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Research Article

Evaluation Of Antioxidant Potential Of Mangifera Indica Ethosomal Gels

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ABSTRACT

Objective: The present study was designed to formulate ethosomal gel of *Mangifera Indica* leaves and investigate the best formulation assessing the antioxidant activity. **Material and methods:** Different formulations of ethosomes using lecithin, cholesterol and ethanol were prepared using different doses of Mangifera Indica leaf extracts. Prepared gels were then evaluated for their physicochemical characteristics and drug content. The gels were tested for their antioxidant activity by DPPH (2,2-Diphenyl-1-1-picrylhydrazyl) scavenging activity. **Results and Discussion**: The p^H of the gel formulation was found to be in the range of 5.4-6.2. Viscosities of gels were ranging between 2250-2399 centipores. The drug content of gels ranged between 74.67- 82.3M1%. The percentage inhibition of DPPH radical of the *Mangifera Indica* leaves extract (MIME) was found to be 498.91µg/ml and of ethosomal gel formulation was found to be 338.91 µg/ml (FN1), 321.76 µg/ml (FN2), 301.06 (FN3). The results obtained were significant when compared with the standard ascorbic acid. **Conclusion:** The results revealed ethosomes of *mangifera Indica* as an efficient drug delivery system for herbal extract. And the ethosomal gel formulation showed significant antioxidant activity than the herbal extract.

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INTRODUCTION:

Free radicals have noxious effects on cells and is believed that the damage caused by the free radicals is reported in the etiology of several diseases.[1]In recent years, there has been an increasing interest in finding natural antioxidants, especially of plant origin, used in the treatment of several human diseases, and their pharmacological and therapeutic properties have been attributed to different chemical constituents isolated from their crude extracts.[2]

The plants that show significant pharmacological activity and low toxicity need extensive screening. The Mangifera Indica leaves belongs to the family plant which belongs to the family Anacardiaceae, grows in tropical and sub-tropical regions. Various parts of the plant were commonly used in folk medicine for a wide variety of remedies like treating diarrhea, asthma, hypertension, insomnia, improves strength and immunity, paralysis, neuropathy, skin self-repair, skin disorders, bleeding disorders, constipation, bloating and used as an astringent.[3] Leaves and bark contain mangiferin, mangiferolic acid, homomangiferin and indicenol. Leaves have tannins, flavonoids, steroids, cardiac glycosides, alkaloids and carbohydrates. Mangiferin is a main constituent is a polyphenolic and a glucosyl amine xanthone with strong antioxidant, wound healing, cardiotonic and antidiabetic acivities. For external application like excessive bleeding, injury and wounds, dry powders of bark, flower, leaves and seeds can be applied.[4]

The effect of free radicals on human beings are closely related to toxicity, disease and ageing. Most living species have an efficient defense system to protect themselves against oxidative stress induced by reactive oxygen species. Over production of various forms of activated species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) and non free radical species is considered to be the main contributors to oxidative stress. The main mechanism of action of antioxidants include radical scavengers and suppressors that neutralize or eliminate reactive oxygen species (ROS)/ nitrogen (RNS) and the binding of metal ions, which are necessary for the production of oxidizing species[5]. Phenolic compounds derived from plant sources were widely studied antioxidant compounds and act in the neutralization of free radicals, helping to control the oxidative stress.[6] Epidemiological data as well as in vitro studies strongly suggest that plants containing phytochemicals with antioxidant potential have strong protective effects against major disease risks caused due to oxidative stress.

As plant drugs are considered safe because of their natural origin, they exhibit promising therapeutic effect. However most of the phyto constituents fail to achieve bioavailability because of poor absorption. The reasons may be the large molecular sizes and low lipid solubility which causes poor absorption of phytoconstituents resulting in reduced bioavailability.[7] Incorporation of these plant actives or extracts into vesicular carriers vastly improves their absorption and consequently bioavailability. There are many reports which revealed the pharmacological activity of the extract but only few of them were found to convert the extracts into suitable dosage forms.

