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Original Article
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Anthelmintic Potential of Discarded *Litchi chinensis* Seeds: a sustainable approach to agricultural by-product utilization

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Objectives: *Litchi chinensis* (Sonn.), belonging to the Sapindaceae family, has historically been used for the treatment of gastrointestinal ailments, including ulcers, gastritis, diarrhea, and infections. Plants in the Sapindaceae family have demonstrated potential anthelmintic effects, while the efficacy of *L. chinensis* remains barely investigated. *L. chinensis* seeds are often discarded as waste; however, utilizing these seeds promotes sustainable practices and may provide a natural alternative to conventional anthelmintics. The study aims to investigate the qualitative phytochemicals and evaluate the anthelmintic efficacy of *L. chinensis* seed ethanolic extract (LCSE).
Methods: Fresh *L. chinensis* fruits were collected from a local market, peeled fruits and removed aril from the seeds and then washed, air-dried and extracted with ethanol. A qualitative phytochemical screening and assessment of the anthelmintic properties of LCSE were conducted using standard procedures. The time required for paralysis and death of adult earthworms (*Eisenia fetida*) was assessed by analyzing each test solution at five distinct dosages (5, 25, 50, 75, and 100 mg/mL). Albendazole served as the standard, while distilled water functioned as the control. Statistical analysis was performed using two-way ANOVA followed by Tukey's multiple comparison test in GraphPad Prism version 10.1.2.
Results: Qualitative analysis revealed that LCSE is rich in phytochemicals, including alkaloids, anthraquinones, carbohydrates, flavonoid, glycosides, proteins and amino acids, phenols, terpenoids, and tannins. The anthelmintic activities of the LCSE and albendazole were dose-dependent, as the time required for paralysis and mortality decreased in proportion to the concentrations increased. LCSE exhibited strong anthelmintic efficacy at a concentration of 100 mg/mL (paralysis: 12.67 ± 0.33, death: 14 ± 0.58), which was significantly greater than that of albendazole (paralysis: 15 ± 1.15, death: 17.67 ± 1.15).
Conclusion: This finding suggests that LCSE holds potent anthelmintic properties, making it a promising natural alternative to conventional treatments like albendazole.

Keywords: albendazole, anthelmintics, *Eisenia fetida*, *Litchi chinensis*, phytochemical

INTRODUCTION

Helminthiasis is a parasitic disease that can occur in both humans and livestock due to infections by worms, including flukes (trematodes), tapeworms (cestodes), or roundworms (nematodes) [1]. These infections result in considerable morbidity and impaired development. It is a substantial global healthcare concern [2]. According to the World Health Organization, helminthiasis affects about 2 billion individuals globally, categorizing this condition as a neglected tropical disease [3]. In

