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Fabrication and Characterization of *Pongamia pinnata* Leaf and Bark Extracts Loaded Nanoemmigel

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Abstract

The type of delivery system is a vital factor on which the efficacy of a drug depends. There have been many newer approaches to increase the therapeutic efficacy of the drug, one among which is nanoemmigel or nanomiemgel. It is an improvised drug delivery system attempted to overcome the disadvantages of the conventional drug delivery system. Nanoemmigel is a delivery system which combines two dosage forms in it viz nanoemulsion and nanomicelle such that they include the advantages of both the systems. The objective of the dosage form is to enhance drug delivery and efficacy by decreasing the particle size to nano and by achieving micellar concentration. Nanoemulsion enhances site specificity and nanomicelle consists of colloidal drug carrier of amphiphilic monomers. Nanoemmigel have been proven with increased efficacy than administered individual system. Thus, taking all these factors into consideration the present work was aimed to fabricate and evaluate a topical nanoemmigel using leaf and bark extracts of *Pongamia pinnata.*

Keywords: Nanoemmigel, nanoemulsion, nanomiemgel, nanomicelle, extract, *Pongamia pinnata*.

INTRODUCTION

Novel drug delivery system has become the need of the hour because of the disadvantages being faced in conventional dosage forms (Thiruvengadam M etal., 2018). Researchers are now pondering towards innovative and combined drug delivery to enhance the efficacy of the drug (Choudary etal., 2017). An individual drug delivery dosing or a delivery system focuses on solving a particular purpose and are not completely effective. Skin is the major part of the body (Sengupta etal., 2017). Topical formulations are affected by the permeation rate of the skin. Delivery of drug via skin is one of the safest routes because of several advantages like it bypasses the presystemic metabolism, long time of action, minimal adverse effects, increase in efficacy, individual variability and the most significant one is patient compliance due to non invasive nature (Goel A etal., 2010).

This study is planned to fabricate a novel topical formulation- Nanoemmigel also termed as nanomiemgel; one of the novel drug delivery systems including all the above mentioned advantages.

Nanoemmigel is a combination dosage form comprising of a nanoemulsion and a nanomicelle thus satisfying the advantages of both the dosage forms. Nanomicelle and nanoemulsion are prepared separately and incorporated into a single dosage form as gel intended for better absorption and permeability than their individual forms (Pratap SB etal., 2012).

Combination topical delivery often offers maximum drug permeation enhancing therapeutic efficacy. The globule size ranges from 20-200nm for nanoemulsion and for nanomicelle it is 10-50nm thus smaller size poses major advantage. There are many advantages associated in using herbal drugs like decreased side effects, effective for chronic conditions, economic and widespread availability (Hussain A etal., 2016).

Pongamia pinnata is a tree of the family Fabaceae commonly called as Pungai in Tamil, Indian beech in English (Baddole SL., 2013). The leaves are used in cold, cough, diarrhea, as antiseptic and also in rheumatism (Arote SR etal., 2010; Al Muqarrabul LM etal., 2013). Bark is used internally for piles (Farahnik B etal., 2017). The tree has a traditional claim of use in psoriasis which is one of the chronic skin conditions typically not curable but managed. It is a hyperproliferative auto immune disorder (Salunke PB etal., 2017; Sanjay Kumar Rout etal., 2017).

The current research focuses on development of a nanoemmigel using leaf and bark extracts of *Pongamia pinnata* for the treatment of psoriasis. The preliminary work on the antioxidant potential of leaf and bark extract has been proven in this study to substantiate the use of the plant in psoriasis (Divakara P etal., 2013; Rahul Deo Y etal., 2011).

MATERIALS AND METHODS

Pongamia pinnata leaf and bark material was collected from in and around Chennai and was authenticated by Prof P. Jayaraman, PARC Tambaram (PARC/2018/3787).

Preparation of Leaf And Bark Extract

The leaves and bark of *Pongamia pinnata* were collected, shade dried, powdered, extract was prepared by maceration process and stored for further use. The leaf and bark extract was evaluated for the presence of phytochemical constituents like flavonoids, alkaloids, phenols, saponin, proteins, amino acids, carbohydrates, glycosides, tannins, coumarins etc (Yin H etal., 2004).

Preformulation Studies

Preformulation studies of the powdered extract of leaf and bark was carried out for the parameters like solubility, density, angle of repose.

