid by hydrogen bond. However mixture of extract and lipid there was no decrease in OH peak show absence of chemical bond.

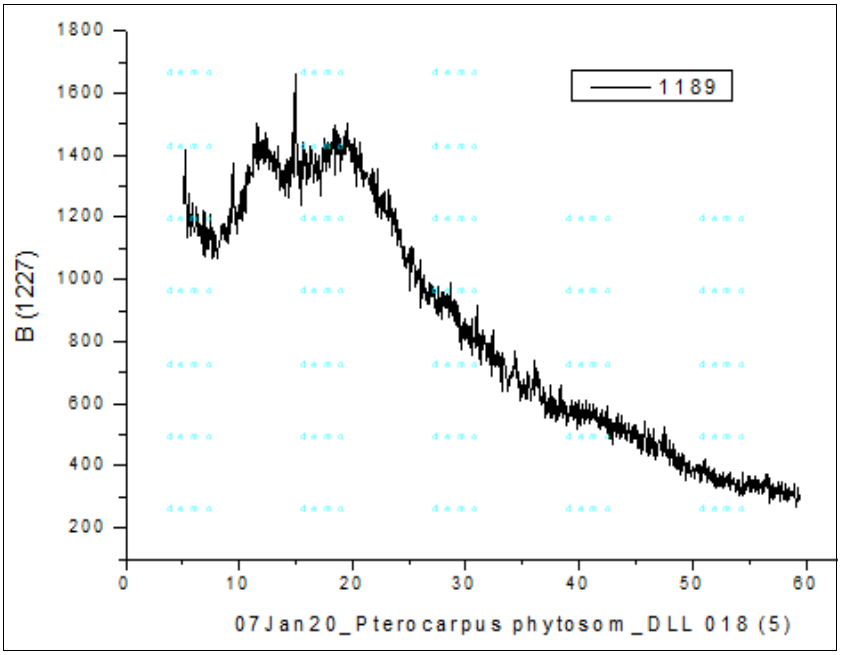

Fig 2: X-ray graph of *Pterocarpus Marsupium* phytosome

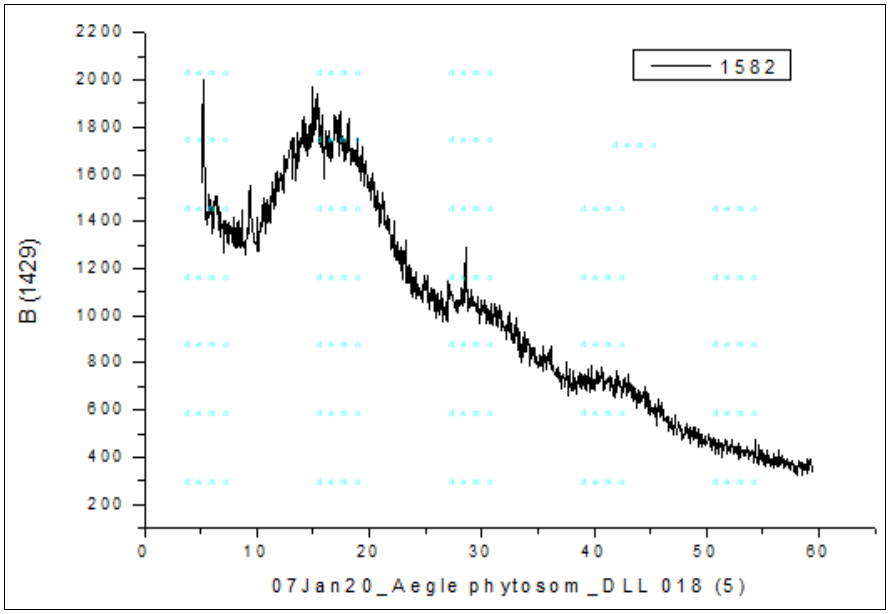

Fig 3: X-ray graph of *Aegle Marmelos* phytosome

Particle Size and Zeta Potential

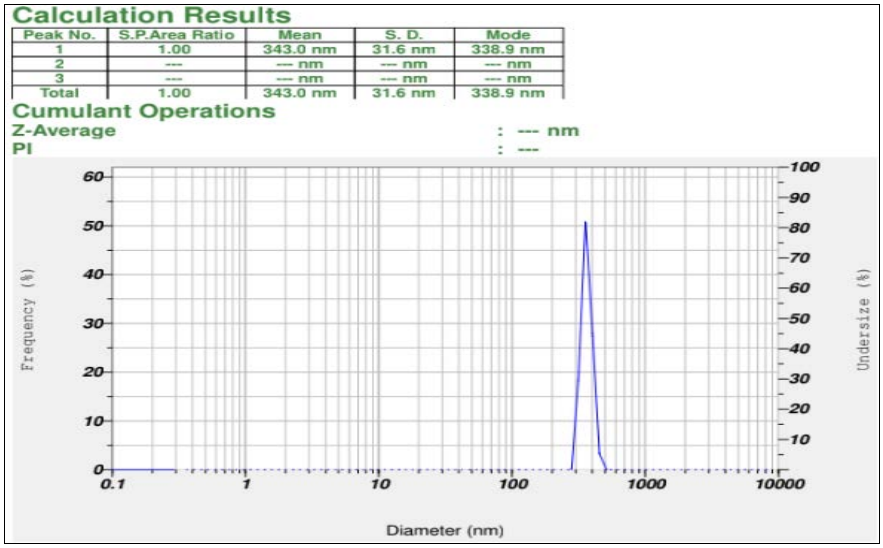

Fig 4: Particle Size *Aegle marmelos* Phytosomes

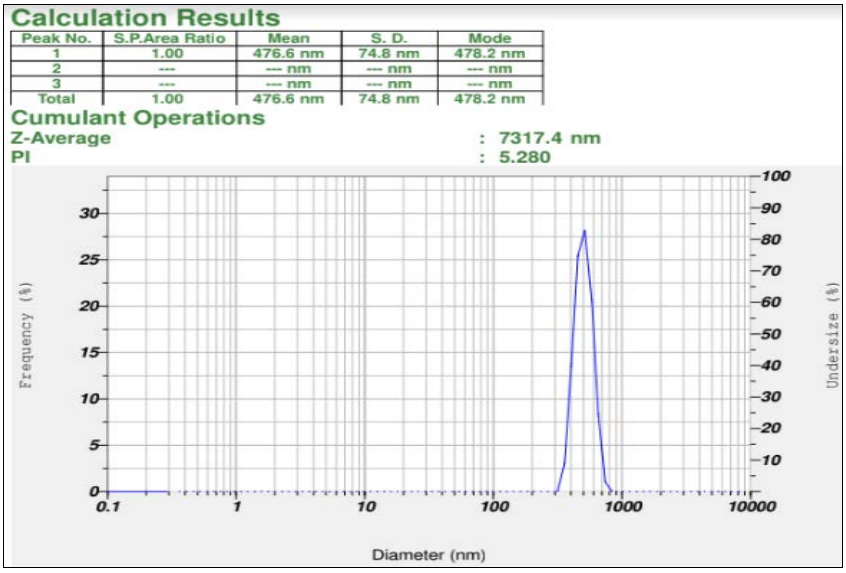

