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PHARMACOLOGICAL PROFILE ANALYSIS OF A VEGETABLE CROP PUMPKIN (*CUCURBITA MAXIMA* LINN.) SEEDS

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ABSTRACT

The seeds of pumpkin (*Cucurbita maxima* Linn.) can be utilized as both a conventional and multifunctional dietary constituent. In this study the antioxidant, antibacterial, and cytotoxic effects of various fractions such as petroleum ether soluble fraction (PESF), chloroform soluble fraction (CFSF), dichloromethane soluble fraction (DCMSF), and aqueous soluble fraction (AQF) were obtained from crude extract (CE) of the *C. maxima* seeds were assessed by using 1,1-Diphenyl-2- Picrylhydrazyl (DPPH) method, disc diffusion technique, and brine shrimp lethality bioassay respectively. The results showed that all the fractions have remarkable antioxidant, antibacterial, and cytotoxic activities. The study revealed that both the AQF and PESF and the CF confirmed significant antioxidant properties. The antibacterial activity of different fractions against Gram-positive and Gram-negative bacteria was examined to determine their significant effect. PESF and CFSF fractions exhibited substantial inhibitory activity against Gram-positive bacteria, particularly *Bacillus cereus*. *Bacillus subtilis* exhibited significant susceptibility to both CFSF and AQF. *Salmonella typhi* and *Shigella dysenteriae* exhibited significant susceptibility to PESF and CFSF, while *Escherichia coli* was the Gram-negative bacterium most effectively inhibited by CFSF. The IC_{50} values revealed that the DCMSF exhibited the highest inhibitory activity, followed by other fractions. The new study emphasizes the significance of pumpkin seeds in terms of their pharmacological and biological properties, which may encourage more research on the potential benefits of this plant for human health.

Keywords: *Cucurbita maxima, Antioxidant, Antimicrobial, Cytotoxicity, Crude extracts.*

Introduction

Humans have relied on nature to provide for their most fundamental needs since the beginning of civilization. Pharmacognosy, the study of *Materia medica* derived from natural sources such as plants, minerals, animals, and fungi, has long been closely associated with the pharmacy field

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(Jamshidi-Kia *et al*., 2018). Medicinal plants have become a crucial element of the contemporary medical system due to their abundance of beneficial natural products. The utilization of natural resources is essential for the research and development of novel medications (Ahmed *et al*., 2013; Akhter *et al*., 2021; Penu *et al*., 2022; Haque *et al*., 2020; Hossain *et al*., 2019; Rahman *et al*., 2020). According to the World Health Organization, around 25% of the drugs now used in the United States are derived from plants (Ahmed *et al*., 2013).

The pumpkin a well-known edible plant, is recognized as a helpful vegetable as a member of the Cucurbitaceae family, which has 800 species and 130 genera. Globally, *Cucurbita maxima* Linn., *Cucurbita pepo*, and *Cucurbita moschata* are the most widely cultivated and utilized pumpkin species (Sharma *et al*., 2020). *C. maxima*, often known as Kumra in Bangladesh, is a rapidly growing species (Shendye and Gurav, 2014). It is mainly cultivated throughout Bangladesh, China, India, Malaysia, Myanmar, Papua New Guinea, and some coastal areas of Africa (Hayat and Khan, 2009). The enormous amounts of seeds and peels of the pumpkin have substantial commercial value. Despite extensive study of the plant's leaves, fruits, and bark, the biological properties and phytochemical composition of *Cucurbita maxima* Linn. still need to be discovered.

Various investigations have been conducted across the globe on several varieties of pumpkin seeds, particularly *C. pepo*, *C. moschata*, and *C. maxima*. It has been claimed that pumpkin seeds contain anti-diabetic, antimicrobial, antioxidant, anti-inflammatory, anti-cancer, anti-tumor, anti-mutagenic, and antiulcer effects (Aziz *et al*., 2023; Sharma *et al*., 2020). Pumpkin seeds are high in protein, fat, antioxidants, fiber, phytochemicals, and vitamins, rendering them an excellent source of nourishment (Agrawal and Shahani, 2021; Hussain *et al*., 2021a). Pumpkin seed-rich meals have been linked to a lower risk of cancer (Varela *et al*., 2022). According to studies, hepatocarcinoma (HepG2), human tumor cell lines, and colon carcinoma (CT26) can no longer spread when exposed to pumpkin seed extracts (Sharma *et al*., 2020).

