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Extraction, Isolation and Physico-Chemical Charaterization of Fenugreek Seed Mucilage

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ABSTRACT

The purpose of this study is to prepare and assess a novel, affordable, and potent natural mucoadhesive agent that may be employed as a successful substitute for conventional mucoadhesive agents. The study's methodology comprised extracting the mucoadhesive agent from Trigonella foenumgraecum (fenugreek) seeds, assessing the mucilage's solubility, testing chemical properties such as presence of saponins and other bioactive constituents of fenugreek include mucilage, volatile oils, and alkaloids as well as physical properties like pH, swelling index, loss on drying, micromeritics property and FT-IR. The study found that the presence of mucilage, alkaloids, proteins and carbohydrate. pH of the polysaccharide was found to be 6.3+0.3,Swelling index was 9+0.2 ,loss on drying was contains 7.54 of moisture content. The Bulk density and the Tapped density were found to be 0.42+0.5gm/cm3 and 0.53+0.2gm/cm3 respectively .Carr's index (%) and Hausner's ratio of given polysaccharide was found to be 20.75 and 1.26, whereas the Angle of repose was 31.79. Polysaccharide shows the maximum solubility in Distilled water and Buffer of pH 6.8 which indicates the polysaccharide was soluble in inorganic solvent whereas in case of organic solvents i.e. Xylene and Petroleum ether it was insoluble. Interpretation of FTIR spectrum of Trigonellafoenum-graecum polysaccharide was:--OH stretch (3656.78), -C-H (3150.50),-C=C (2313.46), -C-H bend (1636.49), -C-H rock (1617.20), -CH bend out of plane (1029.45). FT-IR data is in accordance to that the essential group of polysaccharide was present.

Keywords: *Fenugreek, mucilage, extraction, pH, FT-IR, mucoadhesive agent.*

INTRODUCTION

Trigonella foenum-graecum Linne, generally comprehend as Fenugreek, belongs to the family Fabaceae (Leguminosae) shown in Fig.1, is an herb with medicinal and nutraceutical values which has been used from ancient times in the Indian medicine system. On the behalf of literature reviews, the presence of various bioactive compounds like alkaloids, flavonoids, saponins, fibers, fatty acids, etc. contributes to the therapeutic prospect of the herb (Basch E et al, 2003). Fenugreek has therapeutic capability, anti-biotic, anti-oxidant, anti-carcinogen properties and regulates hyperglycemia in diabetic cases (Ruwali P et al ,2022). Fenugreek seed mucilage are mucoadhesive polysaccharides and having an adhesive property. "Adhesion can be defined as sticking of drug to the membrane by utilizing the sticking property of the water soluble polysaccharides. Adhesion of drug delivery device to the mucosal membrane such as buccal, optical, rectal, nasal etc can be termed as bioadhesion" (Shadab Md. et al ,2012). They give a fairly short term adhesion between the drug delivery system and the epithelial cell surface. Use of this kind of system is to give a drug protection from enzymatic degradation and helps to accelerate the contact duration with the mucosa (N.A. et al, 1985).

Fenugreek seeds are high source of polysaccharide (galactomannan), saponins (such as diosgenin, yamogenin, gitogenin, tigogenin, and neotigogens) and other bioactive constituents including mucilage, volatile oils, alkaloids (such as choline and trigonelline). (Wani S.A. et al,2016; Ahmad A et al 2016)

Natural polymers are biodegradable, biocompatible, and also bio adhesive in nature e.g. starch. Due to its high degree of

swelling property with aqueous medium, biodegradable polymers prolongs the residence time when contact with mucous membrane, results gel formation. The rate and extent of drug release is controlled by concentration of polymer and the release pattern in a sustained manner. Synthetic polymers are extensively used as bulking agent, fillers, embolic particles, drug delivery vehicles etc. and proved to be safe and biocompatible but the main drawback is tend to resettle down from injection site and lead to implicit threat, embolism and further organ damage. (Verma N.V. et al, 2015).

