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Isolation and charaterization of novel bioemulgent from the fruit pulp of prunus insititia

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ABSTRACT

The aim of the research work is to isolate biopolymer and to evaluate its emulsifiability by formulating o/w type of emulsion. The biopolymer was isolated from the fruit pulp of Prunus insititia by collecting and treating with water. The aqueous extract was further treated with 3 vol. of dimethyl ketone and kept aside in refrigeration for 6 hours. The biopolymer was collected by centrifugation and dried and subjected for physicochemical properties like solubility, pH, color, viscosity. Seven different o/w types of emulsions were prepared by using Liquid paraffin oil and biopolymer as an emulsifier in various concentrations ranging from 50mg to 1 gm. The formulated emulsions were subjected for various evaluation parameters like globule size, pH, the effect of freezing and thawing cycle, effect of centrifugation. The results were compared with the standard emulsion which was prepared by using acacia as an emulsifier.The emulsions were not showing any significant stability due to increase in the globule size. The final conclusion was drawn that the biopolymer in lower concentration showed its potential emulsifying property in the formulation FE1 and FE2 containing 50 and 100mg along with uniform globule size ranging from 12 to 30 µm and stable for 3 freezing and thawing cycle. These emulsions are very significantly stable in comparison with standard emulsion.

Introduction

Biopolymers are polymers that are biodegradable in nature. The materials use for the production of these polymers may be either renewable (based on agricultural plant or animal products) or synthetic. Natural gums are mostly used as binders , emulgents, film formers and suspending agents etc.The natural gums are generally the byproducts of metabolic mechanisms of plants. They are either water soluble or produce a viscous solution after absorption of water[1].

Prunus insititia belongs to the family Rosaceae. The fruit pulp consists of minerals, carbohydrate, proteins and fats. Bisoprolol is a drug belonging to the group of beta blockers, which is primarily used in cardiovascular disorders.Specifically, it is a β1 selective type adrenergic receptor blocker. Bisoprolol is beneficial in treatment for hypertension, reduced cardiac ischemia, congestive cardiac failure, preventative treatment before and primary treatment after heart attacks decreasing the chances of recurrence. During hypertension there is an elevation in blood pressure, which is what bisoprolol target.The present study is to isolate biopolymer and to evaluate its emulsifiability by formulating o/w type of emulsion using bisoprolol as a model drug. The results were compared with the standard emulsion which was prepared by using acacia as an emulsifier.

Material and methods

Prunus insititia was obtained from local market, Dehradun. Gum acacia obtained from CDH laboratory reagent, New Delhi. All other chemicals and reagents used were of pharmacopoeal and analytical grade were procured from, Himgiri traders, Dehradun.

Extraction and isolation of biopolymer

The polymer was isolated from the fruit pulp of *Prunus insititia* by collecting 50gm of the pulp and treating with 200ml of water. The aqueous extract was then treated with 3 vol. of dimethyl ketone and kept aside in refrigeration for 6 hrs. The biopolymer was collected by centrifugation for 15minutes at 4000rpm and dried in hot air oven at 40^0C [2-8].

Physicochemical evaluation of biopolymer

The isolated biopolymer was subjected for physicochemical properties like solubility, pH, color and viscosity, surface tension [9-11].

Determination of viscosity

1%w/v solution of the biopolymer was prepared and viscosity was measured using Ostwald viscometer at 25^0C.

Determination of pH

1%w/v solution of the biopolymer was prepared and pH was measured using a Pen pH meter at 25^0C.

Determination of surface tension

1%w/v solution of the biopolymer was prepared and surface tension was measured using stalagmometer at 25^0C by the drop count method.

Chemical characterization

The extracted biopolymer was tested for chemical characteristics like test for carbohydrates, proteins, alkaloids, mucilage, test for chlorides, and test for sulfate [12].

