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Acute Toxicity Studies and Pharmaceutical Effectiveness of Biopolymer from Pulp of *Lageneria siceraria*

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Abstract

The current study was aimed to isolate and characterize a novel mucoadhesive biopolymer from pulp of *Lageneria siceraria*. The biopolymer was isolated from the pulp of *Lageneria siceraria* and characterized for various physico-chemical properties and chemical tests like Molisch's test, Fehling's test, Biuret test, Ninhydrin test and iodine test. It contains high amount of dietary fibers and several vitamins, iron, calcium and magnesium etc. The isolated biopolymer was subjected to shear stress method for determining mucoadhesivity and MS mucoretentive method for determination mucoretaintability and was compared with standard polymers as HPMC and sodium CMC. The spectral analysis were performed and confirmed by IR spectroscopy, 1H NMR spectroscopy, Differential scanning calorimetry, Mass spectroscopy, SEM imaging and Elemental analysis for identification of isolated biopolymer. The single dose acute toxicity study of the biopolymer was performed on rats (Wistar rats, either sex, 200-250g) for two weeks. The isolated biopolymer revealed better mucoadhesive and mucoretentive property than HPMC and Sodium CMC. The results concluded that the isolated biopolymer of *Lageneria siceraria* was biocompatible biodegradable and possessed good mucoadhesivity and mucoretaintability.

Keywords Bioadhesion, *Lageneria siceraria*, Biopolymer, Mucoadhesion, Mucoretaintability

Introduction

Mucoadhesive drug delivery is always a renewed interest in past decades, for prolonging the effect of mucoadhesive drug delivery systems. Mucoadhesion is a term used to define adhesion between two materials, at least one of which holds the mucosal surface for a longer duration of time with the help of interfacial forces. Conversely, bioadhesion is defined as a term which widely includes adhesive interactions to any naturally derived substance. Hence, there are many benefits of the system over the other route of drug delivery such as oral route in which there are certain dilemmas i.e., first- pass metabolism and GI degradation, which ultimately reduces the bioavailability of some drugs (Flavia et al., 2010).

Lageneria siceraria is a climbing plant with hard shelled gourds as fruits; the plant commonly known as bottle gourd from family *cucurbitaceae*. It consists of high amount of dietary fibers and various vitamins such as vitamin A, B1, B2, B5, C, K, B6, and E. It also contains manganese, calcium, iron, magnesium, phosphorus, selenium and protein (Kumar et al., 2012).

The edible portion of fruit of *Lageneria siceraria* is a good source of glucose and fructose. The amino acid composition of the fruit depicts leucine (0.8 mg/g); tyrosine (0.4 mg/g); alanine (0.5 mg/g); glutamic acid (0.3 mg/g); cystine (0.6 mg/g); cysteine (0.3 mg/g); arginine (0.4 mg/g); and proline (0.3 mg/g). The fruit of *Lagenaria siceraria* is a best source of vitamins B and a fair source of ascorbic acid. The fruit also contains triterpenoids (22- deoxo cucurbitacin D and 22- deoxo isocucurbitacin D), oleanolic acid, β-sitosterol, campesterol, isoquercitrin (Kumar et al., 2012, Tyagi et al., 2012).

The medicinal use of *lageneria scieraria* is in the treatment of diabetes, ulcer, piles, jaundice, colitis, skin disease, hypertension and congestive heart failure. The different parts of *lageneria scieraria* shows different properties like fruit pulp as diuresis, flower used as an antidote, plant shows antibiotic property, extract of leaves used in treatment of baldness and juice prevents excessive loss of sodium and useful in diabetes , heart disease digestive and urinary infection(Kumar et al., 2012, Rahman 2003).

Lageneria scieraria has highest content of choline among all the existing vegetables till date, which functions as the precursor of neurotransmitter acetylcholine, this is very essential for retaining and enhancing memory (Minocha 2015). The authors worked in this current research on the isolation of the biomaterial from the pulp of *Lagenaria siceraria* and finally evaluated the intrinsic mucoadhesive and mucoretentive properties, which can be proved a good source of a carrier to deliver the drug for targeting.