Fig.1: Fenugreek seeds

Synonyms:-

Language	Names
French	FenugrecSénegré, Trigonell
German	Bockshornklee, GriechischesHeu
German- Italian	Fieno Greco
Spanish	Alholva, Fenogreco
Indian	Mayti, Methe, Methi
Indian-Tamil	Venthium
Malay	Alba
Sinhalese	Uluhaal

Table 1: Various names of Fenugreek (Indian herbal pharmacopoeia 1999, Mullaicharam A.R *et al* 2013)

2. MATRIAL AND METHODS

The plant materials of *Trigonella foenum-graecum* were collected from local market in Meerut (India). The seeds were identified and authenticated by Department of Horticulture and Food Processing, Meerut, U.P. Castor oil, epichlorohydrin, Isopropyl alcohol and acetone were purchased from the CDH, (New Delhi, India). All other reagents used were of analytical grade.

2.1 Extraction and Isolation of polysaccharide

Method used for the extraction of *Trigonellafoenum-graecum* polysaccharide(Suruse PB *et al*, 2013, Verma S. *et al*, 2014)

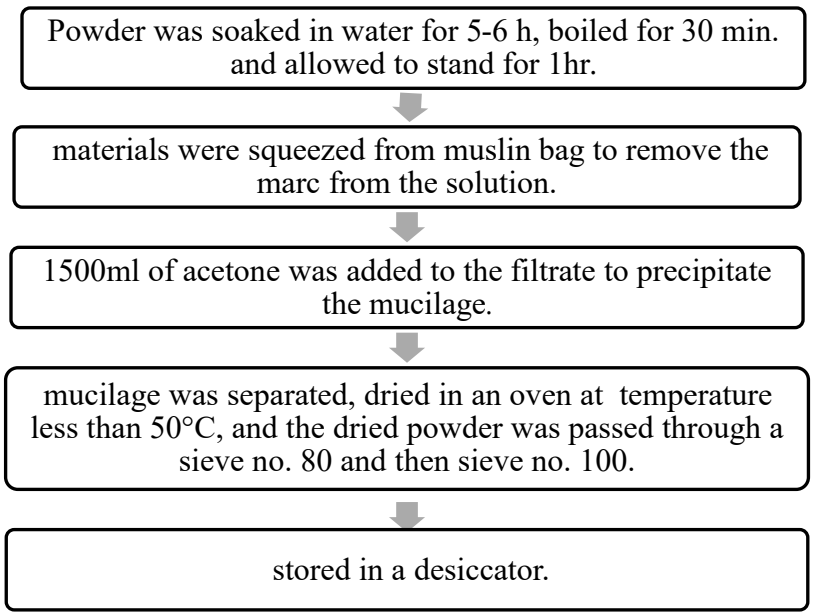

Fig.2- Extraction of *Trigonellafoenum-graecum* polysaccharide

2.2 Characterization of polysaccharide

2.2.1 **Preliminary Chemical Test for Characterization of *Trigonellafoenum-graecum* polysaccharide**

The isolated polysaccharide was evaluated for their chemical and physical characteristics such as its identification and purity test, organoleptic evaluation, solubility, pH, swelling index, loss on drying, micromeritics property etc.(Tiwari P. *et al*,2011; Kokate C.K.,2011, Khandelwal K.R., 2013)

2.2.1.1 **Detection of mucilage**: Powdered drug show used for the identification of mucilage
(Jani G.K. *et al*, 2009)

- **Ruthenium red**: Powdered drug placed on watch glass and few drops of ruthenium red was added then it shows red colour.
- **Polysaccharide Test**: Powdered drug swells in water or aqueous KOH.