underdeveloped nations, tropical diseases including helminthiasis can lead to an enormous public health burden and exacerbate starvation, anemia, eosinophilia, and pneumonia [2]. The gastrointestinal system harbors various helminths. However, some inhabit tissues or have larvae that move to other tissues [4]. Anthelmintic drugs are designed to eradicate or remove parasitic worms from the affected host [5]. Synthetic anthelmintic medications are essential for managing helminth infections in humans because immunizations are largely unavailable for these diseases [6]. The majority of the current anthelmintics are associated with adverse effects, including weight loss, vomiting, nausea, dizziness, bloating, intermittent fever, and skin irritation [7]. Nevertheless, the unchecked global use of anthelmintics has made pathogenic organisms resistant to these anthelmintics. The choice of available treatments for helminth infections has reduced as a result [6]. Innovative strategies are being studied to address anthelmintic resistance by evaluating conventional therapeutic and prospective tropical plants rich in bioactive compounds [8]. The use of plant-based products may potentially diminish our reliance on conventional pharmacological treatments and postpone the emergence of resistance. The pharmaceutical industry has yet to prioritize the development of novel antiparasitic treatments because many parasitic infections primarily affect impoverished countries that lack adequate funds to purchase expensive medications. A promising approach to creating cost-effective and efficient anthelmintic medications is to investigate traditional plant-based medicines. Assessing the efficacy of historically used herbal remedies with established anthelmintic characteristics is receiving heightened interest. The lack of a consistent supply of synthetic anthelmintics, particularly in remote regions, and their high costs have driven the need to rely on plant-based medicines in animal healthcare [9]. Plants produce allelochemicals, with primary metabolites serving as counterparts to various secondary metabolites [10]. Secondary metabolites are biologically active components with analgesic, antibacterial, antihepatotoxic, antioxidant, antiviral, antiparasitic, antitumor, fungicidal, and immunostimulant effects [11]. Plant-derived chemicals often exhibit more biological activity than synthetic chemicals. Plant-based anthelmintics include secondary metabolites, such as saponins, alkaloids, flavonoids, terpenoids, tannins, and polyphenols [12, 13]. The litchi (*Litchi chinensis* Sonn.) fruit, belonging to the Sapindaceae family, is grown commercially in tropical and subtropical regions globally for its delicious, tender, and nutrient-dense arils. Litchi flesh can be consumed directly. Litchi pulp is mostly eaten fresh and may be used to produce juice, vinegar, jelly, liquor, and ice cream. However, litchi seeds (byproducts) are thrown and underused after consumption and industrial processing, resulting in an ecological problem [14]. Traditional medicines worldwide typically utilize this plant to enhance the immune system and address gastrointestinal and reproductive issues [15]. The seed extract can alleviate neuralgic agony and lower the risk of fatty liver disease [16]. In China, litchi seeds are used to treat viral colds and mitigate lethargy. The seed extract can be used as treatment for pain, orchitis, testicular edema, hernia, gastralgia, lumbago, and abdominal pain [17]. Traditional Chinese herbal decoctions with litchi seeds are used to treat urologic cancer [18]. Litchi seed extract is used to manage inflammation, allergies, diabetes, hyperlipidemia, fever, obesity, cardiac problems, and viral infections [15]. Despite its widespread utility, the role of litchi seed in helminthic disease is not reported. Litchi seeds may be an effective treatment targeting helminths. The objective of this investigation was to evaluate the preliminary phytochemicals and determine the potential anthelmintic activity of *L. chinensis* seed (LCS).

MATERIALS AND METHODS

1. Materials

All chemicals and reagents employed in the phytochemical and anthelminthic tests were of excellent analytical quality. Ethanol (Sigma Aldrich, USA) and albendazole (Almex-400 mg, Square Pharmaceuticals PLC, Bangladesh) were procured from authorized vendors.

2. Collection and preparation of LCS crude powder

Fruits of *L. chinensis* were procured from a market near Islamic University, Kushtia campus, Bangladesh in June 2024. The collected *L. chinensis* fruits were manually peeled, and the arils were separated from the seeds. The seeds were subsequently rinsed with distilled water to eliminate impurities, dried with a towel to remove excess moisture, and diced into smaller pieces using a sterilized knife. Subsequently, these components were left to air dry in a well-ventilated, shaded location. The residual water was removed by drying in an incubator at 40℃. The desiccated seeds were further pulverized in an automatic grinder to obtain a coarse powder and kept in tightly sealed containers [19].

3. Worm collection

The earthworm *Eisenia fetida* used for the investigation was obtained from Islamic University, Kushtia, on 19 July 2024. Adult healthy earthworms, averaging 4-6 cm in size, were taken from moisture-laden soil on the Islamic University campus. All waste material was eliminated through washing with distilled water, and the worms were acclimated at 37℃ for 30 min before the test. Worms showing signs of disease or injury were excluded from the experiment. No earthworms were omitted from the analysis; all were included in the final evaluation.