Pseudo Ternary Phase Diagram

Depending on the preliminary evaluation and preformulation parameters the oil, surfactant, cosurfactant and water were selected to prepare the nanoemulsion. Pseudo ternary phase diagram was constructed for different combinations of oil, surfactant, cosurfactant and water (Somekawa S etal.,2015).

Preparation of Nanoemulsion

0.5g of bark and leaf extract, 7ml of isopropyl myristate, 33.75ml of polysorbate and 11.25ml of isopropyl alcohol

were taken separately and stirred continuously on a homogeniser (Tayeb HH etal., 2018)

Preparation of Nanomicelle

0.5g of bark and leaf extract were taken separately, 0.5g of cremophor EL, 50ml of methanol was added and micelle were prepared by solvent evaporation technique. The solvent was fully evaporated to yield dry product (Patel S etal., 2015).

Preparation of Nanoemmigel

Nanoemulsion and nanomicelle preparation were mixed together with the aid of carbopol gel in the ratio 1:1 with uniform mixing. The pH was then neutralized with triethanolamine. Thus prepared nanoemmigel was then evaluated (Somagoni J etal., 2014; Seo YG etal.,2013).

Evaluation of Nanoemmigel

1) Physical appearance: The prepared Nanoemmigel formulations were inspected visually for their colour, homogeneity, consistency, grittiness and phase separation.

2) pH Determination: pH determination of prepared formulations was done using digital pH meter.

3) Rheology of Nanoemmigel: The viscosity of Nanoemmigel was measured using 1% gel at 10 rpm for 5 min at 25^{o}C by Brookfield rotary viscometer with spindle S18.

4) Spreadability: 0.1 g of nanoemmigel was weighed and sandwiched between two slides. 50gm weight load was subjected on the slides. The initial and final diameter of the nanoemmigel was noted.

Characterization of Nanoemmigel

The following characterization methods were carried out for nanoemmigel

SEM (Scanning Electron Microscope) of the nanoemmigel: The SEM analysis of the leaf and bark nanoemmigels were performed (Kathpalia H etal., 2018).

In-vitro drug diffusion study

Drug diffusion study for both leaf and bark nanoemmigel was carried out using Franz diffusion cell with a semi permeable membrane for six hours.

In-vitro anti-oxidant studies of plant extract were carried out by

- Nitric oxide scavenging activity
- Total antioxidant activity
- DPPH assay

***In-Vitro* Anti-Oxidant Studies**

a) Nitric Oxide Scavenging Activity

The nitric oxide scavenging activity of *Pongamia pinnata* was determined as per standard procedures. At physiological pH, nitric oxide gets generated spontaneously with aqueous solution of sodium nitroprusside, the nitrate ions produced interacts with oxygen was measured calorimetrically. 3ml of reaction mixture containing sodium nitro prusside, 10mM in phosphate buffered saline (PBS) and various concentrations of the extracts (10, 50, 100, 200, 400, 800, 1000 µg/mL) was incubated at 37^{0}C for 4 hours. Triplicate of each extract concentration was prepared. Control without test compound was kept in an identical manner, ascorbic acid was added as a standard. After incubation, 0.5ml of Griess reagent was added. At 546nm the absorbance was read. The percentage inhibition of Nitric oxide generation was measured by comparing the absorbance values of control and average absorbance of test compounds (Yildirim M etal., 2003; Dwivedi D etal., 2016).

b) Determination of Total Antioxidant Activity

The total anti-oxidant activity was evaluated by preparing aliquots of 0.1ml of sample solutions (100 and 200 µg/ml) was combined with 1ml of the reagent solution (0.6M H_2So_4, 28mM sodium phosphate and 4mM ammonium molybdate) in each case. Triplicate of each extract concentration was prepared. 0.1ml of methanol was used as blank. The tubes were capped and incubated in a boiling water bath at 95^{0}C for 90 minutes. Cooling the samples to room temperature, at 695nm the absorbance was noted. The standard ascorbic acid was prepared by taking low concentrations (10, 20, 40, 80, 120 µg/ml) in triplicate by replacing the extract in the above aliquot and incubated in same manner at same time. The absorbance values were noted. The standard absorbance versus concentration was plotted on graph to get the linear curve. The sample absorbance values (Y-axis) were extrapolated to the concentration of standard (X-axis), using the linear curve (Wen R etal.,2018; Li J etal.,2015).