Evaluation of acute toxicity

The biopolymer which was isolated from the fruit pulp of Prunus insititia were subjected for Acute oral toxicity was performed as per OECD-423 guidelines and observed the parameters grooming, hyperactivity, sedation, loss of righting reflex, skin rashes and convulsion for 1 hr post-dosing, and at least once daily for 14 days by administering the dose of 5gm/kg body weight. The animal study was approved by Institutional Animal Ethics committee, registration no. 1435/PO/a/11/CPCSEA .Albino mice weight 22-25g selected by

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random sampling technique were used in the study. The animals were fasted overnight, provided only water after which biopolymer solution was administered to the animals orally at the dose of 5g/kg, by oral feeding tubes and the animals were observed individually for mortality and acute toxicity signs. The parameters observed were, sedation, hyperactivity, loss of righting reflex, grooming, skin rashes and convulsion for 1 hr post-dosing, and at least once daily for 14 days [13].

Screening for emulsifiability

The biopolymer was screened for its emulsifiability by preparing emulsions using liquid paraffin as oil, biopolymer as an emulsifier, Bisoprolol as a model drug and other co processing agents like preservatives were used. Eight different emulsions (FE1 to FE8) were formulated by varying proportions of biopolymer by dry gum method. By measuring a specified quantity of mineral oil ,biopolymer and water required for preparing primary emulsion it was calculated based on the ratio of 4:2:0.05 to 10 initially biopolymer was transferred into the motor and triturated with the mineral oil for 3 minutes later specified quantity of distilled water required for primary emulsion was in corporate at once and triturated uniformly in unidirectional until to form primary emulsion later the emulsion was suitably diluted with distilled water containing preservatives and all formulated emulsions were subjected for various evaluation parameters including their stability studies[14].

Evaluation of emulsions

The prepared emulsions were subjected for various evaluation parameters like color, homogeneity, consistency, globule size, pH, the effect of freezing and thawing cycle and effect of centrifugation [15-17].

Globule size determination

The globule size of the prepared emulsion was measured with an optical microscope (x400) equipped with a calibrated eyepiece micrometer. The mean diameter was calculated on the basis of at least 150 droplets with the formula: $dm=\sum d_i n_i/\sum n_i$ where n_i is the number of droplets with diameter d_i.

Centrifugal effect

The emulsions were placed in the centrifuge tubes of centrifuge for 15 min with 4000rpm. The emulsion stability 'S' was determined from the formula:

S= [(V0-V)/V0] X 100%.

Where:-

S=emulsion stability %,

V0-volume of the emulsion undergo centrifugation cm^3

V - Volume of the phase given off cm^3.No phase separation was observed in any case of formulation, indicating 100% stability after centrifugation.

Freezing and thawing cycle

The prepared emulsions were subjected to freezing and thawing cycle. For one cycle the first one hour emulsions were kept at room temperature and for second one hour the emulsions were kept in deep freezer. The volume of creaming or phase separation was observed and reported

Heating and cooling

The prepared emulsions were subjected to Heating and cooling cycles. For one cycle the first one hour emulsions were kept at room temperature and for second one hour the emulsions were kept in a refrigerator. The volume of creaming or phase separation was observed and reported.

Viscosity

The prepared emulsions were subjected to viscosity measurements at room temperature (25 ± 0.1^0C) by using Fungi lab Viscometer and rotated at 12 [min.] and 80 [max.] rpm by using spindle no.61. The corresponding dial reading was noted at each speed.

FTIR of biopolymer

The extracted biopolymer was subjected to FTIR spectroscopy .The sample for the analysis was prepared by solid sampling technique using potassium bromide pellets using FTIR 1601 (Shimadzu, Tokyo, Japan). The scanning range was 500-4000$cm^{-1.}$

HPLC of biopolymer

The extracted biopolymer was subjected to HPLC (Acme 9000, Younglin Instrument, Korea). The flow rate was maintained at 1ml/min and the sample was scanned at 283nm.