However, delivery of herbal drugs also requires modifications with the purpose of better cure for variety of diseases. Now-a-days novel drug delivery systems open the door towards the development of herbal drug delivery systems. Novel drug delivery system is advantageous in delivering the herbal drug at predetermined rate and delivery of drug at the site of action which minimizes the toxic effects with increase in bioavailability of drugs.[8] Ethosomes are soft malleable lipid vesicles composed mainly of phospholipids, alcohol (10-40%) and water. The physicochemical characteristics of ethosomes allow this vesicular carrier to transport active substances more efficaciously through the skin in terms of quantity and depth when compared to conventional liposomes. Ethosomes play an important role in controlling the release rate of drug over an extended time keeping the drug shielded from immune response or other removal systems. In contrast to conventional liposomes, ethosomes shows smaller vesicle size, high entrapment efficiency as well as improved stability. [9] From the above literature, it was decided to develop an ethosomal formulation for *Mangifera indica* extract and its incorporation into gel formulations and to characterize and evaluate the formulations and evaluate the invitro antioxidant potential of the developed formulation.

MATERIALS AND METHODS

Collection of Plant material and Preparation of extract

Mangifera indica leaves were collected from local market, Hyderabad, India and were further authenticated by Dr. Madhava Chetty, Botanist, Tirupati, Andhra

Pradesh. All the other solvents and reagents were of analytical grade. Fresh leaves of the plant were washed with water immediately after collection. These were chopped into small pieces, air dried at room temperature for 10 days, grounded in to fine powder and stored in air tight containers. 650 grams of powder was macerated with 5 litres pure methanol for 7 days at room temperature. Later it was filtered and the extract was concentrated under reduced pressure below 50^0 C in rotary vacuum evaporator. It was kept in petri dish for air drying to remove the traces of methanol and finally a concentrated extract is formed[10,11] .

Preparation of Ethosomes

In this lipid and cholesterol were measured accurately and dispersed in water by stirring it on a magnetic stirrer for 30 minutes with heating at 40°C. Organic phase containing 100mg of extract was added to ethanol and to this propylene glycol was added and kept for stirring separately. Lipid solution was added drop by drop to the organic phase and kept for stirring on a magnetic stirrer for 1 hour. 12 batches of ethosomal formulations were prepared using different concentrations of lipid (100-400mg) and ethanol (10-40%). The optimized formulation was choosen and further ethosomal preparations of other doses (200mg, 300mg) were formulated. The formulations with high entrapment efficiency and drug release were selected to incorporate in to gel formulations.[12]

Preparation Of Ethosomal Gel

The gels were prepared by dispersion method using carbopol 940. Gels were prepared by dispersing gelling agent to the distilled water. Then the mixture was allowed to swell overnight. The mixture was neutralized by drop wise addition of triethanolamine. Then, glycerol was added to gel to balance its viscosity. To this gel solution optimized ethosomal dispersion was added and mixed properly. Mixing was continued until a transparent gel appeared. Paraben was added as a preservative. The prepared gels were filled in glass vials and stored at 4-8 ^{0} C. [13] Ethosomal dispersions F1-F4 (table 1) were prepared by varying the lecithin concentration. The dispersions were evaluated and based on rate of drug release the lecithin concentration was optimized. Ethosomal dispersions F5-F8 (table 2) were prepared by varying cholesterol concentration. Based on drug release the cholesterol concentration was optimized Ethosomal dispersions F9-F12(table 3) were prepared by varying the ethanol concentration and based on the drug release the ethanol.

Evaluation Of Prepared Ethosomes

Amongst all the formulations, F10 formulation was optimized based on % entrapment efficiency and drug release [14-16].

Morphology

The samples are visualised by scanning electron microscopy (Hitachi S-3700N), SEM gives a three-dimensional image of the globules. one drop of ethosomal suspension was mounted on a stub covered with a clean glass. It was then air dried and gold coated using sodium aurothiomalate to visualise under scanning electron microscope 10,000 magnifications.