2.2.1.2 **Detection of carbohydrates**: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

- **Molisch's Test (General test)**: Filtrates were treated with two drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.
- **Fehling's Test**: Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.
- **Benedict's test**: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

2.2.1.3 **Detection of alkaloids**: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

- **Dragendroff's Test**: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.
- **Hager's Test**: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.
- **Wagner's Test**: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

2.2.1.4 **Detection of glycosides**: Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

- **Keller-Killiani test**: To 2ml extract, add 1ml pyridine and 1ml sodium nitroprusside .Pink to red colour appears.
- **Baljet's test**: A thick section shows yellow to orange colour with sodium picrate.
- **Legal's Test**: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.
- **Borntrager's Test**: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 min. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and

treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

2.2.1.5 **Detection of saponins**:

- **Froth Test**: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicates the presence of saponins.

2.2.1.6 **Detection of Proteins**:

- **Biuret test (general test):** Take 5mg of extracted powder than add 3ml of 4% NaOH solution and few drops of 1% CuSO4 solution. Violet or pink colour appears.
- **Warming test**: Take 5mg of powder and mix with 10ml of distilled water and apply heat, then it forms the coagulated protein which shows the presence of protein.

2.2.1.7 **Detection of tannins (phenolic compounds):**

- **Ferric Chloride Test**: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

2.2.1.8 **Detection of amino acids**:

- **Ninhydrin Test**: To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

2.2.1.9 **Detection of starch:**

- **Iodine test**: Mix 3ml test solution and few drops of dilute iodine solution. Blue colour appears; it disappears on boiling and reappears on cooling.

2.2.2 **Preliminary Physical Test for Characterization of *Trigonellafoenum-graecum* Polysaccharide** (British Pharmacopoeia 1988, British Pharmacopoeia 2000)

2.2.2.1 **Organoleptic Evaluation**

Isolated polysaccharide of *Trigonellafoenum-graecum*was evaluated for organoleptic properties such as colour, odour, taste, shape and texture.

2.2.2.2 **pH of Polysaccharide solution**

To determine the pH of the polysaccharide, 1% w/v solution was prepared in distilled water. The pH of the solution was measured during pH meter (pH tutor, Eutech).

2.2.2.3 **Swelling Index of polysaccharide**

Swelling index is defined as the volume in millilitres occupied by 1g of a crude drug, including any adhering mucilage, after it has swollen in an aqueous liquid for 4hrs.

Accurately weighed quantity of finely powdered polysaccharide (1g) was transferred in a 25ml glass-stopper measuring cylinder. Water was added in a measuring cylinder, shaken for every 10min. for 1 hrs .and the solution was then allowed to stand for 3 hrs. at room temperature. The volume in ml occupied by powder, including any sticky mucilaginous portion was measured. The same procedure was repeated thrice and the average value was calculated. Swelling index was calculated from the **Equation 1**(Patil S.B.,2010).

$$\textbf{Swelling index}(\%) = \frac{\textbf{Final volume} - \textbf{Initial volume}}{\textbf{Initial volume}} \times \mathbf{100} \qquad (\mathbf{1})$$

2.2.2.4 **Loss on Drying**

The test was carried out according to the procedure (Indian Pharmacopoeia 1996). One gram of polysaccharide powder was weighed accurately in a tarred glass stopper bottle and dried in a hot air oven at 105°C. The weight of polysaccharide powder was measured at regular intervals of 1hrs, until a constant weighed was obtained. The percentage of weighed loss by the powder was calculated through **Equation 2:**

$$\textbf{Loss on drying}(\%) = \frac{\textbf{Final volume} - \textbf{Initial volume}}{\textbf{Initial volume}} \times \mathbf{100} \qquad (\mathbf{2})$$

2.2.2.5 **Micromeritics properties**

- **Bulk density**

Bulk density of a powder is the ratio of mass of untapped powder sample and its volume including the contribution of the inter-particulate void volume. The bulk density of powder depends mainly on the particle size, particle shape and trend of particles to adhere to one another.

