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Research Article

Biomaterial from Strawberry Fruit Pulp: It's Spectrophotometric and Cell-Line Toxicity Study

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Abstract

The aim of this research was to isolate and characterize the novel biopolymer from Fragaria ananassa fruits. The biopolymer was isolated by simplified and economical process and analysed for different physico-chemical and spectral properties. The isolated biopolymer appears as free flowing powder. The isolation procedures were optimized by repeating it for six times and thus were found to be economical and reproducible in nature with the yield of 13 ± 2%.The different functional groups like presence of hydroxyl, alkanes and alkenes, carboxylic acid which confirms its polymeric nature. The other groups like aromatic ring at, ketone were found to be present in the FTIR spectra, NMR and mass spectra. shows the mean % cell viability ranging from 101.62 ± 0.928 to 81.9 ± 6.265 % with IC50 values (µg/ml) of >500. Thus the cell viability assay data demonstrate that there is no cell death observed in assay. The study finds that the isolated biopolymer from Fragaria ananassa fruits contains unusual inherent stabilizer and cum retardant capabilities that can be exploited to develop a drug loaded delivery systems for long-term administration of drug candidate.

The results of the cell viability assay thus show that no cell death was noticed during the assay. According to the study, the biopolymer that was extracted from Fragaria ananassa fruits has exceptional built-in stabilizer and combustible properties that can be used to create drug-loaded delivery systems for long-term drug candidate administration.

Keywords: Biopolymer; Biomaterial; SEM; Mass Spectra, FTIR Spectra; Economical; Cell Line Toxicity

Introduction

The term "biopolymer" has inspired researchers as a potential advanced biomaterial for the creation and design of custom drug delivery frames [1]. Today, the drawbacks of artificial and semi-synthetic polymers are the complications that become apparent in the manufacture of scaffolds for drug delivery. Artificial and semi-artificial polymers are the preferred choice when it comes to enhancing customized pharmaceutical delivery scaffolds. In any case, these polymers have many unfavorable effects that can lead to inconsistent understanding. Therefore, biopolymers isolated from various characteristic sources such as flowers, seeds, bark and rhizomes have attracted the attention of analysts when planning new drug delivery frameworks [2]. Encapsulated biopolymers featuring novel polymer highlights could be used as an excellent biomaterial for creating stable delivery scaffolds. Since these biopolymers are derived from Common [3], they are essentially biodegradable and biocompatible. Other unique features such as excellent biodegradability, mucosal adhesion, filminess, delay and rate control properties have been demonstrated in the study [4]. These isolated biopolymers have excellent release rate

regulatory properties and can be used to build delayed, controlled, and sustained release drug delivery systems [5,8]. The isolated biopolymers were characterized for a variety of physicochemical and spectrophotometric properties [9,12].

Materials and Methods

The hybrid Fragaria ananassa fruit were purchased from a market in Lucknow, Uttar Pradesh. The rest of the solvents and compounds were analytical reagent-grade.

Isolation of biopolymer

For Separation of biopolymer 200 g of fruit pulp was weighed and soaked in redistilled water overnight. The exposed fruit was crushed with a grinder into a paste. If desired, a small amount of distilled water can be added during the grinding process. A muslin cloth was used to filter the paste. The collected filtrate was centrifuged at 5000 rpm for 10 minutes. A supernatant was obtained after centrifugation. Residues were removed using centrifugation. Next, half of the supernatant was treated with acetone in a ratio of 1: 1, 1: 2, 1: 3. Half of the supernatant was treated with methanol in the same ratio as acetone. Then they were placed in the refrigerator overnight. The mixture was centrifuged at 5000 rpm for 30 minutes. After discarding the supernatant, the biomaterial was collected as a precipitate and air dried. The product was stored in a desiccator for 48 hours. The resulting biomaterial was screened with number 200 before being stored for future use. This technique was optimized by running 6 times and calculating the recovery rate [13].

Characterization of isolated biopolymer

The physico-chemical characteristics of the various biomaterials that were separated from various edible natural sources were described. Different characteristics were used to characterise the isolated biopolymers that were obtained. The separated biopolymers are a free-flowing powder in appearance. The optimisation of the isolation techniques, which involved repeating them six times, led to their discovery as being practical and repeatable.

