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Characterization of selected fruit peel as biofertilizer.

Caracterización de la cáscara de frutos seleccionados como biofertilizante

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ABSTRACT

The primary goal of this study is popularization of probiotic farming in India. A cheap source of Lactic Acid Bacteria produced by fruit peels like pomegranate peel, sweet lime peel and orange peel powder, which are then used as biofertilizer in agriculture. Various characteristics features like observed the growth of lactic acid bacteria by spread plate techniques for 48 hours at 37°C, FTIR, instrumental color analysis and antioxidant DPPH assay of selected fruit peels were analysed and compared. The constant and stable growth of lactic acid bacteria is observed by pomegranate peel powder while compared to sweet lime and orange peel powder. FTIR confirms the presence of functional groups like water, alcohol, carboxylic acid, alkane, amines, aldehyde, anhydride, ester, ketone, amide, NO_2 , fluorine and chlorine in pomegrante peel, orange peel and sweet lime peel powder. The colour analysis L^* a^* b^* shows that highest lightness and redness observed in pomegranate peel and highest range of yellowish color was noticed in orange peel powder. The comparison of fruit peel powder with standard ascorbic acid inferred that pomegranate peel powder extract shows more effective in lowest concentration and orange and sweet lime peel powder extract shows that increase in inhibition % as increase in concentration. The present study concluded that fruit peels with highest content of antioxidant and best source of probiotics can be used as biofertilizer to increase the quality and quantity of the food crops in agricultural field through probiotic farming practices.

Key Words: Probiotic farming, Lactic acid bacteria, Pomegranate peel powder, Sweet lime peel powder, Orange peel powder and Biofertilizer

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RESUMEN

El objetivo principal de este estudio es la popularización del cultivo de probióticos en la India. Una fuente barata de bacterias de ácido láctico producidas por cáscaras de frutas como la cáscara de granada, cáscara de lima dulce y polvo de cáscara de naranja, que luego se utilizan como biofertilizante en la agricultura. Se analizaron y compararon varias características, como el crecimiento observado de bacterias del ácido láctico mediante técnicas de placa extendida durante 48 horas a 37 ° C, FTIR, análisis de color instrumental y ensayo antioxidante DPPH de cáscaras de frutas seleccionadas. El crecimiento constante y estable de las bacterias del ácido láctico se observa en el polvo de cáscara de granada en comparación con el polvo de cáscara de lima dulce y naranja. FTIR confirma la presencia de grupos funcionales como agua, alcohol, ácido carboxílico, alcano, aminas, aldehído, anhídrido, éster, cetona, amida, NO2, flúor y cloro en polvo de cáscara de granada, cáscara de naranja y cáscara de lima dulce. El análisis de color L* a* b* muestra que la luminosidad y el enrojecimiento más altos observados en la cáscara de granada y el rango más alto de color amarillento se observaron en el polvo de cáscara de naranja. La comparación del polvo de cáscara de fruta con el ácido ascórbico estándar infirió que el extracto de polvo de cáscara de granada se muestra más efectivo en la concentración más baja y el extracto de polvo de cáscara de naranja y lima dulce muestra un aumento en el % de inhibición a medida que aumenta la concentración. El presente estudio concluyó que las cáscaras de frutas con el mayor contenido de antioxidantes y la mejor fuente de probióticos se pueden utilizar como biofertilizantes para aumentar la calidad y cantidad de los cultivos alimentarios en el campo agrícola a través de prácticas de cultivo de probióticos.

Palabras clave: cultivo de probióticos, bacterias del ácido láctico, polvo de cáscara de granada, polvo de cáscara de lima dulce, polvo de cáscara de naranja y biofertilizante.

INTRODUCTION

Probiotic farming, also known as biointensive agriculture, integrates different organic farming methods with the goal of improving soil health. Almost every farmer can use the probiotic farming method to supply the nutrients their crops need to thrive. PGPMs are frequently used as crop additives and bio-fertilizers, promoting sustainable agriculture. Whenplants are healthy, they grow and yield is better. (Pini *et al*., 2021).