Zeta Potential

Zeta potential was determined using Zetasizer (HORIBA SZ-100). Measurements were performed on the same samples prepared for size analysis. Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system.

Entrapment efficiency (EE)

Entrapment efficiency of *Mangifera indica* ethosomal vesicles was determined by centrifugation. The vesicles were separated in a high-speed cooling centrifuge at 20,000 rpm for 90 minutes. The sediment and supernatant liquids were seperated, amount of drug in the sediment was determined by lysing the vesicles using methanol. It was then diluted appropriately and estimated using UV visible spectrophotometer at 214nm. From this, the entrapment efficiency was determined by the following equation -

$$EE\% = \frac{(\text{Total drug}) - (\text{free drug})}{\text{Total drug}} \times 100$$

Formulation Of Gels

Gels were prepared by dispersing gelling agent to the distilled water. Then the mixture was allowed to swell overnight. The mixture was neutralized by drop wise addition of triethanolamine. Then, glycerol was added to gel to balance its viscosity. To this gel solution optimized ethosomal dispersion was added and mixed properly. Mixing was continued until a transparent gel appeared. Paraben was added as a preservative. The prepared gels were filled in glass vials and stored at 4-8^0 C [17].

EVALUATION OF PREPARED GELS [18]

Physicochemical properties

Appearance

The appearance was checked visually. They are light greenish in colour.

pH measurement

The pH was checked using pH meter (Systronics digital pH meter). The electrode was submersed in to the formulation at room temperature and the readings were noted.

Spreading diameter

The spread ability of gel formulation was determined by measuring the spreading diameter of 1g of gel between two horizontal plates (20cmx 20cm) after 1 min. The standard weight applied on upper plate was 125 gm.

Viscosity

Viscosity of prepared formulations was prepared carried out by Brookfield Synchro Electric Viscometer (LVDV Pro II), spindle S64 (small sample adaptor) and the angular velocity increased from 5,10,50,100 rpm and values were noted.

Drug content of the formulated gels

Drug content was estimated spectrophotometrically,100mg of the formulation was taken and dissolved in methanol and filtered. The volume was made up to 100ml with methanol. The resultant solution was suitably diluted with methanol and absorbance was measured at 212nm.

In-vitro drug release

The franz diffusion cell consisted of two compartments (cells). Upper one is donor cell, consisting of two open ends and lower one is receptor cell, with one open end capacity of 15 ml. one end of the donor compartment was covered with Himedia dialysis membrane (cut off molecular weight 12000-14000), which was previously soaked in warm water and placed on the receptor compartment. The receptor cell contained a small magnetic bead and was rotated at a constant speed. The temperature in the donor and receptor cells was maintained at 37^0C, with the help of a thermostat. Phosphate buffer 7.4 was placed in the receptor cell. A 5ml of sample of each formulation was transferred to the diffusion cell. 3ml samples were withdrawn from the receptor cell at specified time intervals. Each time immediately after the removal of the sample, the medium was compensated with the fresh media. The samples were analyzed for drug content using a UV-Visible spectrophotometer at 212 nm [19].

Ex vivo drug release studies

After approval of protocol from Institutional Animal Ethics committee permission with reference no. IAEC/SVCP/2016/005, Dated: 27/2/16 the study was conducted. Ex vivo studies were carried out using skin of albino rat. Rats (male albino) 6 to 8 weeks old, weighing 120 to 150g were sacrificed for abdominal skin. After removing the hair, the abdominal skin was separated from the underlying connective tissue with scalpel. The excised skin was placed on aluminum foil and the dermal side of the skin was gently teared off for any adhering fat and / or subcutaneous tissue. The skin was checked carefully to ensure the skin samples are free from any surface irregularity such as fine holes or crevices in the portion that is used for transdermal permeation studies. The skin was mounted between donor and receptor compartment with the stratum corneum side facing upward towards the donor compartment. Phosphate buffer 7.4 was taken in the receptor compartment. Temperature was maintained at 37 ± 0.5^0c. Optimized gel formulation was placed in the donor compartment. Samples were withdrawn at predetermined time intervals over 8 hrs and replaced with fresh buffer solution to maintain sink conditions. The samples were analyzed using UV-Visible spectrophotometer at 212 nm.