The physicochemical characteristics of the isolated biopolymer were established for the following organoleptic qualities tests, including colour, odour, and taste. The biomaterial also underwent chemical and solubility testing.

Solubility

A study on the solubility of the isolated biopolymers was conducted and reported using a variety of solvents, including water, acetone, methanol, ethyl acetate, 10% w/v hydrochloric acid solution, and diethyl ether. The extra isolated biomaterial was gradually added to 10 ml of the appropriate solvent solution in the beaker. To achieve equilibrium, the solution was evenly disseminated and put on an orbital shaker for 24 hours. After 10 minutes of centrifuging at 400 rpm, the solution was filtered to obtain the clear solution. The filtrate was then permitted to be measured using a UV spectrophotometer (Mapada). For each extracted biopolymer, the method was carried out three times [14-16].

Biochemical tests for bioconstituents

A test tube was filled with 1ml of freshly made biomaterial solution (5% w/v prepared biomaterial solution in double distilled water). Molisch reagent should be added two drops. In the test tube, add 1-2 ml of concentrated sulfuric acid and look for the formation of purple colour at the junction of the two layers that have formed. Test results were reported [17].

The extracted biomaterial was treated with a 0.1% ninhydrin reagent solution and a 10% tannic acid solution to test for the presence of protein. Precipitate that is blue and yellow in colour shows the presence of protein. The results of the test were reported.

The biuret test was used to verify the presence of proteins. In a test tube, 2 ml of biomaterial (5 percent biopolymer solution in distilled water) was added together with 1 ml of sodium hydroxide solution and a few drops of copper sulphate solution. Five minutes were given for the mixture to rest while any colour changes were monitored. The presence of proteins is confirmed by the emergence of violet colour. A test was run and the results reported [17].

Spectrophotometric characterization

The isolated biopolymer was characterised using SEM analysis, DSC testing, IR spectroscopy, mass spectroscopy, and NMR spectroscopy.

SEM analysis

The isolated biopolymer was studied with a scanning electron microscope. SEM testing was used to analyse the internal structure

and external surface. Aluminium studs were treated with a small amount of biopolymer before being coated with gold using a coater sputter while under vacuum. Images of the biopolymer under study were captured using scanning electron microscopy.

FTIR

It was done to get the KBr discs ready for FTIR spectroscopy. 1mg of separated biopolymer was mixed with 100mg of dried and dehydrated solid KBr. The substance was ground in a mortar and pestle and exposed to an IR lamp to eliminate any moisture. The prepared disc was put in a disc holder and positioned in the path of the IR radiation. The 4000-200cm^{-1} region of the spectrum was recorded [17].

Differential scanning calorimetry (DSC) analysis

Thermal analysis method called DSC testing measures the amount of heat that enters or leaves a sample in relation to temperature. It has been established what temperature the glass transition occurs at. The heat flow could occur at temperatures between 50 and 300 °C. The DSC technique was used to take the thermogram [18]

Nuclear magnetic resonance

NMR spectroscopy was used to analyse the spectra of the isolated biopolymer. The substance was dissolved using a certain solvent, such as $CDCl_3$. The combination was delivered into the device at a rapid flow rate. The valve switch was used to stop the flow. The measurement is now finished. After the measurement was finished, the spectrum was processed and looked at on an automated computer [19].

Results and Discussion

Biopolymer isolation

Repeating the determinations six times and calculating the means standard deviation (SD) allowed for the optimisation of the isolation process. The Fragaria ananassa biopolymer was discovered to be brownish-cream in colour and had a yield of 120.4%.

The physico-chemical characteristics of the biomaterial, which was separated from several edible natural sources including fruit, were described. Different metrics were used to characterise the isolated biopolymer that was obtained. The biopolymer is separated as a free-flowing powder. The optimisation of the isolation techniques over six repetitions led to its discovery as being practical and repeatable ([17-19]).

The process's capacity to be optimised and scaled up supported the yield percentage numbers. The yield was discovered to be consistently repeatable in nature with no notable variance.

The biopolymer was discovered to have an amorphous form and a flaky surface. The pH analysis establishes that the biopolymers are non-irritating since they are similar to the physiological pH.

Thus, the existence of various components, such as proteins and carbohydrates, in chemical tests supported its polymeric nature [17-19].