DSC of biopolymer

The glass transition temperature (TG) and melting temperature (Tm) of the biopolymer was analyzed with the (Perkin Elmer, Jade DSC, Temp- 50-250 ºC, at the rate of 10 ºC/min; Nitrogen gas flow rate- 20ml/min).

Drug content

Drug concentration in emulsion was measured by spectrophotometer. Bisoprolol content in emulsion was measured by dissolving known quantity of emulsion in solvent (methanol) by sonication. Absorbance was measured after suitable dilution at 224 nm in UV/VIS spectrophotometer (UV-1700 CE, Shimadzu Corporation, Japan).

In vitro drug release study

The in vitro release study of bisoprolol from the emulsions was performed through the surface of egg membrane. The egg membrane was clamped between the donor and the receptor chamber of diffusion cell, using Franz diffusion cell, with a diffusional area of 1.767 cm2 (Microette-Hanson, 57-6AS9 Model). 0.3 g of the emulsion was placed on the membrane surface in the donor compartment which was sealed from the atmosphere with a plastic film, in order to ensure sink conditions during the experiment. The receptor compartment of the cell was filled with 7 ml of phosphate buffer (pH 7.4).During the study, the solution in the receptor side was kept at 37±1ºC and it was stirred magnetically at 600 rpm. The samples from the receptor compartment were withdrawn at 30, 60, 120, 180, 240, 300 and 360 minutes intervals and immediately replaced by an equal volume of fresh buffer solution (to keep the diffusion medium constant). The collected samples were analyzed for the bisoprolol assay spectrophotometrically at a wavelength of 224 nm. All release studies were carried out in triplicate.

Accelerated stability studies of emulsions

Stability studies were performed according to ICH guidelines. The formulations were stored in hot air oven at 37 ± 2°, 45 ± 2° and 60 ± 2° for a period of 3 months. The samples were analyzed for drug content every two weeks by an UV-Visible spectrophotometer at 224 nm. Stability study was also carried out by measuring the change in pH of emulsion at regular interval of time.

Results

The biopolymer isolated from *Prunus insititia* has displayed its inbuilt emulsifying property which was confirmed by formulating suitable drug loaded emulsions and all the formulations emulsions displayed promising stability towards globule size, freezing and thawing cycle, heating and cooling cycle.

The proposed mechanism for stabilizing the formulated emulsions by the biopolymer may be having ability to form a fine coat around the dispersed phase globules and assists to reduce the interfacial tension between the two phases and making the disperse system stable for a significant period.

Preparation of emulsions

Eight different emulsions (FE1 to FE8) were formulated by varying proportions of biopolymer by dry gum method. Bisoprolol was used as a model drug. By measuring a specified quantity of mineral oil ,biopolymer and water required for preparing primary emulsion it was calculated based on the ratio of 4:2:0.05 to 10 initially biopolymer was transferred into the motor and triturated with the mineral oil for 3 minutes later specified quantity of distilled water required for primary emulsion was in corporate at once and triturated uniformly in unidirectional until to form primary emulsion later the emulsion was suitably diluted with distilled water containing preservatives

The formula for the preparation of emulsions is given in table.1.

Table 1: - Preparation of emulsions

FORMULATION	FE 1	FE 2	FE 3	FE 4	FE 5	FE 6	FE7	FE 8
Drug(mg)	10	10	10	10	10	10	10	10
OIL(ml)	30	30	30	30	30	30	30	30
BIOPOLYMER(mg)	50	100	200	400	600	800	1000	-----
ACACIA(gm)	-----	-----	-----	-----	-----	-----	-----	10
SODIUM BENZOATE(%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
WATER UPTO(ml)	100	100	100	100	100	100	100	100

Physicochemical characterization of the biopolymer

The biopolymer was subjected to various physicochemical characteristics. the results of which are given in table 2.