INVITRO ANTIOXIDANT STUDIES:

DPPH radical scavenging method

The free radical scavenging activity of prepared ethosomal gel was measured by 1,1- diphenyl-2-picryl-hydrazil (DPPH) using the method of Sreejayan method.[20] 1ml of different concentrations 50, 100, 200, 300, 400 and 500ug/ml of the test substance and standard were taken in different test tubes. To this, add 3ml of methanolic solution of DPPH and incubated at 37C for 20 min. The absorbance was measured at 517nm on a spectrophotometer (UV-spectrophotometer). Ascorbic acid is used as standard antioxidant agent. The concentration of the test extracts required to decrease the initial concentration by 50% (IC_{50}) was calculated.

RESULTS AND DISCUSSIONS:

The microscopic evaluation showed the surface morphology of ethosomes. It was observed that most of the vesicles were spherical in shape and its smooth surface was further confirmed by SEM. The vesicular size of the ethosomes significantly increased with increase in phospholipid concentration and decreased with increased concentration of ethanol. The zeta potential of the ethosomes was determined using zeta sizer. The value of the optimized ethosomal formulation – was found to be -8.8mv which indicated that ethosomes were stable. The entrapment efficiency of ethosomes was

found to be in the range of 65.31-89.38%. In the *in vitro* drug release, the cumulative percentage drug release from various ethosomal formulations were done. The formulation F10 showed higher drug release of 87.79 % in 8 hrs In the evaluation of ethosomal topical gel, all the formulations were found to be opaque, light greenish in color, odorless, semi solid in nature and had smooth appearance.

The pH for all the formulations exhibited in the range of 5.4-6.2. The formulations were analyzed Spectro photometrically at 212 nm. All the formulations were found to possess uniform drug content. The viscosity of all the gel formulations ranged from 2250- 2574 cps. The viscosity of the formulations decreased on increasing the shear rate i.e. pseudo plastic behavior was noted. In the *in vitro* drug release, the cumulative percentage drug release after for 8 hrs was highest for all the three doses of extracts using 1% carbopol. The drug content of the gels ranged between 74.67-82.31 %.

Evaluation Of Antioxidant Activity Of Mangifera Indica Gel Ethosomal Gel Preparation

Table 1 Optimization of concentration of lecithin

Formulation code	Drug concentration (mg)	Lecithin(mg)	Cholesterol (mg)	Ethanol(ml)	Propylene glycol(ml)
F1	100	100	20	10	3
F2	100	200	20	10	3
F3	100	300	20	10	3
F4	100	400	20	10	3

Table 2: Optimization of concentration of cholesterol

Formulation code	Drug concentration (mg)	Lecithin(mg)	Cholesterol (mg)	Ethanol(ml)	Propylene glycol(ml)
F5	100	300	20	10	3
F6	100	300	30	10	3
F7	100	300	40	10	3
F8	100	300	50	10	3

Table 3: Optimization of ethanol concentration

Formulation code	Drug concentration (mg)	Lecithin (mg)	Cholesterol (mg)	Ethanol (ml)	Propylene glycol(ml)
F9	100	300	40	10	3
F10	100	300	40	20	3
F11	100	300	40	30	3
F12	100	300	40	40	3

Table 4: Gels prepared by dispersion method using Carbopol 940 in different ratios

S NO	Formulation	Carbopol 940(%w/v)	Amount of extract
1	EG1	1	100
2	EG2	1	200
3	EG2	1	300

Table:5 Invitro Antioxidant activity of methanolic extract of Mangifera Indica leaves

Groups	Concentrations(µg/ml)	% inhibition	IC_{50}
MEMI	50	5.10±0.70	
	100	10.05±0.05	
	200	27.35±0.45	[b]498.91µg/ml
	300	32.52±1.07	
	400	41.85±2.01	
	500	50.98±1.50	
Ascorbic acid	50	16.08±0.25	297 µg/ml
	100	32.45±0.54	
	200	42.08±0.87	
	300	56.07±0.85	
	400	67.54±0.97	
	500	94.45±1.05	

[b]p<0.001 considered as significant, compared with corresponding standard.