Antimicrobial proteins are also found in pumpkin extracts (Dowidar *et al*., 2020). Earlier studies have revealed that phenolic compounds in fruits and vegetables play a significant role in antibacterial activity because their polar isopropyl functionality may play a role in bacteriostatic activities (Asif, 2015; Hussain *et al*., 2021b). Pumpkin seed oil has antibacterial capabilities against a variety of microbes, including *E. coli*, *Bacillus subtilis*, *Xanthomonas campestris*, and *Proteus mirabilis* (Amin *et al*., 2020; Leichtweis *et al*., 2022). According to Hussain *et al.* (2021b) the seed oil can also kill several fungi, such as *Rhizopus stolonifera*, *Trichoderma herzianum*, *Pythium ultimum*, and *Paecilomyces lilacinus*.

Pumpkin extracts are thought to possess antioxidant properties beneficial to those with vascular disease, diabetes, and prediabetes. Local healers suggest consuming a crude aqueous extract of pumpkin fruits as a treatment for type 2 diabetes or non-insulin-dependent diabetes mellitus (Adams *et al*., 2014; Larner, 2002). More research showed that pumpkins significantly lowered blood sugar levels in alloxan-induced diabetic rabbits, people with type 2 diabetes, and rabbits with temporary hyperglycemia (Adams *et al*., 2014; Yoshinari *et al*., 2009). Pumpkin, specifically *Cucurbita ficifolia*, contains d-chiro-inositol, which has been recognized as a modulator of insulin

action, sometimes known as an insulin sensitizer (Larner, 2002). So, pumpkin seeds need to be scientifically tested to see if they can kill bacteria that are resistant to antibiotics. The goal is to develop new plant-based drugs or antimicrobials that can be used in the food industry. Despite the acknowledged capacity of pumpkin seeds to combat diseases, a comprehensive evaluation of the antioxidant, cytotoxicity, and antibacterial properties of *C. maxima* Linn. seeds across different varieties still needs to be improved. Consequently, this study examines pumpkin seeds' antioxidant cytotoxicity and antibacterial properties.

Materials and Methods

Plant materials

Seeds of *Cucurbita maxima* Linn. were collected in and around Chawkbazar in Old Dhaka, Bangladesh, in January 2023. The specimen was identified with the help of a pant taxonomist from the Bangladesh National Herbarium, Dhaka, Bangladesh. The seeds were separated from the fruit, foreign materials removed, then dried at 60-70°C and pulverized into powder using a grinder. The powdered material was preserved in an airtight container or stored at 4°C until further use.

Extraction and fraction procedure

The plant material was extracted using the modified Kupchan partition method, following the procedure outlined by VanWagenen *et al*. (1993). The crude extract (CE) is separated using the modified Kupchan partition technique, using petroleum ether, chloroform, and dichloromethane as solvents. The petroleum ether soluble fraction (PESF), chloroform soluble fraction (CFSF), dichloromethane soluble fraction (DCMSF), and aqueous soluble fraction (AQF) were obtained from CE. This commonly employed extraction method involves a series of extractions with progressively more polar solvents, starting with a non-polar solvent and ending with a more polar solvent (methanol). The purpose is to extract molecules with different degrees of polarity.

Chemicals and Reagents

Dimethyl sulfoxide (DMSO, Merck, Germany), methanol, petroleum ether, chloroform, dichloromethane, Tert-butyl-1-hudroxy toluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), acid casein hydrolysate, starch, Whatman No. 1 filter paper, ascorbic acid (Sigma Aldrich, Germany), agar, nutrient agar media, and nutrient broth media (Hi Media, India) were used.