Isolated biopolymer characterization

The biopolymer that was extracted appeared to be brownish-white. It was revealed that the biopolymer had a particular flavour and was odourless. The colour and smell were noted to be distinctive. It was found to be very marginally soluble in water. Tests for protein and carbohydrates also revealed their presence. Its polymeric character is confirmed by the presence of these macromolecular components [17].

Color

It was discovered that the biopolymer powder had a brownish-white colour.

Odor

The biopolymer powder was discovered to have a Characteristic smell.

The biopolymer powder was discovered to have a distinctive flavour. Table 1 provides a summary of the results.

Solubility

The solubility investigation (n = 3) was carried out in triplicate. The biopolymer was discovered to be somewhat soluble in methanol and water. Table 2 provides an overview of the biomaterial's solubility studies in several solvents.

Extracted biomaterial	Observed Color	Observed Odor	Observed Taste	Observed Shape	Observed Color changing point (℃)	Observed pH (in aqueous solution)
Fragaria ananassa	Brownish- white	Characteristic	Characteristic	Irregular	229 ± 5	6.60 ± 0.31

Table 1: Organoleptic characteristics of the isolated biomaterial.

Biomaterial Used	Solvents used					
	Double distilled water	Acetone	Ethyl acetate	Methanol	Diethyl ether	10%HCl
Fragaria ananassa	Soluble	Insoluble	Insoluble	soluble	Insoluble	Partial soluble

Table 2: Solubility study of extracted biomaterial.

Biomatrial particle size

The vast particle size distribution is revealed by optical microscope analysis of the particle size of all isolated biomaterial. The range of the obtained size distribution was discovered to be 76.56 - 650.91. The isolated biopolymer's particle size evaluation reveals the considerable particle size distribution [18]. Table 3 provides an overview of the biomaterial's particle size in various solvents.

Isolated biomaterial	Particle size range (µm)
Fragaria ananassa	76.56 - 650.91

Table 3: Evaluation of particle size of isolated biomaterial.

Chemical analysis

The presence of a violet colour ring at the interface, as revealed by the Molisch test of isolated biopolymers, indicated the existence of carbohydrates.

Protein presence was determined by the formation of blue and yellow coloured precipitate following treatment with the Ninhydrin reagent. Since all biopolymers displayed the violet colour, the Biuret test also supported the existence of proteins [17-20].

SEM

The isolated biopolymer's SEM investigation reveals a rough and flaky structure, as seen in Figure 1. Additionally, granular and amorphous structure was seen in the SEM picture, pointing to the biopolymer's polymeric nature [20].

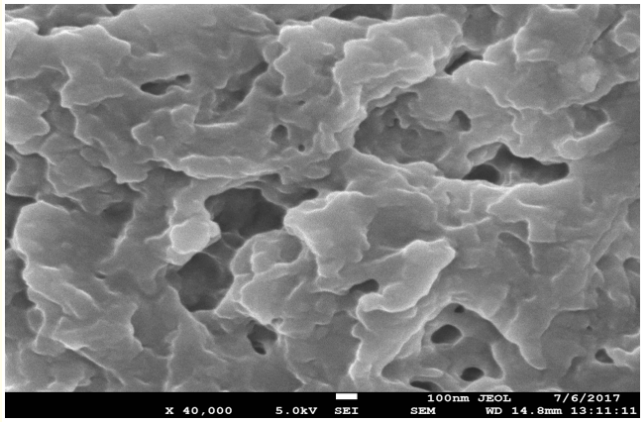

Figure 1: SEM of biomaterial from Fragaria ananassa.

Infrared spectroscopy (IR spectroscopy)

FTIR spectroscopy of Fragaria ananassa isolated biopolymer shows the presence of different functional groups in IR spectra which are responsible for the polymeric nature of the isolated biopolymer. The FTIR spectra shows the presence of different functional groups like hydroxyl (3396.15 cm^{-1}), alkynes (669.43 cm^{-1}), carboxylic acid (1410.35 cm^{-1}) which confirms its polymeric characteristics. The other groups like amide at 1639.02 cm^{-1}, alkane at 2925.45 cm^{-1}, tertiary alcohol at 1215.89 cm^{-1},were found

to be present in the IR spectra. Presence of these functional groups is responsible for the retardibility in drug release. FTIR spectra of isolated biomaterial has been given in figure 2.