Experimental section

Material and Methods

Lagenaria siceraria was obtained from the local market. Acetone was purchased from CDH Pvt. Ltd. Sodium dihydrogen phosphate, potassium dihydrogen phosphate, and sodium hydroxide were purchased from Qualigen Chemicals Pvt. Ltd. Double distilled water was prepared from the institutional laboratory. All chemicals used were of analytical grade.

Isolation of Biopolymer and physicochemical characterization of isolated biopolymer

The procedure for isolation of biopolymer from *Lagenaria siceraria* is demonstrated in Figure1.

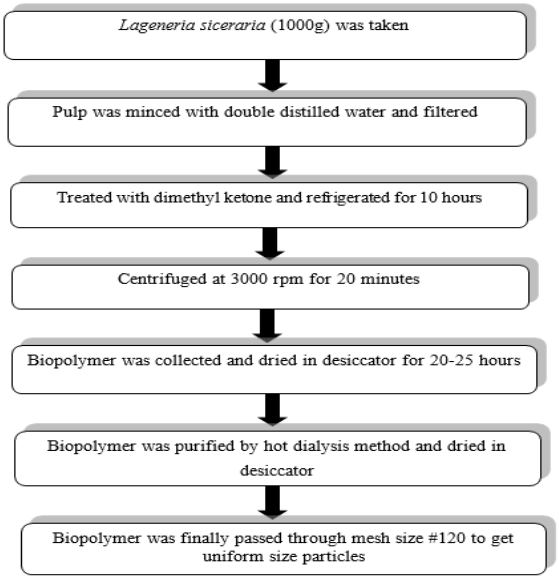


Figure 1: Method of Isolation Biopolymer

Various physicochemical properties were examined for the isolated biopolymer such as colour, odour, solubility, color changing point and chemical tests (Martin 2001, Subramanyam 2014). Isolated biopolymer was visually inspected for colour and odour and the solubility was determined by dissolving the biopolymer in various solvents. Color changing point was determined using melting point apparatus using glass capillary. The fine powder form of isolated biopolymers was taken in a glass capillary and then sealed one side of glass capillary by flame. The sealed glass capillary was placed inside the melting point apparatus. A thermometer was placed in the melting point apparatus and point at which isolated biopolymer changed its colour and state was reported and record.

Various chemical tests were performed like Molisch's Test, Fehling's Test, Biuret Test, Ninhydrin Test and Iodine test (Kokate 2014, Gupta 2009).

A. Molisch's Test: The isolated biopolymeric solution was mixed with the molisch's reagent which is an alcoholic solution of alpha naphthol, and concentrated sulphuric acid; then red to purple colour at the junction of two layers was observed.
B. Fehling's Test: Firstly Fehling's solution was prepared by mixing 3.46 g copper sulphate and dissolved in distilled water and volume was made up to 50 ml (solution A). 17.3 g of potassium sodium tartarate and 5 g of sodium hydroxide were dissolved in purified water and volume was made upto 50 ml (solution B). Then both the solution were mixed in equal proportion and mixed with isolated biopolymer and appearance of brick red precipitate was observed.
C. Biuret Test: Biopolymeric solution was mixed with 10% NaOH solution, mixture was warmed and few drops of aqueous copper II sulphate were added. Reddish violet color was observed.
D. Ninhydrin Test: Biopolymeric solution was treated with 0.1% ninhydrin solution in n-butanol, boiled and cooled, bluish color was observed.
E. Starch Test: Biopolymeric solution was mixed with few drops of iodine solution, deep blue to violet color was observed.

Ex-vivo mucoadhesion studies of isolated biopolymer

The mucoadhesion property of isolated biopolymer was determined by shear stress method (Figure 2). In this method biopolymeric solution was prepared using water as a solvent in various concentrations from 1 - 5% w/v and prepared solution was subjected for measuring in vitro bond breaking strength at various contact intervals (5, 10, 15, 20, 25, and 30 minutes). And same method was adopted for the standard polymers like sodium CMC and HPMC (Varshney et al., 2018).