4. Preparation of ethanolic extracts of LCS

LCS powder (50 g) was added to 400 mL of laboratory-grade ethanol in an amber vial, which was subsequently wrapped with aluminum foil. The solution was then agitated in a shaking incubator at 37℃. After 96 h, the solution was passed through

Table 1. Preliminary phytochemical analysis of *Litchi chinensis* seed ethanolic extract (LCSE)

Phytochemicals	Test	Procedure	Observation
Alkaloids	Mayer's Test	2 mL extract + 1-2 drops of Mayer's reagent (Along the sides of test tube)	A creamy white/yellow precipitate
	Dragendorff's test	2 mL extract + 1-2 mL Dragendorff's reagents	A reddish-brown precipitate
	Hager's test	2 mL extract + few drops of Hager's reagent	A creamy white precipitate
Anthraquinones	Borntrager's test	3 mL extract + 3 mL Benzene + 5 mL NH_3 (10%)	A pink, violet or red coloration in ammoniacal layer
Carbohydrates	Molisch's test	2 mL extract + 10 mL H_2O + 2 drops Ethanolic α-naphthol (20%) + 2 mL H_2SO_4 (conc.)	A violet ring
	Benedict's test	0.5 mL extract + 0.5 mL Benedict's reagent + Boiled for 2 min.	A green/yellow/red color
	Fehling's test	1 mL each of Fehling's solution A & B + 1 mL extract + boiled in water bath	A red precipitate
Flavonoid	Alkaline reagent test	1 mL extract + 2 mL of 2% NaOH solution (+ few drops dil. HCl)	An intense yellow color, becomes colorless on addition of diluted acid
	Shinoda's test	5 mL extract + Fragments of magnesium ribbon + few drops of conc. HCl	A pink to crimson colored solution
Glycosides	Keller Kiliani test	1 mL extract + 1.5 mL glacial acetic acid + 1 drop of 5% ferric chloride + conc. H_2SO_4 (along the side of test tube)	A blue-colored solution
Phlobatannin	Hydrochloric acid test	2 mL extract + 2 mL 1% HCl (boiled)	A red precipitate
Steroids	Salkowski test	2 mL extract + 2 mL $CHCl_3$ + 2 mL H_2SO_4 (conc.)	A reddish-brown ring at the junction
Proteins and Amino Acids	Biuret test	2 mL extract + 1 drop of 2% copper sulphate sol. + 1 mL of 95% ethanol + KOH pellets	A pink colored solution
	Ninhydrin test	2 mL extract + 2 drops of Ninhydrin solution (10 mg ninhydrin + 200 mL acetone)	A purple-colored solution
Phenols	Ferric chloride test	2 mL extract + few drops 5% ferric chloride sol.	Dark green/bluish black color
	Lead tetra acetic acid test	5 mL extract + 3 mL of 10% lead acetate sol.	A white precipitate
Quinones	Alcoholic KOH test	1 mL extract + few mL alcoholic potassium hydroxide	Red to blue color
Terpenoids	Horizon test	2 mL extract + 2 mL $(CH_3CO)_2O$ + 2-3 drops conc. H_2SO_4	A Deep red coloration
Tannins	Ferric chloride test	2 mL extract + 2 mL H_2O + 2-3 drops $FeCl_3$ (5%)	A green precipitate
	Lead tetra acetic acid test	1 mL extract + 3 drops of lead sub acetate solution	A creamy gelatinous precipitate
Saponins	Foam test	20 mL water in measuring cylinder + 50 gm extract (vigorously shaken for 15 min.)	Formation of 2 cm thick layer of foam

Solution A: 34.66 gm copper sulphate + distilled water to make final volume 100 mL; Solution B: 173 gm potassium sodium tartarate + 50 gm NaOH + distilled water to make 100 mL.

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Whatman filter paper No. 1, and the filtrate solutions were subsequently air-dried in an incubator at 50-55℃. The parched extracts were kept in sterilized vials and stored at 4℃ until required [20].

5. Phytochemical screening of LCS extract

The allelochemicals in the LCS extract were analyzed using standard protocols [21, 22] as shown in Table 1.