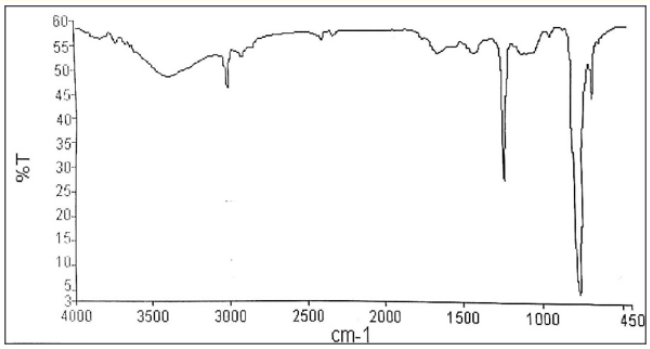

Figure 2: FTIR Spectra of biomaterial from Fragaria × ananassa.

The biomaterial's FTIR spectroscopy revealed many functional groups, including the presence of hydroxyl, alkanes and alkenes, and carboxylic acid, which supports its polymeric properties. The FTIR spectra revealed the presence of additional groups such as aromatic ring at and ketone. As with ordinary polymers, the presence of these functional groups is what causes the drug release to be delayed. This made its polymeric nature apparent [17].

Mass spectra of Fragaria ananassa biomaterial reveals that the isolated biopolymer is polymeric in nature due to presence of large molecular weight structure. It indicates the presence of protein. HRMS spectra of isolated biopolymer showed the parent peak at m/z 456.3355 which confirmed its large molecular weight structure like polymer.

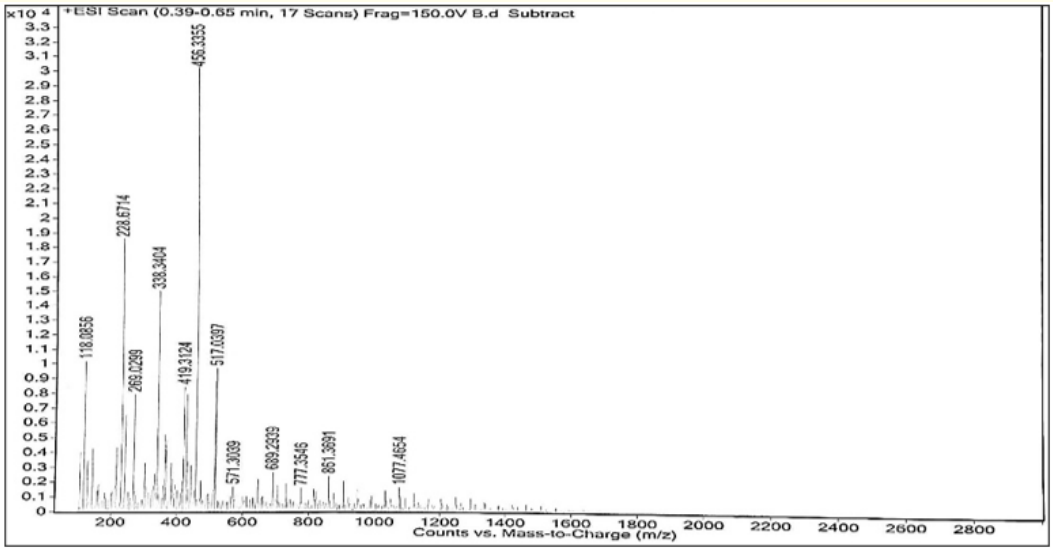

Figure 3: Mass Spectra of biomaterial from Fragaria ananassa.

The proton NMR spectra of Fragaria ananassa biomaterial show the presence of different peaks like multiplet at 0.829-0.902ppm which reveals the presence of primary alkyl group, peaks at 1.232ppm confirms the presence of methylene group, at 1.255ppm shows the presence of hydroxyl group. The presence of these groups confirmed its polymeric nature (Figure 4). These function groups' existence in biomaterials provided evidence for their polymeric nature [17-20].