The mucoretaintability of the isolated biopolymer was performed using MS mucoretaintability method by Capra aegagrus labium as mucosal substrate using thin film of polymer with phosphate buffer pH 6.5 as shown in figure 3. The dislodgement time of the biopolymer film from mucosa; surface was reported at specific time interval and this results was compared with the standard film of HPMC and sodium CMC polymer (Ojha et al., 2014).

Spectral Analysis of Isolated Biopolymer

The isolated biopolymer was subjected to various spectral analysis such as IR spectroscopy, 1H NMR spectroscopy, Differential scanning calorimetry, Mass spectroscopy, SEM imaging and Elemental analysis. The results were analyzed for interpretations (Chatwal et al., 2014, Silverstein et al., 2014)

Acute Toxicity Study

As per OECD guidelines, the single dose acute toxicity study of the isolated biopolymer was performed on rats (Wistar rats, either sex, 200-250 g) for two weeks. The acute toxicity study protocol was approved by the Institutional Animal Ethical Committee (Registration Number 1156/AC/07/CPCSEA). The biopolymeric solution was prepared according to 5 g/kg body weight. The prepared biopolymeric solution was given orally and rats were observed for two weeks regarding any change in body weight or physical behavior. Rats were also observed for any changes like itching, lacrimation, swelling, inflammation, redness or drowsiness due to biomaterials (Madhav et al., 2017, CDER 1996).

Results and Discussion

Physicochemical characterization of isolated biopolymer

The biopolymer of Lageneria siceraria was of whitish green color with color changing point 190^{0}C. The isolated biopolymer was soluble in water and insoluble in methanol and acetone. As well as 1% solution of isolated biopolymer showed viscosity of 1.0 cps, and pH found to be 7.4. The surface tension of isolated biopolymer was found to be 68.12dyne/cm.

The biopolymer showed positive reaction with Molisch's and Fehling's reagent so it was observed that isolated biopolymer was carbohydrate in nature, The positive test was shown by biopolymer for Biuret and Ninhydrin reagent indicating the protein content in biopolymer. The positive result with iodine test showed presence of starch.

Ex-vivo mucoadhesion studies of isolated biopolymer

Shear stress method

The mucoadhesive property of the isolated biopolymer was determined by shear stress method and the result revealed the bond strength of 3% w/v with HPMC and sodium CMC, while it showed the bond strength of 5% w/v with *Lagenaria siceraria* biopolymer. It was observed that increase in the contact time as 5, 10, 15, 20, 25, 30 minutes showed a slight improvement in the bioadhesive bond strength between polymer solution and glass substrate. So mucoadhesivity of biopolymers increased with time as shown in Table I and Figure 2.

Table I: Determination of mucoadhesivity by shear stress

Solvent/Biopolymer/ Synthetic polymer (wt in gm)	Contact time (minutes)					
	5 min	10 min	15 min	20 min	25 min	30 min
Water	3gm	7gm	12gm	13gm	15gm	17gm
Biopolymer 1%	144gm	151gm	160gm	173gm	182gm	200gm
Biopolymer 2%	156gm	160gm	172gm	190gm	210gm	218gm
Biopolymer 3%	185gm	195gm	201gm	211gm	218gm	239gm
Biopolymer 4%	196gm	199gm	209gm	223gm	228gm	246gm
Biopolymer 5%	201gm	208gm	218gm	230gm	239gm	250gm
HPMC 3%	201gm	211gm	220gm	232gm	238gm	252gm
Sodium CMC 3%	198gm	214gm	226gm	231gm	242gm	250gm

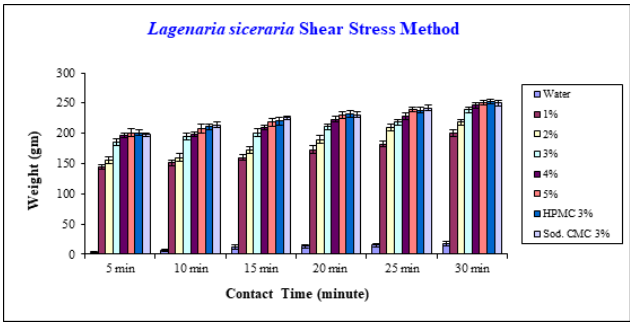


Figure 2: Determination of mucoadhesive property by shear stress method

Mucoretaintability method

The *Lageneria siceraria* biopolymer showed dislodgement time 194 ± 1.00 minutes from labial mucosal substrate. This was more than HPMC (190 ± 1.00 minutes) and Sodium CMC (165 ± 2.00 minutes). Thus it reveals that *Lagenaria siceraria* biopolymer has better mucoretentive property than HPMC and Sodium CMC as shown in Figure 3.