Table 2:- Physicochemical characterization of the biopolymer

PH(1.0 %w/v sol^{n})	6.9
COLOR	Brown
SOLUBILITY	Soluble in water, methanol, dil.Hcl &20% NaoH
VISCOSITY(1.0 %w/v sol^{n})	1.093cps
SURFACE TENSION(1.0w/v % sol^{n})	67.012 dynes/cm2

Chemical characterization

The extracted biopolymer was tested for chemical tests and it shows positive test for carbohydrates, proteins, mucilage and negative test for chlorides, and test for sulfate [9-10]. The results are shown in table. 3

Table 3 Chemical test

S.NO	TESTS	OBSERVATION
1.	Test for Carbohydrates	+
2.	Test for proteins	+
3.	Test for alkaloids	_
4.	Test for mucilage	+
5.	Test for chlorides	_
6.	Test for sulphates	_

FTIR of biopolymer

The FTIR spectrum of biopolymer obtained from *Prunus insititia* showed characteristic peaks at 2934.82, 2363.87, 1731.19, 1638.60, 1419.67, 1260.54, 1078.25, 892.12, 817.85, 778.31 and 668.36cm^{-1}.The FTIR spectrum of biopolymer obtained from *Prunus insititia* is shown in Fig.1

HPLC of biopolymer

The HPLC spectra of the extracted biopolymer revealed that the polymer showed a sharp peak at 224nm with a RT (min) at 3.5500.this characteristic can be used to identify its retention time of the polymer using mobile phase methanol. The graph is shown in fig.2

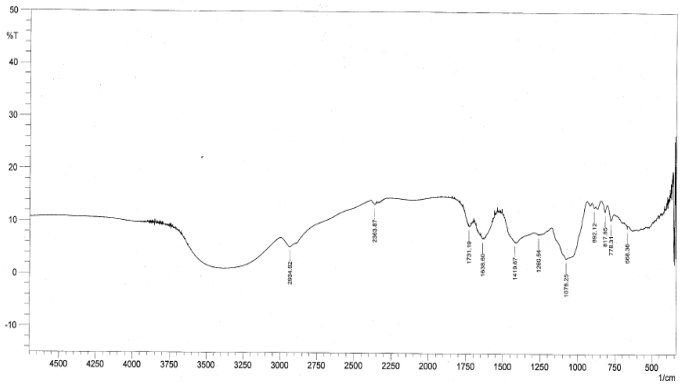


Fig:-1 FTIR of biopolymer

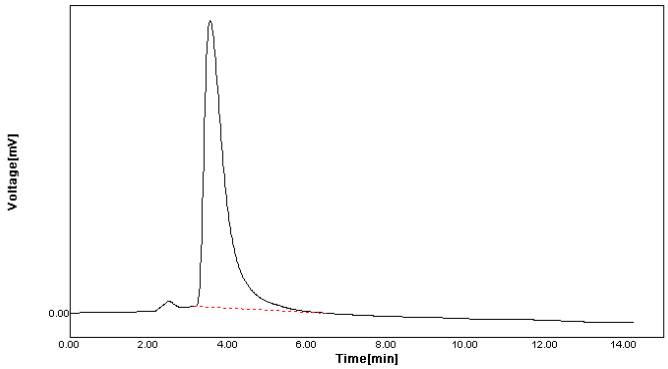


Fig:-2 HPLC of biopolymer

DSC of biopolymer

The DSC spectra of the extracted biopolymer revealed that the polymer started changing its state at 120.76° and ended at 151.81° and the GTT of the polymer was determined at 137.82°C.at this temperature the behavior of the polymer started changing at 137.82°C from glassy state to rubbery state .at this temperature the polymer undergoes enthalpy of relaxation which appears as a positive deviation from DSC base line that results in increase in the specific heat above Tg[18]. The DSC spectrum is shown in fig.3