DSC (Differential scanning calorimetry) thermogram of isolated biomaterial from Fragaria ananassa shows peaks at 83.794Cel,

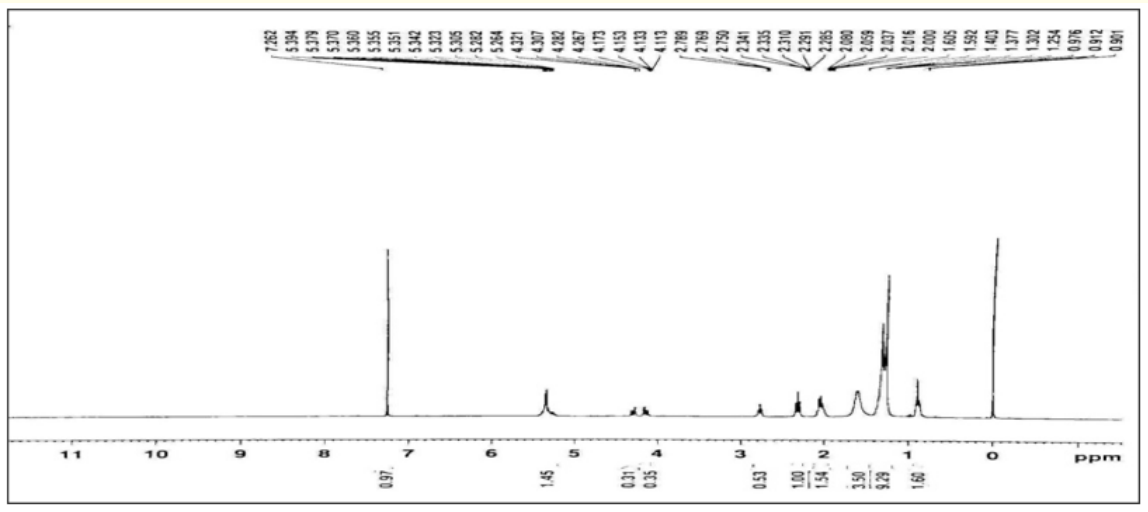

Figure 3: Mass Spectra of biomaterial from Fragaria ananassa.

161.035Cel and 208.709Cel. The area was found to be 229 mj/mg, 30.5 mj/mg and 10.3 mj/mg respectively. The DSC spectra with broad endothermic peak revealed about the amorphous nature of biopolymer. The DSC spectra is given in Figure 5. The obtained result reveals its polymeric nature [18].

Figure 5: DSC of biomaterial from Fragaria ananassa.

NMR spectroscopy

The proton NMR spectra of Fragaria ananassa biomaterial show the presence of different peaks like multiplet at 0.829-0.902 ppm which reveals the presence of primary alkyl group, peaks at 1.232 ppm confirms the presence of methylene group, at 1.255 ppm hows the presence of hydroxyl group (Figure 6). The presence of these groups confirmed its polymeric nature [18].

Figure 6: NMR Spectra of biomaterial from Fragaria ananassa.

Biomaterial toxicity in cell lines

The cell line toxicity study of biopolymer from Fragaria ananassa in the concentration of 31.25,62.25, 125,250 and 500 (µg/ml) shows the mean % cell viability ranging from 108.82 ± 5.33% to 81.21 ± .85% with IC50 values (µg/ml) of >500. Thus the cell viability assay data demonstrate that there is no cell death observed in assay. Along with this the IC50 (Inhibitory concentration) value of the biopolymer was observed above 100 µg/ml. So the obtained data revealed that isolated biopolymer was safe and non-toxic in nature. So it can be safely used for the preparation of drug loaded bionanosuspension [17]. Cell-line toxicity graph between concentration of biopolymer Fragaria ananassa v/s Mean % of cell viability is shown in figure 7.

Figure 7: Cell-line toxicity graph between concentration of biopolymer Fragaria ananassa v/s Mean % of cell viability. The results has been expressed as Mean ± SEM (n = 3).

A drug-loaded delivery system for long-term administration of a drug candidate can be developed using the isolated biopolymer from Fragaria ananassa fruit, according to the study, which reveals that it has exceptional inherent stabiliser and cum retardant characteristics. Since isolated biopolymers are naturally biodegradable, biocompatible, bioretardant, and biostabilizers, they can be employed as an alternative to regular polymers and are still being investigated for their distinct inherent features in drug delivery. From the various edible natural sources, the biopolymers can be economically separated [17-20]. These isolated biopolymers can be employed to create delayed, regulated, and sustained release drug delivery systems because they have good release rate regulation features. Numerous physical, chemical, and spectrophotometric characteristics of the isolated biopolymers were examined.

Conflict of Interest

The authors of the article do not have any conflict of interest.

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