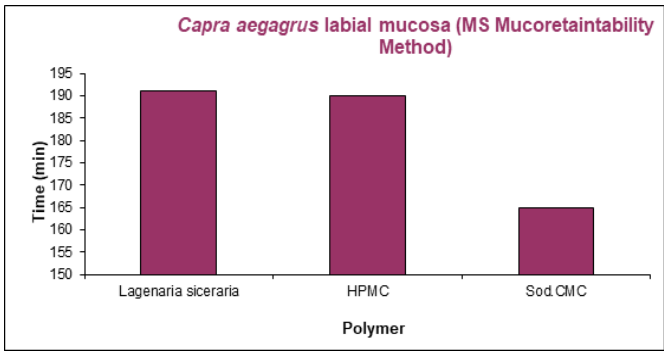


Figure 3: Determination of dislodgement time by MS mucoretaintability method

Spectral Analysis of Isolated Biopolymer

The IR spectrum of *Lagenaria siceraria* biopolymer showed peaks at 3410 cm^{-1} (OH stretching), 2928 cm^{-1} (CH stretching alkane), 1627 cm^{-1} (C=O stretching of carboxylic acid), 1423 cm^{-1} (CH bending alkane), 1242 cm^{-1}; 1060 cm^{-1} (C-N stretching aliphatic amine) and 619 cm^{-1} (CH bending aromatic ring) as shown in Figure 4. The 1H NMR spectrum of *Lagenaria siceraria* biopolymer showed chemical shift values at δ 1.22 ppm (-CH saturated proton), δ 2.1 ppm (-C≡CH, acetylenic proton), δ 3.2-3.7 ppm (-CH3OR, ether proton) and δ 4-5.1 ppm (R-OH, hydroxyl proton) as shown in Figure 5. The mass spectroscopy revealed the parent peak of *Lagenaria siceraria* biopolymer at m/z value 205.1 as shown in Figure 6.

The DSC thermogram of *Lagenaria siceraria* showed primary glass transition temperature at 128.99^{0}C and secondary glass transition temperature at 153.17^{0}C. Primary peak height was observed at 1.0015 mW and peak area was found to be 86.885 mJ. The value of delta H for primary peak was 8.6885 J/g. Secondary peak height was observed at 1.9630 mW peak area was 61.878 mJ. The value of delta H for secondary peak was 6.1878 J/g as shown in Figure 7.