Bulk density of *Trigonellafoenum-graecum* polysaccharide powder was determined by measuring the volume of a known weight of powder sample that may have been passed through a sieve no.25 into a graduated cylinder. Mathematically, bulk density can be represented by **Equation 3:**

$$\textbf{Bulk density}(\rho) = \frac{\textbf{Weight of powder}(W)}{\textbf{Bulk volume}\ (Vb)} \qquad (3)$$

- **Tapped density**

Tapped density is determined by mechanically tapping a graduated measuring cylinder containing a powder sample. Tapped density of *Trigonellafoenum-graecum* polysaccharide powder was determined by placing a graduated cylinder into a Mechanical tapper apparatus. After observing the volume, the cylinder was mechanically tapped for 100 times or until the powder achieves a constant volume. Mathematically, tapped density can be represented by **Equation4:**

$$\textbf{Tapped density}(\rho t) = \frac{\textbf{Weight of powder}(W)}{\textbf{Bulk volume}(V)} \times 100 \qquad (4)$$

2.2.2.6 **Powder flow properties**

- **Angle of Repose**

The frictional forces in a loose powder can be measured by the angle of repose (ɵ). It is defined as the maximum angle possible between the surface of a pile of powder and the horizontal plane.

Angle of repose was determined by using funnel method (by keeping a funnel vertically in a stand at a specified height above a graph paper placed on a horizontal surface). Approximately, 2 g of polysaccharide powder was transferred into the funnel keeping the orifice of the funnel blocked by the thumb. Then the orifice of the funnel was opened to release the powder on the paper to form a smooth conical heap. The radius of the heap (r) and the height of the heap (h) were measured. Angle of repose can be represented by **Equation 5:**

$$\textbf{Angle of repose}(\theta) = \tan^{-1}\frac{\textbf{Height}\ (h)}{\textbf{Radius}(r)} \qquad (5)$$

Table 2: Values of Angle of repose

Flow character	Angle of repose (ɵ)
Good	31-35
Fair	36-40
Passable	41-45
Poor	46-55
Very poor	56-65
Very very poor	More than 66

- **Carr's index**

Compressibility index is used as an important parameter to determine the flow behavior of the powder. It is indirectly related to the relative flow property rate, cohesiveness and particle size. Carr's index can be represented by **Equation 6** and then flow behavior was determined from the **Table 3:**

$$\textbf{Carr's index}(\%) = \frac{\textbf{Final volume} - \textbf{Bulk density}}{\textbf{Tapped density}} \times 100 \qquad (6)$$

- **Hausner's ratio**

Hausner's ratio is used to predict the flow ability of the powder. This method is similar to compressibility index. Hausner's ratio can be represented by **Equation7** and then flow behaviour was determined from the **Table 3:**

$$\textbf{Hausner's ratio} = \frac{\textbf{Tapped density}}{\textbf{Bulk density}} \qquad (7)$$

Flow character	Carr's index	Hausner's ratio
Excellent	≤10	1.0-1.11
Good	11-15	1.12-1.18
Fair	16-20	1.19-1.25
Passable	21-25	1.26-1.34
Poor	26-31	1.35-1.45
Very poor	32-37	1.45-1.59
Very very poor	>38	>1.60

Table 3: Values of compressibility index and Hausner's ratio (Kumar R,2009)

2.2.2.7 **Solubility behaviour**
The solubility of polysaccharide is in different solvent was observed visually according to (Indian Pharmacopoeia,1996). In this solubility is dissolved in respective solvents (1ml each) and solubility (mg/ml) was determined. Which shows the drug is soluble in distilled water, phosphate buffer pH 6.8, ethanol and insoluble in acetone, chloroform and ether etc.

2.2.2.8 **Infrared spectra of isolated mucilage**
100mg of the powdered pectin was mixed with KBr (400mg) and was compressed in a hydraulic press to form a pellet at 15tons pressure. The pellet was scanned from 4000 to 400cm-1 in Shimadzu FT-IR.