c) Anti-Oxidant Activity by DPPH Method

DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of 25mg of DPPH (200µM) was prepared in 100ml of ethanol, 0.05ml of extract of different concentrations (10, 50, 100, 200, 400, 800, 1000 µg/mL) were dissolved in ethanol. Triplicate of each extract concentration was prepared. An equal amount of ethanol was added to the control, ascorbic acid was used as the standard. The reaction was allowed to be completed in the dark for about 20 minutes. Then the absorbance of test and standard mixtures was read at 517nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical (Ghosh A etal.,2018).

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening of *Pongamia pinnata* Extracts

S. No.	Parameters	Inference	
		Leaf	Bark
1.	**Test for alkaloids**		
	Dragendroff test	-	-
2.	**Test for flavonoids**		
	Shinoda test	+	+
3.	**Test for phenols**	-	+
4.	**Test for saponins**	-	-
5.	**Test for proteins**		
	Biuret test	-	-
6.	**Test for amino acids**		
	Ninhydrin test	-	-
7.	**Test for Carbohydrates**		
	Fehling's test	-	-
8.	**Test for coumarins**	+	-
9.	**Test for tannins**	+	+
10.	**Test for glycosides**	+	+

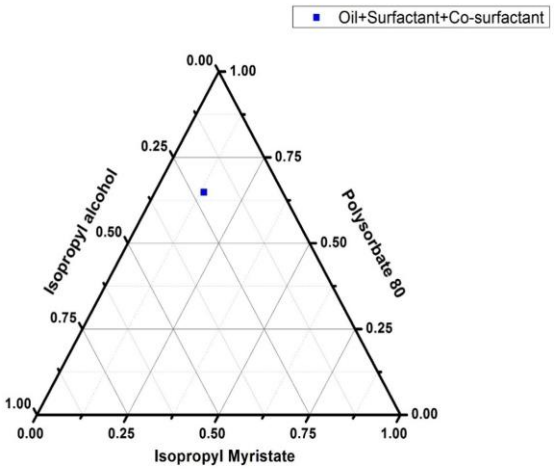
The leaf extract showed the presence of flavonoid, coumarins, tannins and glycosides and the bark extract showed the presence of flavonoid, phenols, tannins and glycosides. The anti oxidant activity of the bark and leaf may be attributed due to the presence of flavonoids.

Preformulation Evaluation

S.no	Parameters	Observation	
		Leaf	Bark
1.	Solubility		
	Water	sparingly	Partially
	Methanol	Not soluble	Soluble
	Chloroform	Soluble	Not soluble
	IPA	Not soluble	Not soluble
	Pet Ether	Not soluble	Not soluble
2.	Bulk Density	-	0.491
3.	Hausner's Ratio	-	1.055
4.	Angle Of Repose	-	24°18'

Preformulation studies showed the above results and since the leaf extract was not obtained as a powder the flow property parameter like bulk density, angle of repose Hausner's ratio was not carried out.

Ternary Phase Diagram



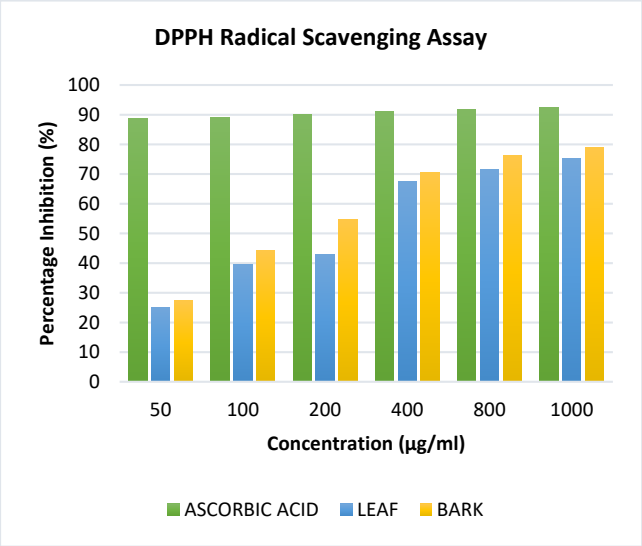
Ternary Phase Diagram

The blue area denotes the emulsion region for the formulation. The quantity of surfactant mix, oil and water was determined by the phase diagram