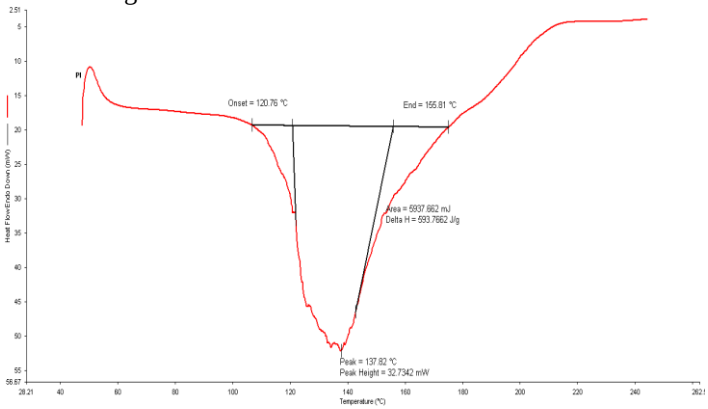


Fig:-3 DSC of biopolymer

Evaluation of formulated emulsion

The prepared emulsions were white, viscous creamy preparations with a smooth and homogeneous appearance.the results of various evaluation parameters are shown in table-4. The viscosity and drug content of the formulated emulsions were also determined the results are shown in fig. 4 and 5 respectively.

Table 4: - Physical evaluation of formulated emulsions

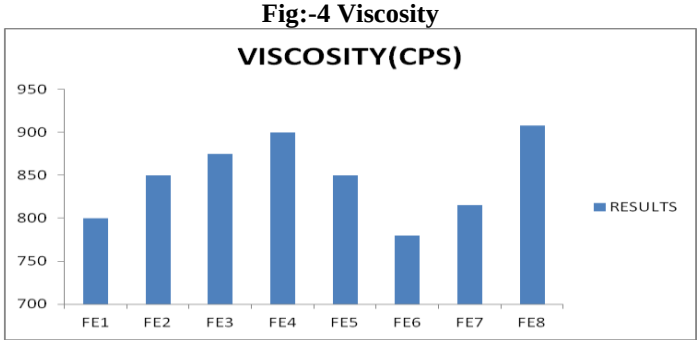
FORMULATION	pH	Globule size (µm)
FE1	8.4	8-11
FE2	8.4	12-18
FE3	7.2	9-13
FE4	7.5	10-18
FE5	8.1	12-23
FE6	7.4	17-28
FE7	8.3	12-25
FE8	8.4	10-30

Effect of centrifugation (At 4000 rpm) -all the formulations were stable for 60 mins

Effect of freezing and thawing cycle (1cycle=15mins)-all the formulations were stable for 3 cycles

Effect of heating and cooling cycle (1cycle=15mins) -all the formulations were stable for 4 cycles

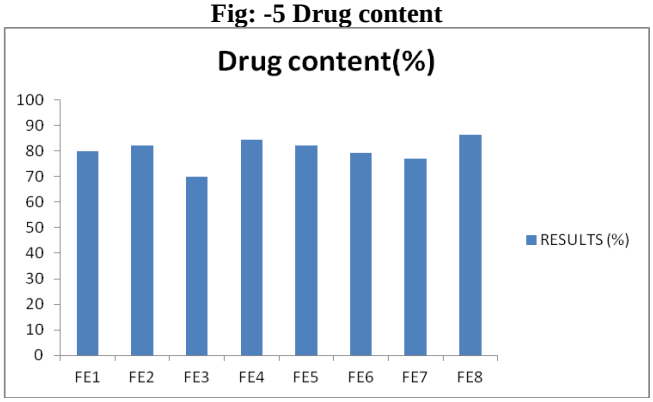
Fig:-4 Viscosity



Acute toxicity studies

The limit dose of 5g/kg did not cause any mortality or signs of acute toxicity in the mice tested during the observation period. This indicates that the biopolymer is relatively safe when administered orally in mice.