3. RESLUT

3.1 Preliminary chemical test for characterization of Trigonellafoenum-graecumpolysaccharide

S.NO	CHEMICAL TEST	COLOUR	INFERENCE
1	**Test for Mucilage**		
	Ruthenium red	Pink	Present
	Polysaccharide test	Gelatinous mass	Present
2	**Carbohydrate test**		
	Molisch's test	Reddish violet ring	Present
	Fehling's test	Brick red	Present
	Benedict's test	Green colour	Present
3	**Alkaloid test**		
	Dragendroff's test	Orange brown	Present
	Hager's test	Orange yellow precipitate	Present
	Wagner's test	Orange yellow precipitate	Present
4	**Test for Glycosides**		
	Borntrager's test	Reddish pink	Absent
	Killer-killiani test	Reddish brown	Absent
	Baljet test	Orange	Absent
	Legal test	Orange brown	Absent

5	**Test for Saponins**		
	Froth formation test	Froth formed	Present
6	**Test for Protein**		
	Biuret test	Violet or pink colour	Present
	Warming test	Protein coagulated	Present
7	**Test for Tannins(phenolic compounds)**		
	FeCl3 test	Formation of green and blue colour	Absent
8	**Test for Amino acids**		
	Ninhydrin test	Purple colour	Present
9	**Test for Starch**		
	N/50 Iodine solution	Blue colour	Absent

Table 4: Preliminary chemical test for characterization of *Trigonellafoenum-graecum* polysaccharide

From the above study it was concluded that the extracted polysaccharide of *Trigonellafoenum-graecum* have shown the presence of mucilage, alkaloids, proteins and carbohydrate.

3.2 Preliminary physical test for characterization of Trigonellafoenum-graecum polysaccharide

3.2.1 **Organoleptic Evaluation**

Test	**Observation**
Colour	Light brown
Odour	Characteristic
Taste	Mucilaginous
Shape	Irregular
Texture	Rough

Table 5: Organoleptic evaluation of *Trigonellafoenum-graecum*

Odour of isolated polysaccharide was found to be characteristic which indicating that they have no taste and odour. Rough texture shows the mucoadhesive nature of the polysaccharide.

3.2.2 **pH of Polysaccharide Solution**
The pH of the polysaccharide was found to be 6.3$\pm$0.3.This result show the extracted polysaccharide was non-irritant during application.

3.2.3 **Swelling index**
Swelling index of the polysaccharide was found to be 9$\pm$0.2, which indicating that polysaccharide have good water holding capacity.

3.2.4 **Loss on drying**
Loss on drying was done to identify the amount of water content or moisture which was entrapped within the particles, the given polysaccharide was contains 7.54 of moisture content.
3.2.5 **Micromeritics properties**
Bulk density and Tapped density of *Trigonellafoenum-graecum* polysaccharide were found to be-

Value expressed as Mean+ SD, n=3

Properties	Values
Bulk density(gm/cm3)	0.42±0.5
Tapped density(gm/cm3)	0.53±0.2

Table 6: Bulk density and tapped density

The Bulk density and the Tapped density were found to be 0.42±0.5gm/cm3 and 0.53±0.2gm/cm3 respectively. Micromeritics properties of powdered mucilage depend mainly on the particle size, particle shape and trend of particles to adhere to one another.

3.2.6 **Powder flow property**

Carr's index(%), Hausner's ratio and Angle of repose of *Trigonellafoenum-graecum* polysaccharide were found to be –

Properties	Flow characters
Carr'sindex(%)	20.75(passable)
Hausner's ratio	1.26(passable)
Angle of repose(ɵ)	31.79(good response)

Table 7: Carr's index, Hausner's ratio and Angle of repose

Powder flow properties are directly affect the particle size and surface area of a polysaccharide, which means they influence the release of drug from a dosage form. The Carr's index (%) and Hausner's ratio of given polysaccharide was found to be 20.75 and 1.26, which means the dry powder of polysaccharide are passable. Angle of repose was 31.79 that indicating the good response.