Table:6 Invitro Antioxidant activity of methanolic extract of Mangifera Indica Ethosomal gel preparation.

Groups	Concentrations(µg/ml)	% inhibition	IC_{50}
FN 1	50	10.90±0.70	
	100	21.65±0.35	
	200	40.55±0.45	338.91µg/ml
	300	52.02±1.07	
	400	61.65±2.01	
	500	80.18±1.50	
FN 2	50	13.76±0.32	
	100	18.98±1.76	
	200	41.09±0.11	[a]321.76 µg/ml
	300	54.34±1.20	
	400	66.21±1.11	
	500	83.65±2.01	
FN 3	50	10.02±0.16	
	100	18.80±0.34	
	200	31.45±0.61	[a]301.06 µg/ml
	300	52.67±0.12	
	400	72.13±0.31	
	500	91.83±0.76	
Ascorbic acid	50	16.08±0.25	297 µg/ml
	100	32.45±0.54	
	200	42.08±0.87	
	300	56.07±0.85	
	400	67.54±0.97	
	500	94.45±1.05	

[a]P<0.0001, [b]p<0.001 considered as significant, compared with corresponding standard.

This study has assessed Mangifera Indica Ethosomal gel preparation for its invitro antioxidant activity.The invitro antioxidant studies ie, DPPH assay was conducted for mangifera indica methanolic leaf extract (MIME). DPPH

is unchanging free radical at room temperature and its drop or to receive an electron or a hydrogen radical from antioxidants is determined by determining the reduction in its absorbance values at 517nm spectrophotometrically. DPPH radical scavenging activity of MIME have shown significant antioxidant activity. The percentage inhibition improved with increasing concentration of the extract (Table 5). At the highest concentration of 500 µg/ml of MIME the percentage inhibition was found to be 50.98% ($IC_{50,}$ 498.91µg/ml).

Mangifera indica ethosomal gel preparations containing 100mg (FN 1), 200mg (FN 2), 300mg (FN 3) were formulated to perform the invitro studies. Ethosomal gel formulations FN1(100mg), FN2(200mg), FN3(300mg) were dissolved in water to obtain desired dilution and were subjected to the test. The results obtained showed that the radical scavenging activity found to increase with increase in concentration (Table 6). At the highest concentration 500 µg/ml concentration FN1, FN2, FN3 have shown the significant percentage inhibition of 80.18%, 83.65%, 91.83 % and IC_{50} of 338.91, 321.76, 301.06 µg/ml correspondingly. The results were compared with standard ascorbic acid (IC_{50} 297 µg/ml).Phyto constituents present in the plant parts are the sources of natural antioxidants. Formulating them in appropriate dosage form is very important to make them available for the body. The herbal extract of *Mangifera indica* have shown significant antioxidant activity henceforth it was formulated it into ethosomal gel preparation. In the ethosomal gel preparation the presence of ethanol allow the drug to penetrate into deeper tissues and make the drug available for more better action. As per the results obtained the ethosomal gel formulation have shown more significant percentage inhibition than MEMI.

CONCLUSION:

Oxidative stress also play a role in various dermatological disorders like aging of skin example deep wrinkles, psoriasis, pigmentation, alopecia, melanoma's. The transdermal drug delivery system is a noninvasive route of administration which helps the drug to penetrate into deeper layers of the skin. It gives an alternative to oral drug delivery for treating skin diseases. Hence treating the dermatological disorders by this route is more beneficial. In the present study it can be concluded that development of formulation for herbal extract helps in enhancing the activity of phyto constituents of *Mangifera indica* leaves.

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