6. Anthelmintic activity of LCS extract

Anthelmintic activity was assessed according to standard protocols [23]. Adult earthworms (*Eisenia fetida*) were chosen due to their accessibility and their morphological and physiological similarities to the human intestinal roundworm infection [24, 25]. The freshly prepared extract and standard drug solutions were formulated in different concentrations of 5, 25, 50, 75, and 100 mg/mL [26]. Freshly produced extracts and standard drug solutions were used. Albendazole was used as the standard reference solution, and distilled water was used as control. Earthworms were collected and categorized into 12 groups, each of which contained three (n = 3) earthworms. Different concentrations of the extract and the reference drug were added to Petri dishes containing earthworms [27]. Group I was treated with distilled water (control), group 2 with a 10% ethanol, and groups III-VII with albendazole at concentrations of 5, 25, 50, 75, and 100 mg/mL, respectively. Groups VIII-XII were treated with LCS extract at the same concentrations of 5, 25, 50, 75, and 100 mg/mL, respectively. The earthworms were meticulously monitored, and the time at which the worms were paralyzed and the time at which the worms died were documented. Paralysis was defined as the absence of any observable movement in earthworms, except when they were subjected to excessive shaking [23]. The death of the worms was confirmed by their immobility in 50℃ water and the lightening of their body hue.

7. Statistical analysis

The anthelmintic activity test was conducted in triplicate, and the findings are expressed as mean ± standard error of the mean. GraphPad Prism (version 10.1.2) was used for the statistical analyses. Two-way analysis of variance, followed by Tukey's post hoc test, was used for multiple comparisons. Statistical significance was determined at $p < 0.05$, with $p < 0.0001$ indicating a high significance level.

RESULTS

1. Qualitative phytochemical screening of LCS extract

LCS extract was found to contain alkaloids, anthraquinones, carbohydrates, flavonoids, proteins and amino acids, phenols, terpenoids, tannins, and glycosides in the preliminary phytochemical analysis (Table 2). The (+) symbol indicates the presence of phytochemicals, whereas the (–) symbol indicates their absence.

2. Anthelmintic activity of LCS extract

Fig. 1 depicts the anthelmintic efficacy of the ethanolic LCS extract on mature earthworms. The anthelmintic activity of LCS

Table 2. Results of the phytochemical analysis of *Litchi chinensis* seed ethanolic extract (LCSE)

Phytochemical tested	Reagent used	LCSE
Alkaloids	Mayer's test	+++
	Dragendroff's test	+++
	Hager's test	+++
Anthraquinones	Bontrager's test	+
Carbohydrates	Molisch's test	++
	Benedict's test	++
	Fehling's test	++
Flavonoid	Alkaline reagent test	+
	Shinoda's test	+
Glycosides	Keller Kiliani test	+
Phlobatannin	Hydrochloric acid test	–
Steroids	Salkowski test	–
Proteins and Amino Acids	Biuret test	+++
	Ninhydrin test	
Phenols	Ferric chloride test	+++
	Lead tetra acetic acid test	+++
Quinones	Alcoholic KOH test	–
Terpenoids	Horizon test	+++
Tannins	Ferric chloride test	+++
	Lead tetra acetic acid test	+++
Saponins	Foam test	–

+++, abundance amount; +, small amount; –, absent.

Figure 1. Comparative anthelmintic efficacy of *Litchi chinensis* seed ethanolic extract (LCSE) and Albendazole against earthworms (*Eisenia fetida*). Different letters indicate varying concentrations of both test samples. Group-I, is the control group (DW), group-II represents 10% ethanol, and group III, IV, V, VI, and VII represents Albendazole at increasing concentrations of 5, 25, 50, 75, and 100 mg/mL, respectively. Additionally, Group VIII, IX, X, XI, and XII denotes LCSE at corresponding concentrations of 5, 25, 50, 75, and 100 mg/mL.

extract and albendazole was proportional to their concentration, i.e., the time taken for paralysis and death decreased proportionally with increasing concentrations, as shown in Fig. 2 and 3, respectively. In the control group, distilled water showed no effect, whereas the 10% ethanol solvent induced paralysis and death at 7.2 ± 0.37 min and 9.4 ± 0.51 min, respectively. Albendazole showed significantly lower ($p < 0.001$) anthelmintic activity than LCS extract at lower concentrations. For instance, at 5 mg/mL, albendazole induced paralysis at 57 ± 2.31 min and death at 63.33 ± 2.31 min, whereas LCS extract induced paralysis at 47 ± 1.15 min and death at 50.33 ± 1.45 min, respectively, as shown in. However, at higher concentrations, both LCS extract and albendazole exhibited comparable activity, particularly at 100 mg/mL, at which albendazole caused paralysis at 15.0 ± 1.15 min and death at 17.67 ± 1.15 min, whereas LCS extract showed similar effects at 12.67 ± 0.33 min and 14.0 ± 0.58 min. The differences in the time of paralysis and death between the two treatments at higher doses were not statistically significant