3.2.7 **Solubility behaviour**

Solubility of *Trigonellafoenum-graecum* polysaccharide in different solvents were found to be-

S.no	Solvents	Solubility
1	**Distilled water**	Viscous solution
2	**Xylene**	Insoluble
3	**Buffer pH6.8**	Soluble
4	**Petroleum ether**	Insoluble

Table 8: Solubility behaviour of *Trigonellafoenum-graecum* polysaccharide

Polysaccharide shows the maximum solubility in Distilled water and Buffer of pH 6.8 which indicates the polysaccharide was soluble in inorganic solvent whereas in case of organic solvents i.e. Xylene and Petroleum ether it was insoluble.

3.2.8 **Infrared spectra of isolated mucilage**

FT-IR spectrum of isolated mucilage showed all the peaks corresponding to the functional group present in the structure of *Trigonellafoenum-graecum.* Interpretation of FTIR spectrum of *Trigonellafoenum-graecum* polysaccharide was:--OH stretch (3656.78), -C-H (3150.50),-C=C (2313.46), -C-H bend (1636.49), -C-H rock (1617.20), -CH bend out of plane (1029.45). FT-IR data is in accordance to that the essential group of polysaccharide was present.

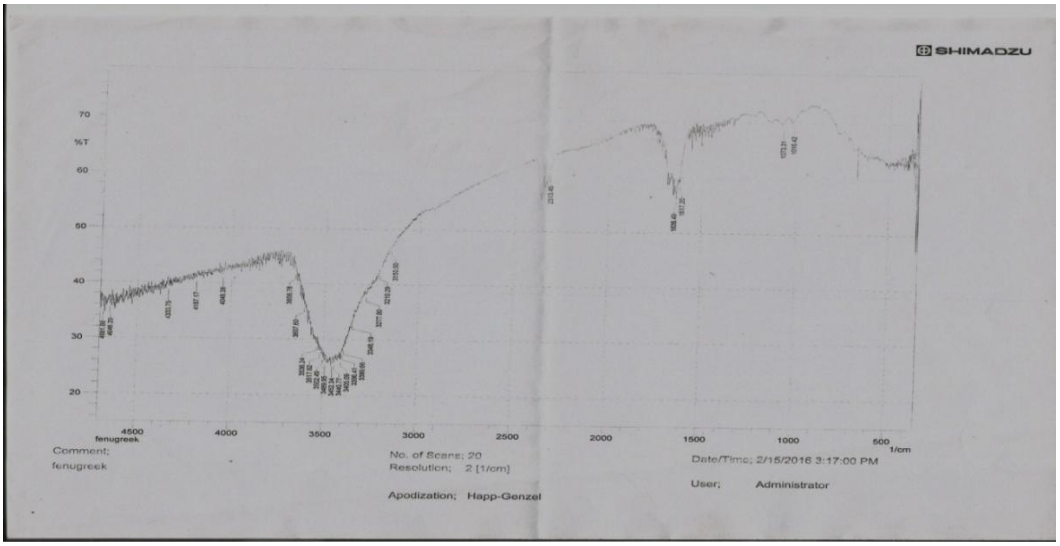

Fig.2- FT-IR of *Trigonellafoenum-graecum* polysaccharide

4. CONCLUSION

From the above study it seen that the extracted mucilagenous substance of Trigonellafoenumgraecum is edible, has the potential as a mucoadhesive agent even at lower concentration. They are biodegradable, biocompatible, and also bio adhesive in nature. Easy to prepare a formulation with low toxicity and high viscosity. The polysaccharide derived from *Trigonellafoenum-graecum L*. can be used as a platform for drug delivery in a target site. The time of drug release could be controlled by varying concentration of polysaccharide, as well as stirring rate. By selecting and evaluating the process parameters it might be possible to prepare microspheres and nanoparticles with desired properties; such as uniform particle size, high entrapment efficiency and improved surface properties. Further the drug release and other properties can also be modified either by chemical modification of polysaccharide or using the combination of some other suitable polysaccharide.

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6. DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no competing financial interests.

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