India is the world's biggest fruit producer. Fruit peels, which constitute a solid waste after consumption, are a burden to the environment. The Fruit peels are separated and disposed into municipal landfills after the edible portion has been consumed. This results in significant pollution and garbage disposal as solid-waste management (Sachin *et al*., 2017). Fruit peel waste have numerous nutritional and functional properties. Utilizing this fruit peel waste as biofertilizer increase the quality and quantity of the crop production and also raise the income of the farmers too. Peels from three varieties namely Pomegranate (PP), Orange (OP) and Sweet Lime (SLP). Nutrients such as dietary fibre, vitamins, minerals phytochemicals, antioxidants and culinary elements including pectin,

natural hues, antifungal agents, and antibacterial compounds, among other things, are abundant in fruit waste.

Like all other living things, plants need food to grow and develop. Plant development is influenced by disease control, and pest control. Domestic waste comes in two different varieties: dry waste and wet waste. Domestic waste contains some organic materials that can be used to create agricultural fertilizer. Each plant has a unique range of nutritional requirements. Plants begin to develop nutrient shortage symptoms when they go below this required level. Soil pH has an indirect impact on nutrient availability and toxicity. For most plants, soil pH levels from 6.0 to 7.5 are appropriate as nutrients (Khairnar and Nair., 2019).

A total of 16 essential nutrients are needed by the plant. From the atmosphere, soil water, and hydrogen and oxygen are supplied to the plant. The remaining elements that must be present are chlorine, nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, iron, zinc, manganese, and copper. To keep the soil's nutrient content constant plants are fed soil minerals, organic matter, or fertiliser. One of the most important sources of nourishment in the soil is fertiliser. Because of the high source of nourishment, it also promotes soil immunity. (Khairnar and Nair., 2019).

Since they do not contain any hazardous materials and improve the quality of soil, biofertilizer and biological waste are utilized to replace the use of chemical fertilizers. Utilizing organic materials like biofertilizers when cultivating crops will help to protect the soil's health and the quality of the crop products (Vidhya Devi and Sumathy, 2017).

MATERIALS AND METHODS

Collection of Samples: Fruit peel waste were collected from the fruit juice shop. The three different fruit peels used for the present study are pomegranate, sweet lime, and orange Figure 1. Fruit peels were divided into small pieces, dried in the shade for 96 hours, and then ground into a fine powder.

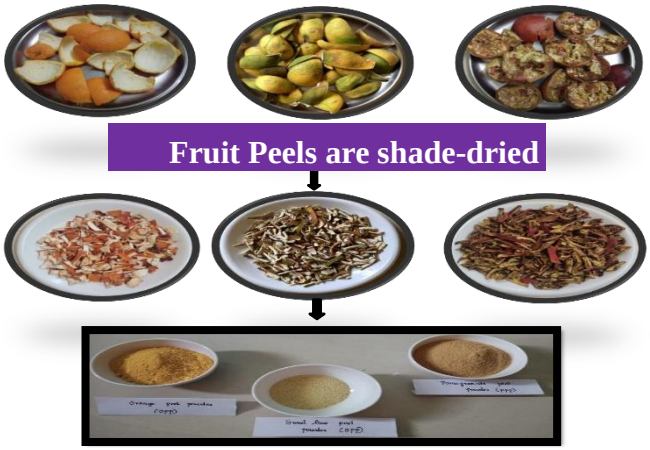

Figure-1 Process of Preparation of Fruit Peel Powder

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Instrumental Color Analysis: A digital color meter was used to evaluate the powdered fruit peel's color. after calibration with a white and black plate. Units L*, a*, and b* were used to define color. L* stands for lightness (0 = black, 100 = white), a* for color on a green (-) to red (+) axis, and b* for color on a blue (-) to yellow (+) axis. (P.G.I. Dias *et al*., 2020).

FTIR of Selected Fruit Peel Powder: Pomegranate, orange, and sweet lime peel powder functional groups were identified using SHIMADZU Miracle spectrophotometer (FTIR 820IPC) KBr methods and Fourier Transform Infrared Spectroscopy. Infrared spectra between ranges of 4000 and 400 cm-1 werecaptured. The functional group region is between 1500 and 4000 cm-1, while the finger print region is between 500 and 1500 cm-1 shown in the Figure 2.