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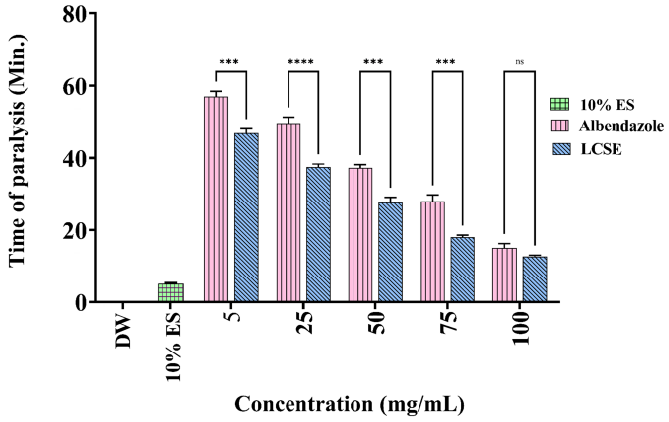

Figure 2. Time required for paralysis of earthworms after administration of *Litchi chinensis* seed ethanolic extract (LCSE). Data are reported as mean ± SEM (standard error mean), n = 3. ****p < 0.0001 (Albendazole vs. LCSE, Con. 5-100 mg/mL); ***p < 0.001 (Albendazole vs. LCSE, Con. 5-100 mg/mL). Two-way ANOVA with Tukey's multiple comparisons test.

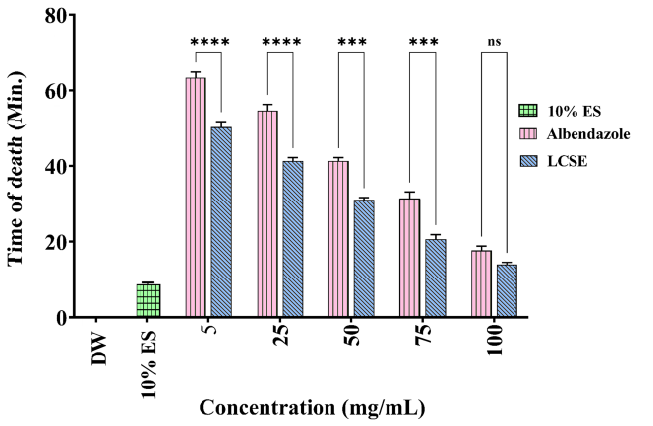

Figure 3. Time required for death of earthworms after administration of *Litchi chinensis* seed ethanolic extract (LCSE). Data are reported as mean ± SEM (standard error mean), n = 3. ****p < 0.0001 (Albendazole vs. LCSE, Con. 5-100 mg/mL); ***p < 0.001 (Albendazole vs. LCSE, Con. 5-100 mg/mL). Two-way ANOVA with Tukey's multiple comparisons test.

($p > 0.05$), indicating that LCS extract possesses comparable efficacy to the standard drug at elevated concentrations.