Figure 4: IR spectrum of *Lagenaria siceraria* biopolymer

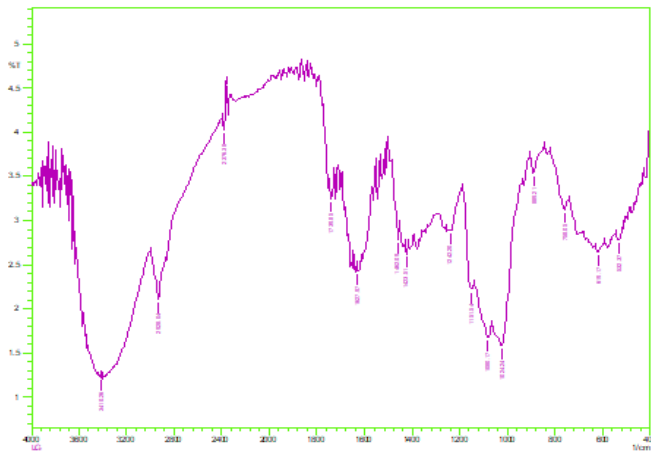


Figure 5: ^{1}H NMR spectrum of *Lagenaria siceraria* biopolymer

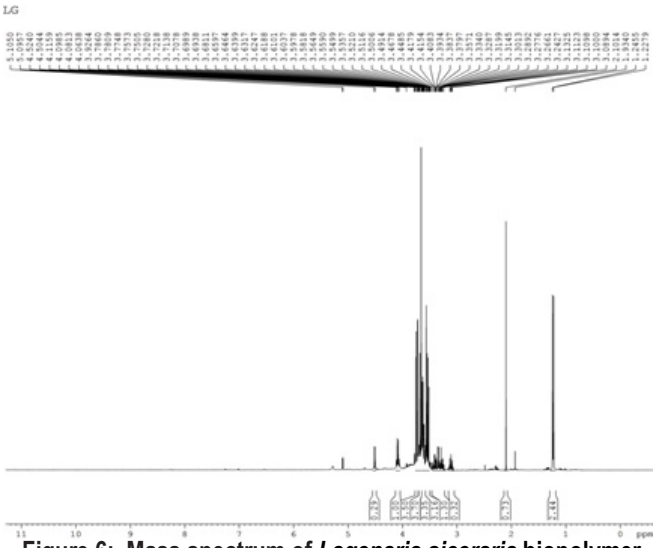
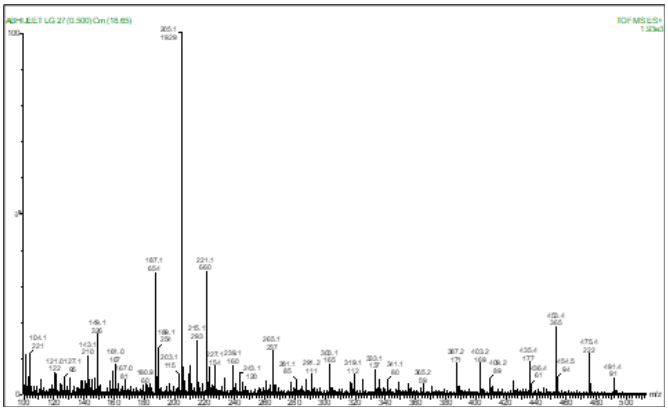


Figure 6: Mass spectrum of *Lagenaria siceraria* biopolymer



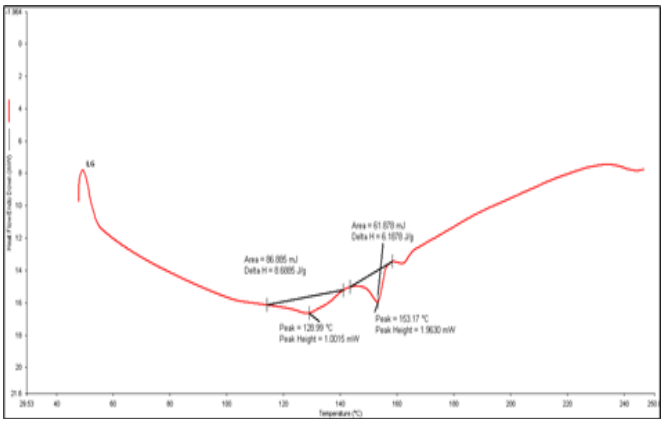


Figure 7: DSC thermogram of *Lagenaria siceraria* biopolymer

SEM image of biopolymer revealed smooth and irregular topography as shown in Figure 8. The particles were irregular in shape with larger surface area and confirmed that the isolated biopolymer was amorphous in nature. The elemental analysis showed that the biopolymer had carbon and hydrogen as the major elements. The isolated biopolymer was devoid of metal contamination like arsenic, lead, mercury etc. confirmed by SEM as shown in Figure 9.

Acute toxicity studies

The experimental animal's studies did not signify any change in rat's body weight, skin reaction, corneal reflex, respiratory rate, autonomic symptoms, salivation, diarrhea, lethargic conditions, sleeping

conditions, and behavioral patterns and there was no symptom of convulsion. So it was inferred that the isolated biopolymer were devoid of toxicity due to their edibility, biocompatibility and biodegradability properties.