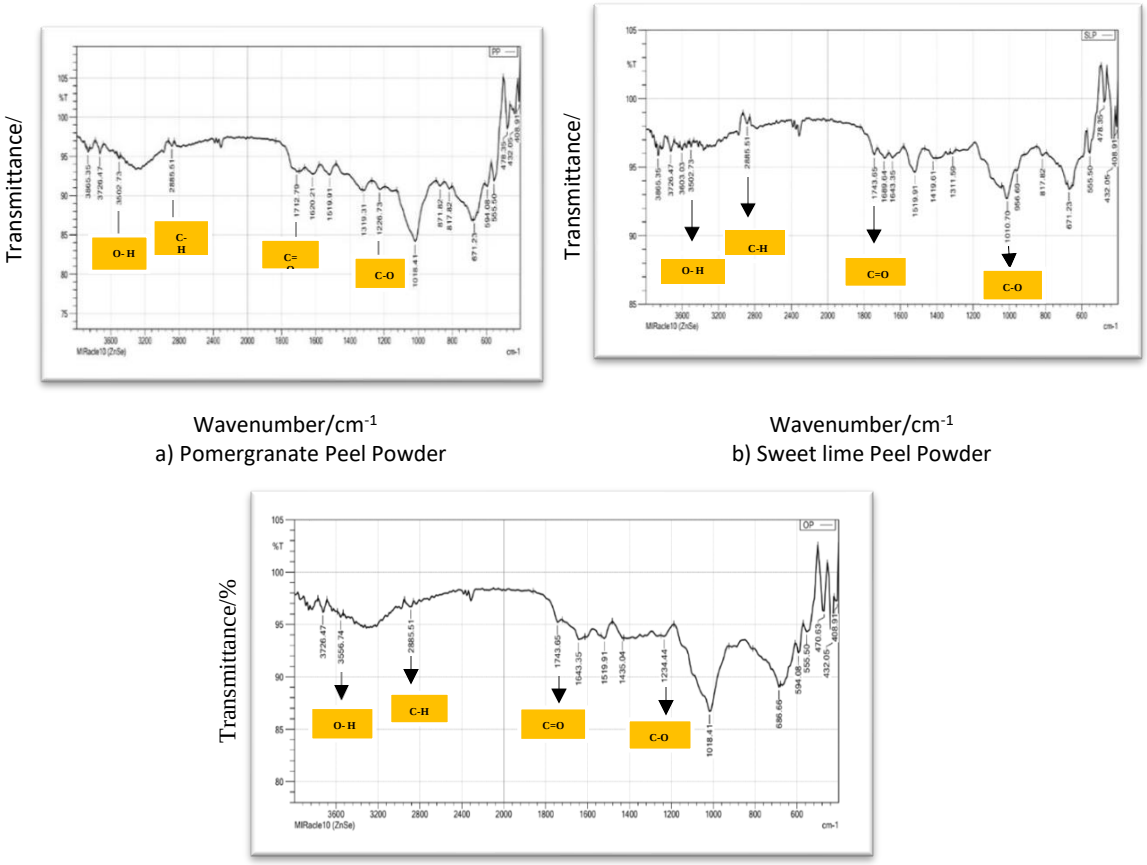

Figure-2 FTIR Spectra of Fruit Peel Powder (a) Pomegranate Peel Powder (b) Sweet lime Peel Powder (c) Orange Peel Powder

Antioxidant DPPH Assay: The fruit peel powders are extracted using an aqueous method in the following proportion of 3 grams of fruit peel powder, added 120 ml of distilled water for 20 minutes at 20°C to 30°C in water

bath. The solution was chilled to 4°C and filtered using Whatman filter paper. The aqueous extraction of pomegranate, orange and sweet lime peel powders are testedfor quantitative analysis of antioxidant by DPPH assay. In DPPH assay ascorbic acid was takenas standard and pomegranate, orange and sweet lime peel powder aqueous extracts were compared with the same standard shown in the Figure 3.