Biological investigation

The crude extract has been examined for its biological activity using various methods. For instance, the antioxidant activity can be assessed using the DPPH method. The Muller-Hinton agar plate can test the antimicrobial properties (20 ml of a crude extract, acid casein hydrolysate, starch, and agar are added to a 100-mm plastic Petri dish). The cytotoxicity can be determined through the Brine shrimp lethality bioassay. Lastly, the anti-inflammatory activity can be measured using a well-established method (Brand-Williams *et al*., 1995)

Antioxidant activity testing

The study used the DPPH Free Radical Scavenging Assay to see how well different *Cucurbita maxima* extracts got rid of free radicals. (Szabo *et al*., 2007). The IC_{50} value is used to measure

antioxidant activity. This is the amount of antioxidant-containing material needed to eliminate 50% of the original DPPH radicals (Vimala and Adenan, 1999). A lower IC_{50} value indicates greater effectiveness in scavenging DPPH radicals, thereby reflecting higher levels of antioxidant activity. This method provides insight into the potential antioxidant properties of *C. maxima* Linn. extracts, offering valuable information for further research and potential applications in various fields. The ascorbic acid and test samples were dissolved in a methanol solvent. Each sample, with a volume of 500 µL, was mixed with 9.5 mL of freshly prepared DPPH solution (2,2-diphenyl-1-picrylhydrazyl) at a concentration of 50 µg/mL in pure methanol. As a positive control, ascorbic acid was utilized, while a negative control consisted of 10 mL of 50 µg/mL DPPH solution in pure methanol. The mixtures were thoroughly combined and then allowed to incubate at room temperature in darkness for 10 minutes. Absorbance readings were taken at 517 nm using a spectrophotometer. All experiments were conducted in triplicate. The percentage of DPPH free radical scavenged was determined using the following formula:

Percentage Inhibition = $(A_0-A_1)/A_0\times100$. Where, A_0 is the initial absorbance and A_1 is the absorbance after incubation with DPPH.

Antimicrobial activity testing

The disc diffusion method, also known as the Kirby-Bauer method, is a standard laboratory technique used to assess the efficacy of antibiotics or other antimicrobial agents against bacterial pathogens with modification (Rasool *et al*., 2016). The antibacterial activity of the Kupchan fractions was tested using the disc diffusion technique. Mueller-Hinton agar was prepared with approximately 20 ml per 100-mm plastic petri dish (Dewees *et al*., 1970). With a diameter of 5 mm, the Whatman No. 1 filter paper discs were placed into a small vial and sterilized using an autoclave. Subsequently, the discs were thoroughly dried in a drying oven at 60°C. The samples (CE, PESF, CFSF, DCMSF, and AQF) were all dissolved in chloroform separately. They were then put on sterile discs at 400 µg/disc and left to dry completely. The samples were then used for the antibacterial assay. For this purpose, pure cultures of Gram-positive and Gram-negative bacterial strains were collected from the Microbiology Laboratory of the Bangladesh Council for Scientific and Industrial Research (BCSIR), Dhaka. Subculture the bacteria from stock culture onto Mueller-Hinton agar plates and incubate overnight at 37°C to obtain well-isolated colonies. A single colony was isolated from the overnight culture and inoculated into a 100-µL Mueller-Hinton broth tube. Incubate the broth culture at the appropriate temperature with shaking (1-2 hours) until it gets cloudy, which is equal to almost 1-2 × 10^8 colony-forming units (CFU) per milliliter for most bacterial species. Then, 50 µL of each bacterial suspension was spread uniformly over the surface of the Mueller-Hinton agar plate. Allow the plate to dry for a few minutes to ensure even distribution of the bacterial culture. Using sterile forceps, place the antibiotic discs and discs containing test samples onto the surface of the agar plates, ensuring they are evenly spaced and pressed gently onto the agar to ensure contact. Incubate the plates at the appropriate temperature (usually 37°C) for 18–24 hours. After incubation, examine the plates for inhibition zones around the antibiotic disc and the samples. Measure the diameter of each zone of inhibition using a ruler in millimeters. The diameter of the zone of inhibition is inversely proportional to the susceptibility of the bacteria to the antibiotic and samples, with larger zones

indicating greater susceptibility. All the experiments were repeated three times. In every antibacterial test, blank discs were used as the negative control and a standard kanamycin disc (30 µg/disc) as the positive control.