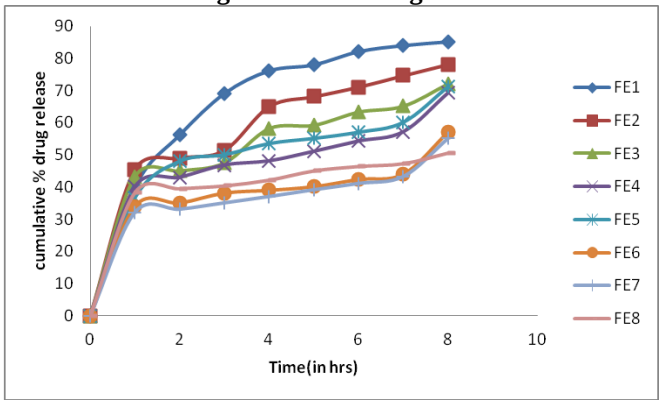
Fig: -5 Drug content



In vitro drug release study

All the formulated emulsions were subjected to in vitro drug release study. FE1 and FE2 showed good release profile after 8 hrs.The in vitro drug release profile of all the formulations is shown in fig. 6

Fig :-6 In vitro drug release



Accelerated stability studies of emulsions

The accelerated stability studies were performed according to ICH guidelines for 3 months and the results were found to be stable in varying temperature as shown in Table.5

Table 5: Accelerated stability study of optimized emulsion formulation FE1 &FE2

Storage Temp. 0C	Period of studies in week FE1			Period of studies in week FE2		
	1 month	2 months	3 months	1 month	2 months	3 months
37 ± 2	91.2%	90.3%	90.2%	82.1%	82.02%	83.15%
45 ± 2	92.0%	92.11%	94.06%	83.21%	81.05%	82.24%
60 ± 2	93.1%	95.03%	93.05%	81.12%	83.20%	84.11%
PH	8.4	8.2	8.3	7.4	7.8	8.1

Discussion

The biopolymer was isolated from the fruit pulp of *Prunus insititia* by simplified economical process and it was subjected for various physicochemical properties and the study revealed that it was brown in colour, having characteristic odor and taste with pH 6.9, soluble in water and methanol.

The acute toxicity study revealed that there is no any sign of toxic reactions and behavioral changes observe in experimental animals as the polymer is isolated from the fruit pulp of *Prunus insititia* which is edible and generally recognize as a safe and comprises of mainly carbohydrates, proteins, fats and minerals having edible value.

The inbuilt property of the polymer was screened by formulating emulsions using liquid paraffin oil, water and Bisoprolol as a model drug and along with biopolymer as a bioemulgent. Our experimental results revealed that the formulated emulsion (FE1 and FE2) was showed significant stability along with the promising in vitro release profile with a pattern of Zero order and the drug release was extended for a period of 8 hrs. The proposed mechanism of the biomaterial for making stable emulsion between oil and water is due to forming a thin film coat over the dispersed phase globules and assist to reduce the interfacial tension between two phases and the polymer potentiality was compared with the emulsion that was prepared by acacia as an emulsifier in the ratio of 1:10. In comparison with the standard the biopolymer displayed significant stability in lower concentration 1:0.5 and 1:1 where as the standard polymer displayed it's at 1:10 ratio. This results confirm that at very lower concentration it posses potential emulsifiability property for stabilizing the drug loaded biphasic system.

Hence the conclusion was drawn that the isolated biopolymer can serve as a potent bioemulgent for formulating various stable drug loaded emulsions.

Conclusion

This biopolymer can serve as a potential bioemulgent for preparing various drug loaded formulations or drug loaded colloidal dosage forms. The biopolymer in lower concentration showed its potential emulsifying property in the formulations FE1 and FE2 containing 50 and 100 mg along with uniform globule size ranging from 12 to 30 µm and stable for 3 freezing and thawing cycle. These emulsions are very significantly stable in comparison with standard emulsion.

Conflicts of interest

All authors have none to declare.

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