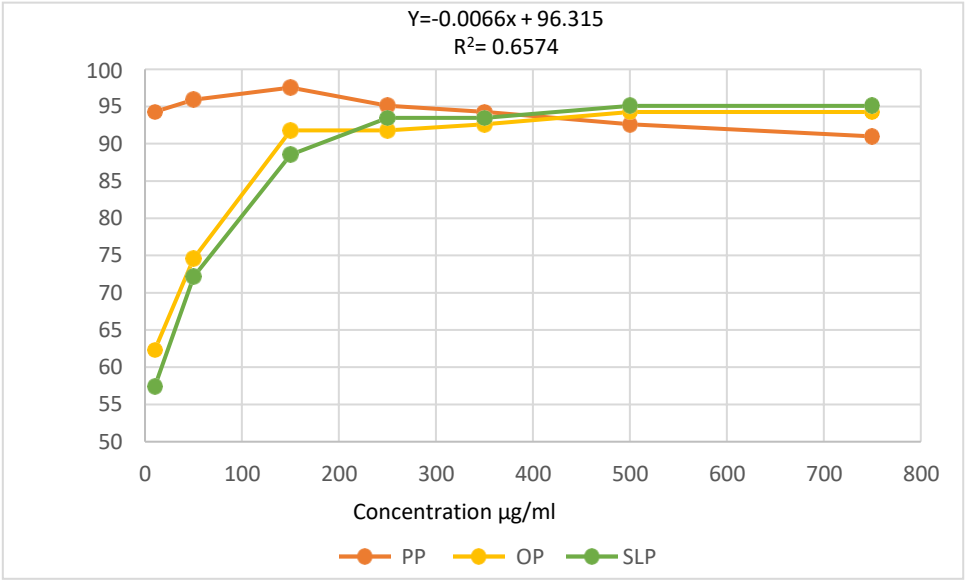

Figure- 3 DPPH Assay of Fruit Peel Powder

Production of Probiotics in Fruit Peel Powder: The spread plate technique was used to separate the lactic acid bacteria from raw cow'smilk. For the formation of lactic acid, as the culture media, Man Rogosa Sharpe (MRS) agar isselected. At 37 °C, the plates were incubated for 24 hours.

The spread plate count technique was utilized to determine the total amount of Lactic Acid Bacteria generation in the powdered fruit peel. Fruit peel powder samples (10g) were blended with 90 ml of sterilised water and serially diluted from 10^{-1} to 10^{-5} in sterilised water.(Sanders, 2012).

Under anaerobic conditions, agar containing Lactobacilli de Man Rogosa Sharpe(MRS) was incubated at 37 °C for 24 hours and the total number of LAB was counted using the spread plate method. The quantity of bacterium colonies was calculated as the number of colony-forming units of viable microorganisms per gram of fresh materials.

RESULTS AND DISCUSSION

Instrumental Color Analysis: Fruit peels' color is one of the crucial quality factors. Table I shows that the sweet lime peel powder has the highest lightness (50.13 ± 4.92) compared to pomegranate peel and orange peel powder, a^* indicates 2 colors like green and red. If values are in (-) it indicates the presenceof green color and if values

are in (+) it indicates the presence of red color. Pomegranate (11.19 ± 0.33) and orange (5.16 ± 0.64) peel powder shows the presence of red color and sweet lime peel (-9.64 ± 5.71) shows the presence of green color. b^* indicates 2 colors like blue and yellow.If values are in (-) it indicates the presence of blue color and If values are in (+) it indicates the presence of yellow color. Pomegranate (35.34 ± 0.98) and orange (42.55 ± 0.12) peel powder shows the presence of yellow color and sweet lime peel powder (-27.84 ± 3.75) shows the presence of blue color.

Table-1 Digital Color Analysis of Selected Fruit Peel Powder

S. No	Sample	Color Analysis		
		L^*	a*Green (-)Red(+)	b*Blue (-) Yellow (+)
1.	Pomegranate Peel Powder	36.25 ± 0.56	11.19 ± 0.33	35.34 ± 0.98
2.	Sweet lime Peel Powder	50.13 ± 4.92	-9.64 ± 5.71	-27.84 ± 3.75
3.	Orange Peel Powder	49.75 ± 0.59	5.16 ± 0.64	42.55 ± 0.12