Brine shrimp cytotoxicity assay

Brine shrimp lethality bioassays evaluated the cytotoxic effects of extracts (Meyer *et al*., 1982). The eggs of brine shrimp were hatched for 24 h and screened to determine LC50 values against varying concentrations (200, 100, 50, 25, 12.50, 6.25, 3.125, 1,563, 0.781, and 0.390 µg/ml) of extracts diluted in DMSO by the serial dilution method. Vincristine sulfate was used as the positive control, while DMSO was the negative control. Ten living nauplii of *Artemia salina* were transferred to each vial holding 4 mL of simulated seawater with the help of a Pasteur pipette. The test was performed at 25 ± 1 ℃ and 35% salinity by normal operating procedure (Kester *et al*., 1967).

Results and Discussion

Antioxidant assay

The DPPH radical test is one method for determining the antioxidant activity of a substance by assessing its capacity to scavenge free radicals. The discoloration of the solution is attributed to the antioxidants present in the extracts, which effectively respond to the violet DPPH radical in this assay. This study estimated the DPPH free radical scavenging activity of different soluble fractions of the crude methanol extract with various solvents to increase polarity. The DPPH free radical scavenging activity was evaluated using a spectrophotometric method. The effectiveness of each fraction, including the overall methanolic extract and its divisions into petroleum ether, chloroform, dichloromethane, and water-based fractions, was quantified by their IC_{50} values, indicating the concentration required to reduce by 50% of the DPPH activity. These values are detailed in figure 1. It was found that the aqueous and petroleum ether fractions, along with the crude methanol extract itself, exhibited high antioxidant activities. Meanwhile, the chloroform and dichloromethane fractions showed moderate abilities to scavenge free radicals, with IC_{50} values of 27.1 µg/ml and 45.2 µg/ml, respectively. Anti-oxidant activity of fractions were significantly different from the anti-oxidant activity of and standard (Ascorbic acid) and the level of significance was designated as *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. The antioxidant capacity of *Cucurbita pepo* was shown to be higher when ethanol and n-butanol were used as solvents. On the other hand, *Cucurbita maxima* demonstrated a more vital ability to scavenge DPPH radicals in the case of methanol extracts (Yadav *et al*., 2016). The researchers noted that the antioxidant activity of leaf extracts from *Cucurbita pepo* L. showed that the ethyl acetate extract exhibited the maximum inhibition of DPPH radicals followed by the n-butanol extract and the aqueous acetate extract. Chloroform and n-hexane extracts had the lowest antiradical capabilities (Dar *et al*., 2017). Saavedra *et al*., (2015) discovered that extracts derived from pumpkin peel exhibited approximately 2-3 times more antioxidant activity than those obtained from pumpkin seeds.

Overall, the study revealed that the methanolic extract and its fractions possess significant antioxidant properties, with the aqueous and petroleum ether fractions showing the highest

activity. These findings suggest that further research on these fractions could potentially lead to the development of natural antioxidants for various applications.