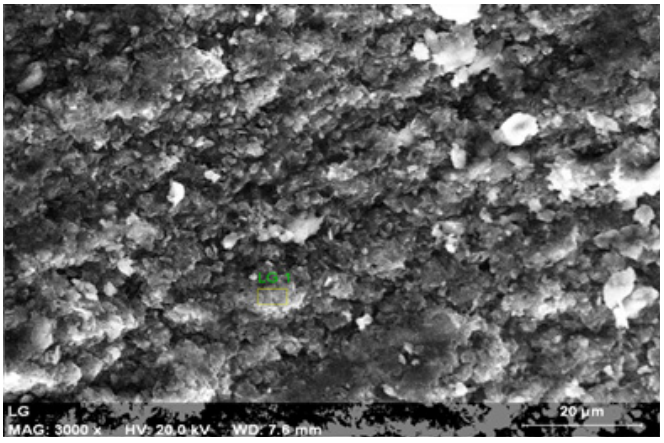
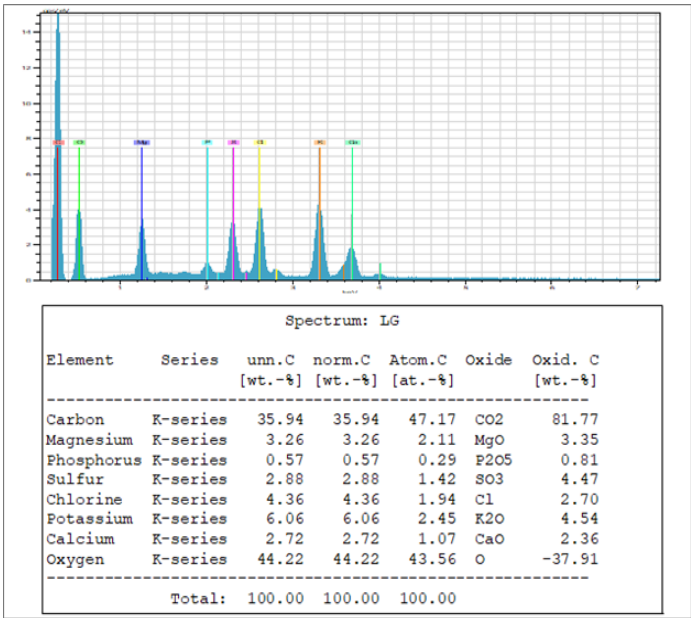


Figure 8: SEM image of *Lagenaria siceraria* biopolymer



Spectrum: LG

Element	Series	unn.C [wt.-%]	norm.C [wt.-%]	Atom.C [at.-%]	Oxide	Oxid. C [wt.-%]
Carbon	K-series	35.94	35.94	47.17	CO2	81.77
Magnesium	K-series	3.26	3.26	2.11	MgO	3.35
Phosphorus	K-series	0.57	0.57	0.29	P2O5	0.81
Sulfur	K-series	2.88	2.88	1.42	SO3	4.47
Chlorine	K-series	4.36	4.36	1.94	Cl	2.70
Potassium	K-series	6.06	6.06	2.45	K2O	4.54
Calcium	K-series	2.72	2.72	1.07	CaO	2.36
Oxygen	K-series	44.22	44.22	43.56	O	-37.91
	Total:	100.00	100.00	100.00		

Figure 9: Elemental analysis of *Lagenaria siceraria* biopolymer

Conclusion

In the particular research work, it was concluded that the isolated biopolymer of *Lageneria siceraria* possesses good mucoadhesivity, mucoretaintability and mucoretention properties which increased with the increase in time. The acute toxicity studies performed for the biopolymer did not show any significant changes in rat such as body weight, corneal reflux, salivation, diarrhea etc. and thus reveal that isolated biopolymer does not contain any toxicity. Being edible in nature, biocompatible and nontoxic, the biopolymer of *Lageneria siceraria* can be used for preparing mucoadhesive films and also provides a targeted effect by drugs which get incorporated in the film. This approach can be a novel scope as a substitute for oral route or other conventional route of drug delivery and can act as a benchmark in the field of mucoadhesive dug delivery in near future.

Acknowledgement

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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