FTIR Analysis: In Table II shows confirm the presence of functional groups like water, alcohol, carboxylic acid, alkane, amines, aldehyde, anhydride, ester, ketone, amide, NO_2 , fluorine and chlorine in pomegrante peel, orange peel and sweet lime peel powder. The peak frequency ofwater and alcohol OH stretch absorbed at 3502.73 cm^{-1}, 3726.47 $cm^{-1}$and 3502.73 cm^{-1} in pomegrante, orange and sweet lime peel powder. The absorption peak centred at 3602.73, 3556.74 cm^{-1} and 3502.73 cm^{-1} indictaes the presence of (N-H stretch) amines. The aldehyde and anhydride, ester, ketone and amide presences observed at 1712.79 cm^{-1}, 1743.04 cm^{-1} and 1743.65 cm^{-1} in pomegrante peel, orange peel and sweet lime peel powder. Presence of alkene,aromatic compounds are confirmed at the peak of (1620.21 cm^{-1} and 1519.91 cm^{-1}), 1643.35 cm^{-1} and 1643.35 cm^{-1} in pomegrante peel, orange peel and sweet lime peel powder. NO_2 vibrant presence confirmed at the peak of 1319.31 cm^{-1}, 1435.04 cm^{-1} and 1311.59 cm^{-1} in pomegrante peel, orange peel and sweet lime peel powder. The presence of fluorine, chlorineare obeserved in the peak region of finger print between 1500-4000 cm^{-1} it is shown in the figure 3. Good source of nitrogen in the fruit peel is confirmed by amine and amide presence.

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Table-2 Peak Absorbance Values of Pomegranate, Orange and Sweet Lime Peel PowderUsing FTIR Spectroscopy

*Strong - indicates low transmittance; Weak - indicates high transmittance; Variable – indicates varying transmittance

FunctionalGroup	Frequency Standard (cm^{-1})	Frequency Obtained(cm^{-1})					
		PP	Intensity	OP	Intensity	SLP	Intensity
Water OHstretch	3700-3100	3502.73, 3726.47, 3865.35	Weak	3726.47	Weak	3502.73, 3603.03, 3726.47, 3865.35	Weak
Alcohol OH stretch	3600-3200	3502.73	Weak	3556.74	Weak	3603.03	Weak
Carboxylic acid OHstretch	3600-2500	3502.73, 2885.51	Weak	3556.74	Weak	2885.51	Weak
N-H stretch Amines	3500-3350	3502.73	Weak	3556.74	Weak	3502.73	Weak
?C-H stretch	?3300	3502.73	Weak	3556.74	Weak	3502.73	Weak
-C-H stretch Alkane	2950-2840	2885.51	Weak	2885.51	Weak	2885.51	Weak
-C-H aldehydic	2900-2800	2885.51	Weak	2885.51	Weak	2885.51	Weak
C=O aldehyde	1740-1720	1712.79	Weak	1743.04	Weak	1743.65	Weak
C=O anhydride	1840-1800, 1780-1740	1712.79	Weak	1743.04	Weak	1743.65	Weak
C=O ester	1750-1720	1712.79	Weak	1743.04	Weak	1743.65	Weak
C=O ketone	1745-1715	1712.79	Weak	1743.04	Weak	1743.65	Weak
C=O amide	1700-1500	1712.79	Weak	1743.04	Weak	1689.64	Weak
C=C alkene	1680-1600	1620.21	Weak	1643.35	Weak	1643.35	Weak
C=C aromatic	1600-1400	1519.91	Weak	1643.35	Weak	1643.35	Weak
C-O-C stretch	1250-1050 Several	1319.31	Weak	1234.44	Weak	1010.70	Medium
NO_2 stretch	1600-1500, 1400-1300	1319.31, 1620.21, 1519.91	Weak	1435.04, 1519.91	Weak	1311.59, 1419.61, 1519.91	Medium
C-F	1400-1000	1018.41, 1226.73	Medium	1018.41	Medium	956.69, 1010.70	Medium
C-Cl	800-600	671.23	Medium	686.66	Medium	817.82	Weak
C-Br	750-500	555.50, 594.08, 671.23	Medium	555.50, 594.08	Weak	555.50, 671.23	Medium
C-I	?500	408.91, 432.05, 478.35	Weak	408.91, 432.05, 470.63	Weak	408.91, 432.05, 478.35	Weak