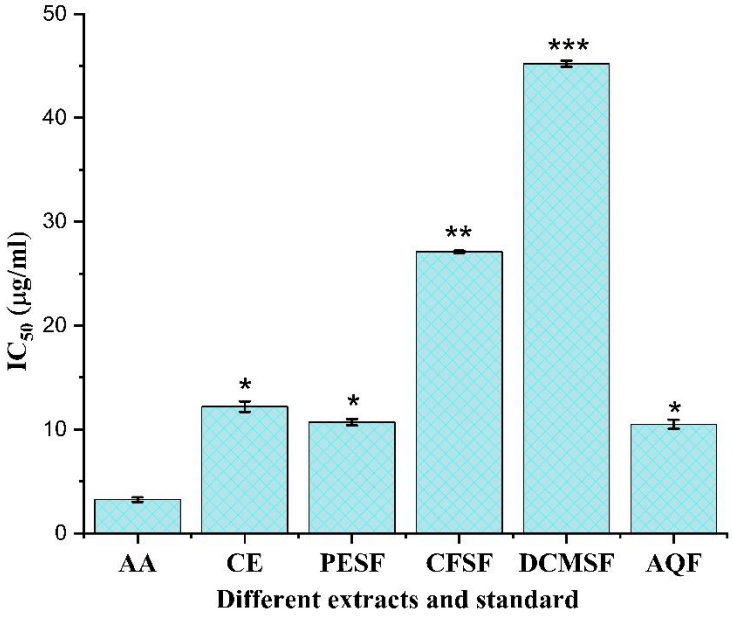

Fig. 1. The antioxidant activity of the seeds of *Cucurbita maxima* Linn. Values of the IC_{50} for different partitions of seeds of *Cucurbita maxima* and standard. CE = Crude extract, PESF = Petroleum ether soluble fraction, CFSF = Chloroform soluble fraction, DCMSF = Dichloromethane soluble fraction, AQF = Aqueous soluble fraction, BHT= Tert-butyl-1-hudroxy toluene and AA= Ascorbic acid (standard). Values were expressed as mean ± SD of three independent experiments.

Antimicrobial activity

The petroleum ether soluble fraction (PESF) and chloroform soluble fraction (CFSF) had strong inhibitory effects against Gram-positive bacteria, especially *Bacillus cereus*, outperforming other fractions in this regard. *Bacillus subtilis* also demonstrated high sensitivity to CFSF and the aqueous fraction (AQF). *Salmonella typhi* and *Shigella dysenteriae* showed notable sensitivity to PESF and CFSF, although *Escherichia coli* was the Gram-negative bacterium most efficiently suppressed by CFSF. Notably, *Vibrio mimicus* demonstrated a significant sensitivity to the dichloromethane soluble fraction (DCMSF). The antimicrobial activity of standard (Kanamycin) and different partitions are presented in Table 1. This study suggests that pumpkin seeds exhibit antibacterial properties. The findings of Saavedra *et al*., (2015) contradict these results. In their study, pumpkin shells and seeds were extracted using various solvents (70% ethanol, 70% methanol, 70% acetone, water, and dichloromethane), but they did not exhibit any antibacterial

activity against several bacteria. In a separate study conducted by Kabbashi *et al*., (2014), it was found that *C. maxima* exhibited significant antimicrobial properties against various microorganisms, including *P. aeruginosa*, *A. niger*, *C. albicans*, *E. coli*, and *B. subtilis*. In a study conducted by Dubey *et al*., (2010), it was demonstrated that the methanolic extract derived from the fruit of *C. pepo* exhibited varying degrees of antibacterial activity against several bacterial strains, including *Bacillus subtilis*, *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Cryptococcus meningitis*. Researchers are investigating the antibacterial properties of natural herbs to combat the rise of bacterial resistance (Alviano and Alviano, 2009). Seed extracts containing powerful antimicrobial chemicals have the potential to treat numerous infectious illnesses caused by resistant bacteria. These results point to bioactive chemicals with strong antibacterial activity in *Cucurbita maxima* seed extracts, suggesting possible uses in creating new antimicrobial drugs.

Table 1. Antimicrobial Activity of standard (Kanamycin, 30 µg/ Disc) and different partitions (400 µg/ Disc) of seeds of *Cucurbita maxima* Linn.

Test microorganisms	Zone of Inhibition (in mm)					
Gram-positive bacteria	CE	PESF	CFSF	DCMSF	AQF	Kanamycin
Bacillus cereus	10.2±0.21	15.3±0.23	17.1±0.41	9.8±0.31	7.3±0.23	35±0.22
Bacillus subtilis	10.2±0.32	16.5±0.34	18.2±0.33	10.2±0.42	6.4±0.33	35.2±0.31
Staphylococcus aureus	10.3±0.43	18.1±0.23	17.4±0.45	9.6±0.33	7.1±0.43	36.3±0.42
Gram-negative bacteria						
Escherichia coli	9.4±0.08	11.2±0.18	18.2±0.32	7.3±0.23	NA	35±0.22
Salmonella typhi	10.3±0.30	12.5±0.04	17.2±0.21	6.4±0.33	NA	35.2±0.31
Shigella dysenteriae	8.5±0.14	10.1±0.22	19.2±0.14	8.2±0.14	NA	36.1±0.40
Vibrio mimicus	7.2±0.23	6.4±0.14	13.2±0.08	9.2±0.30	NA	37.4±0.32
Vibrio parahemolyticus	11.2±0.04	11.2±0.16	11.2±0.16	8.3±0.43	8.2±0.14	35.3±0.32