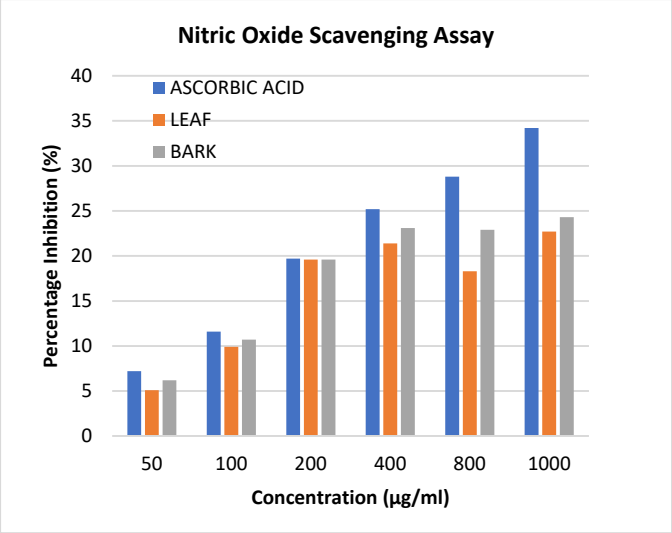
Evaluation of Nanoemmigel

Sl.no	Parameters	Observation	
		Leaf	Bark
1.	pH	6.2	6.7
2.	Spreadability	40mm	42mm
3.	Viscosity	46.9 cP	24.1 cP
4.	Consistency	good	good
5.	Gritty particles	no	no
6.	Appearance	Pale green	Light brown
7.	Washability	washable	washable

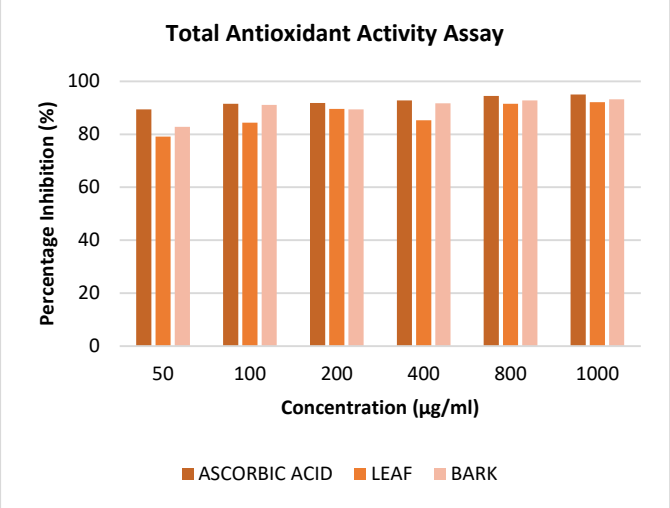
DPPH Radical Scavenging Assay



Nitric Oxide Radical Scavenging Assay

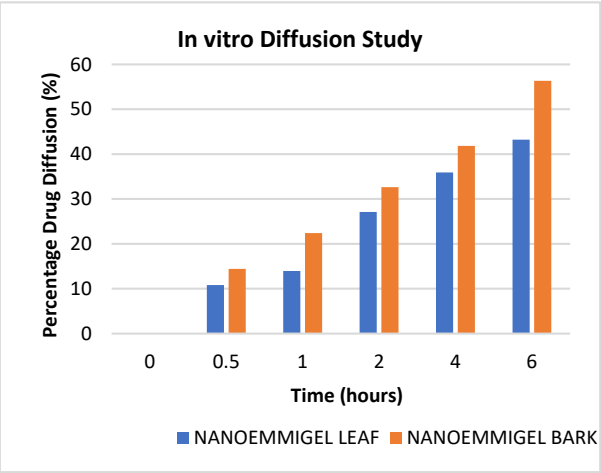


Total Antioxidant Activity Assay



The antioxidant activity of leaf and bark extract was carried out by total antioxidant activity, DPPH radical scavenging assay and nitric oxide radical scavenging assay showed that the bark extract had increased antioxidant potential than the leaf extract and it had comparable activity as that of the standard ascorbic acid.