Antioxidant DPPH Assay: In Table **3**, DPPH assay inferred that the pomegranate peel powder extract was more effective in lowest concentration of 10 µl it shows highest inhibition of 94.26 %. The concentration has increased in 50 µl, 150 µl, 250 µl,350 µl, 500 µl and 750 µl it shows the inhibition of 95.90 %, 97.54 %, 95.08 % 94.26 %, 92.62 % and 90.98 % so it is concluded that lowest concentration has highest inhibition in pomegranate peel powder. Meanwhile, the DPPH inhibition of standard ascorbic acid in 12 µl shows the 90.98 % of inhibition. In Orangepeel powder extract was more effective in highest concentration of 500 µl it shows the highestinhibition of 94.26 %. The concentration increases in 50 µl, 150 µl, 250 µl,350 µl, 500 µl and 750 µl it shows the inhibition of 62.30 %, 74.59 %, 91.80 %, 91.80 %, 92.62 %, 94.26 % and 94.26 % respectively.

Table-3 DPPH Assay of Standard Ascorbic Acid and Fruit Peel Powder

Concentration (µl)	% Inhibition		
	Pomegranate Peel	Orange Peel	Sweet lime Peel
10	94.26	62.30	57.38
50	95.90	74.59	72.13
150	97.54	91.80	88.52
250	95.08	91.80	93.44
350	94.26	92.62	93.44
500	92.62	94.26	95.08
750	90.98	94.26	95.08

In sweet lime peel powder extract, concentration increases the % of inhibition also increased. At their highest concentration, the extracts highest percentage of DPPH inhibition was noted. The concentration has increases in 10 µl, 50 µl, 150 µl, 250 µl,350 µl, 500 µl and 750 µl it shows the inhibition of 57.38 %, 72.13 %, 88.52 %, 93.44%, 93.44 %, 95.08 % and
95.08 % respectively. The comparison of fruit peel powder extract with standard ascorbic acid shows that concentration of 3 µl, 6 µl, 9 µl, 12 µl and 15 µl shows the inhibition of 57.38 %, 71.31 %, 77.87 %, 90.98 % and 96.72 % respectively.

Production of Probiotics in Fruit Peel Powder: *Lactobacillus* species appearance as small, white, creamy colonies of selected fruit peel powder like pomegranate peel powder, orange peel powder and sweet lime peel powder shownin the Figure 4. Production of lactic acid bacteria is determined by 48 hours of monitorizationat 37°C of incubation The growth of lactic acid bacteria is higher in orange and sweet lime fruit peels while compared to

pomegranate fruit peel in first 24 hours is observed through spread plate method. On second 24 hours of there is a decline in the growth lactic acid bacteria in sweet lime and orange peels. The constant and stable growth of lactic acid bacteria is observedby pomegranate peel.

a b c

Figure -4 Growth of probiotics in fruit peel powder (a) Pomegranate peel powder (b) Orange peel powder (c) Sweet lime peel powder.

CONCLUSION

The findings of the current research show that fruit peels are rich in antioxidant and it is a good and cheap source of Lactic Acid Bacteria. Fruit peels are good substrate for the growth of the probiotics. Highest content of dietary fibre in fruit peels enhance the growth of Lactic Acid Bacteria. Production of Lactic Acid Bacteria in fruit peel waste is economically viable to farmers to use fruit peel as biofertilizers. Lactic Acid Bacteria enhance the quality and quantity of the crops and highest yield increases the farmers income.

In various country, probiotic farming is implemented to enhance their food supply and quality of the food crops. In Italy 2018 and Australia from 2015 to still they are working on the probiotics which act as biofertilizer to boost their crops to be healthier. Spain 2019 country usethe probiotics as tool to increase the functional properties of the crops. In England, probiotics are used to fight plant diseases without pesticides. In united states 2020, microorganisms are applied for sustainable agriculture. In Belgium 2021, microorganism used in farming practices.In Portugal 2020 plant probiotics are used for the enhance the effects on agro economically valuable crops. Recent studies in 2021 Rome, Italy, Germany and Finland countries are involved in microbes as biofertilizer for sustaining the agriculture growth. But in India there are no more research in the field of probiotic farming to enhance the probiotic farming for sustainable agriculture this study plays crucial role.

Conflict of Interest: The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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