Fig 5: Particle Size *Pterocarpus marsupium* Phytosomes

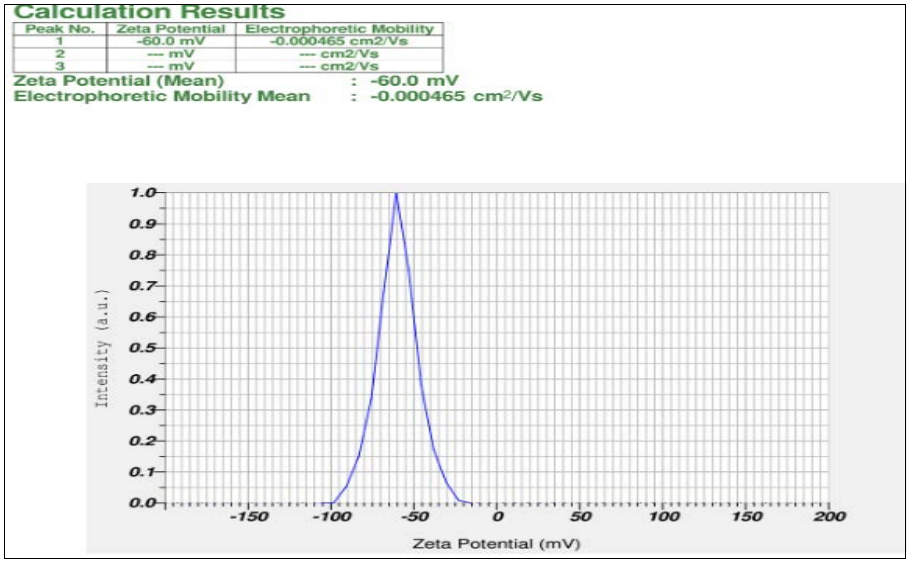

Fig 6: Zeta Potential *Aegle marmelos* Phytosome

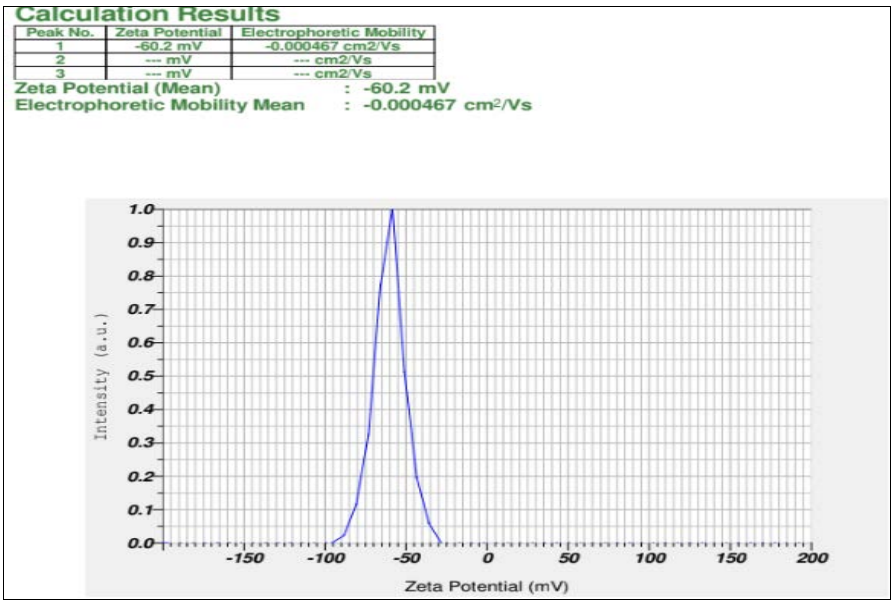

Fig 7: Zeta Potential *Pterocarpus marsupium* Phytosome

Conclusion

Phytochemical investigation was done for estimation of phytoconstituent the phytosomev complex was prepared by standardised extract and phospholipid the microscopic study show presence of spherical structure complex. The FTIR spectra show intense peak the physical mixture give confirmation about purity and no interaction showned XRD spectra show sharp crystaline peak. Particle size of formulation was 150nm and 127 nm and zeta potential was -60.02mv and -60 mv for aegle marmelos and pterocarpus marsupium respectively and From above study we are concluded that phytosome showed better physical characteristics and bioavailability characterstic.

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