In Vitro Diffusion Study

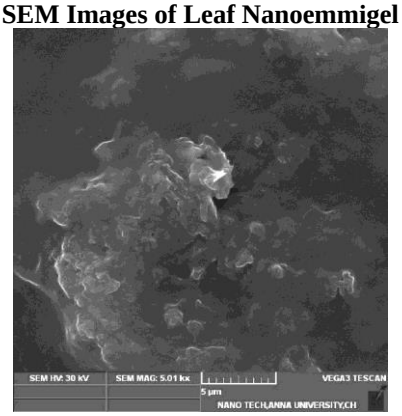


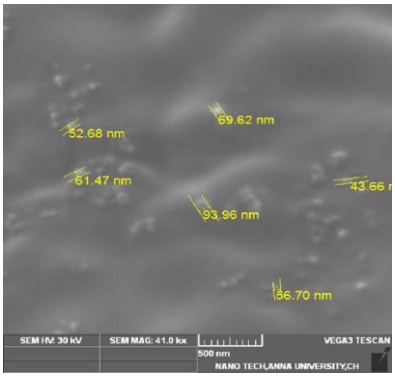
***In vitro* Drug Diffusion Study**

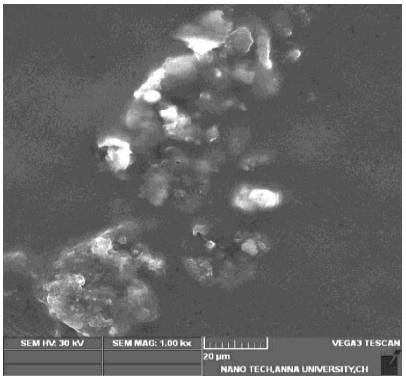
In-vitro drug diffusion study was carried out for the bark and leaf nanoemmigels. The results showed that the gel containing leaf extract showed more penetration compared with bark nanoemmigel.

SEM Analysis

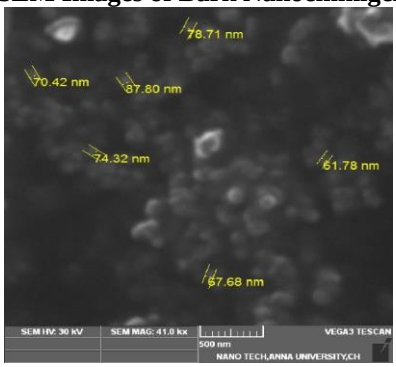
SEM Images of Leaf Nanoemmigel

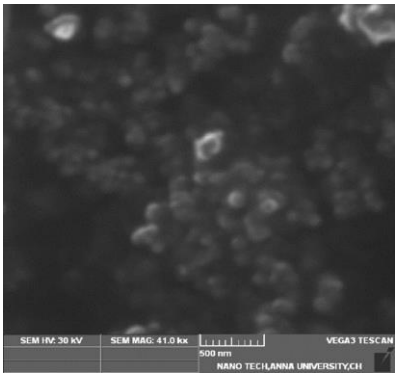


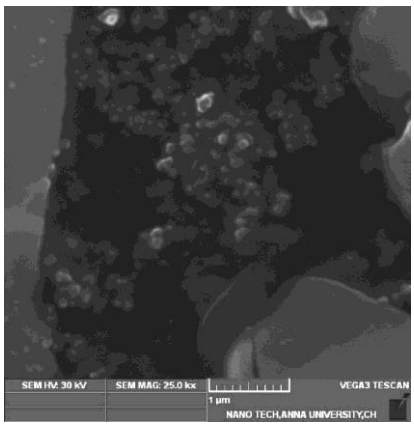




SEM Images of Bark Nanoemmigel







SEM images of the leaf and bark nanoemmigel shows the presence of nano sized particles in the gel. Thus, it can be substantiated for its use in enhanced skin penetration.

CONCLUSION

This study concludes that the prepared nanoemmigel of bark and leaf extract of *Pongamia pinnata* had enhanced skin permeation into the deeper layers of skin by improving contact time with the skin, hydration of the skin and by forming a monolayer over the skin. Increase in therapeutic efficacy can be achieved by increased permeation into the skin. The presence of antioxidant efficacy can substantiate the use of this formulation for treating psoriasis and this research work can be used as a supportive study for the traditional claim of the plant for treating psoriasis. The future work of the study is to evaluate the formulation for its anti-psoriatic activity.

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