Cytotoxicity by brine shrimp lethality bioassay

The IC_{50} values of petroleum ether, chloroform, dichloromethane, and aqueous soluble partitions were found to be 20.13µg/ml, 6.51µg/ml, 1.2µg/ml, and 11.52µg/ml, respectively (Fig. 2). These values indicate the potency of each partition in inhibiting the growth of the target organism. It can be observed that the dichloromethane partition exhibited the highest inhibitory activity, followed by chloroform, aqueous soluble, and petroleum ether partitions. These values indicate the lowest IC_{50} value, indicating the highest potency against the target. On the other hand, petroleum ether has the highest IC_{50} value, suggesting lower efficacy in inhibiting the target. The brine shrimp lethality bioassay results show that the crude extract and Kupchan fractions have considerable

cytotoxic potency. In contrast, the pumpkin byproducts were verified by assessing their toxicity in a primary culture of non-tumor porcine liver cells (PLP2), where they did not get any cytotoxic properties (Leichtweis *et al*., 2022). This suggests that the crude extract and Kupchan fractions may have potential as cytotoxic agents, while the pumpkin byproducts may be safer for non-tumor cells. Further studies are needed to understand the mechanisms behind these results fully and to explore the potential applications of these findings in drug development.

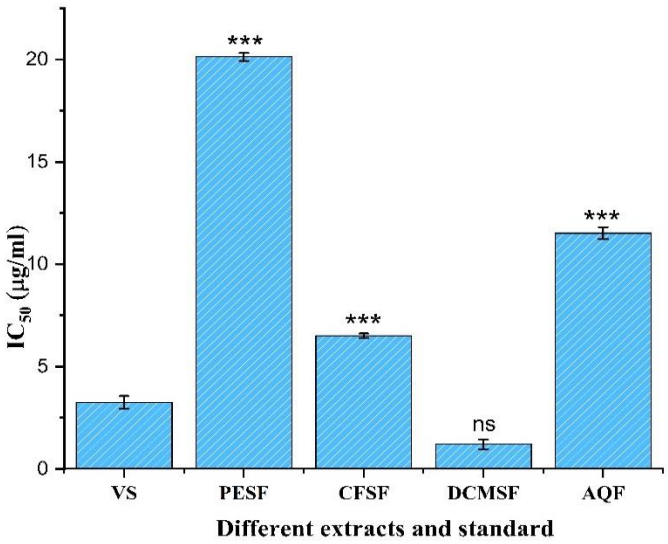

Fig. 2. Brine shrimp lethality bioassay of seeds of *Cucurbita maxima*. Values of the IC_{50} for different partitions of seeds of *Cucurbita maxima* and standard. VS= Vincristine sulphate (standard), PESF = Petroleum ether soluble fraction, CFSF = Chloroform soluble fraction, DCMSF = Dichloromethane soluble fraction, and AQF = Aqueous soluble fraction. Values were expressed as mean ± SD of three independent experiments. ns = not significant, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ the level of significance.

Conclusion

Cucurbita maxima, a multipurpose plant, is recognized for its various secondary metabolites that may have medicinal properties. The plant's fruits, leaves, and bark have traditionally been used for therapeutic benefits. This study aimed to investigate the pharmacological activity of chemicals found in *Cucurbita maxima* seeds. The research includes extracting various fractions using organic and aqueous solvents, followed by qualitative studies to determine the phytochemical makeup. The seed extracts' antioxidant, antibacterial, and cytotoxic properties were substantial. The antioxidant assay revealed significant free radical scavenging activity, whereas the antibacterial activity test revealed encouraging results against bacteria and fungus. The seed extracts were also found to have antibacterial action against Gram-positive bacteria, with

inhibition zones equivalent to those of the conventional drug kanamycin. The crude extract and Kupchan fractions showed significant cytotoxic efficacy in the brine shrimp lethality test, with the dichloromethane soluble fraction (DCMSF) suggesting potential for cytotoxic uses. The findings demonstrate the pharmacological potential of *Cucurbita maxima* seed extracts, supporting traditional usage and paving the way for further research into their medicinal applications.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of the manuscript.

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