DISCUSSION

Helminthiasis is a global health concern because it is a chronic illness and reinforces cycles of poverty, particularly in vulnerable groups. Therefore, effective preventive and curative treatment strategies are critical. Albendazole is a wide-range antiparasitic drug used for treating parasitic infections [28]. Albendazole attaches to the β-subunit of tubulin and inhibits microtubule assembly in the parasitic cells. This leads to the disintegration of the parasite cytoskeleton, disruption of glucose transportation, and consequent depletion of glycogen stores. These effects eventually result in the immobilization and death of helminths [28, 29]. Albendazole has a 75% efficacy in eliminating mature parasitic worms and diminishing helminth larvae [30]. This study demonstrated the potent anthelmintic activity of LCS extract in comparison with the standard drug albendazole. LCS extract was significantly more potent ($p < 0.001$) than the standard medication albendazole at smaller concentrations (5-75 mg/mL). At 5 mg/mL, the LCS extract induced paralysis at 47.0 ± 1.15 min and death at 50.33 ± 1.45 min, whereas albendazole induced paralysis at 57.0 ± 2.31 min and death at 63.33 ± 2.31 min. However, at the highest concentration of 100 mg/mL, LCS extract caused paralysis at 12.67 ± 0.33 min and death at 14.0 ± 0.58 min, making its effects apparent slightly earlier than the effects of albendazole (paralysis: 15.0 ± 1.15 min and death: 17.67 ± 1.15 min) but not significantly ($p > 0.05$). The anthelmintic activity of both LCS extract and albendazole positively correlated with their concentration. Mumed et al. [31] reported that higher extract concentrations are associated with better anthelmintic activity, consistent with the present results. Numerous medicinal plants have been documented for their efficacy as deworming remedies due to the presence of various secondary metabolites [32]. Every bioactive compound possesses a distinct mode of anthelmintic action. Alkaloids exert anthelmintic effects by blocking acetylcholine receptors and inhibiting glucose absorption, resulting in helminth starvation and death [33]. Flavonoids suppress the nicotinamide adenine dinucleotide (NAD+)-catabolizing enzyme, disrupting calcium homeostasis and impairing worm motility [34]. Glycosides disrupt the transport of sodium and potassium ions and induce death in several nematode species [35]. Tannins impede the metabolic mechanisms of worms by forming complexes with parasite proteins or inhibiting crucial enzymes, thus reducing motility, food ingestion, and sexual reproduction [36]. Terpenes inhibit parasites' tyramine receptors, and alkaloids produce nitrated compounds and free sugars to impair host-gut interactions [37]. Polyphenolic compounds also have good anthelmintic activity [38]. The anthelmintic activity of phytochemicals is usually the result of synergistic effects of individual phytochemicals [39]. Preliminary phytochemical analysis of LCS extract (Table 2) revealed the presence of secondary metabolites

including alkaloids, anthraquinones, carbohydrates, flavonoids, glycosides, proteins and amino acids, phenols, terpenoids, and tannins. The presence of these components makes LCS extract a viable alternative to albendazole, especially in cases resistant to synthetic anthelmintics. This finding opens new avenues for the development of cost-effective and efficient anthelmintic drugs. The superior efficacy of LCS extract highlights its potential to contribute to the advancement of more accessible and potent treatments for parasitic infections. Further investigations are required to isolate and quantify the specific bioactive compounds in LCS extract to elucidate the relationship between its phytochemical content and anthelmintic effects. Such analyses will enhance the understanding of its mechanisms. Furthermore, comprehensive toxicity evaluations at different concentrations using human cell lines are vital to determine the safety profile of LCS extract, which is an important consideration before its therapeutic use.

CONCLUSION

The study demonstrated the anthelmintic activity of ethanolic LCS extract against *Eisenia fetida*, with this activity being directly proportional to its concentration. The isolation and quantification of bioactive constituents and evaluation of the safety profile of LCS extract using human cells will substantiate its therapeutic potential as a natural anthelmintic drug.

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AUTHORS' CONTRIBUTIONS

Conceptualization: Mustakin Ahmed Shohel, Md. Humayan Kabir, Ivvala Anand Shaker, Md. Abul Kashem Tang; Methodology: Mustakin Ahmed Shohel, Md. Humayan Kabir, Ivvala Anand Shaker, Md. Abul Kashem Tang; Validation: Md. Humayan Kabir, Nirmal Chandra Mahat, Sumaiya Siddika, Sheikh Arafat Rahman; Investigation: Mustakin Ahmed Shohel, Md. Humayan Kabir, Nirmal Chandra Mahat, Sumaiya Siddika, Sheikh Arafat Rahman, Md. Rakibul Hasan Rahat, Tarique Muhammad Tawabul Islam; Formal analysis: Md. Humayan Kabir; Data Curation: Sheikh Arafat Rahman, Sumaiya Siddika, Md. Rakibul Hasan Rahat, Tarique Muhammad Tawabul Islam; Writing – Original draft: Mustakin Ahmed Shohel, Md. Humayan Kabir; Writing – Review & Editing: Mustakin Ahmed Shohel, Md. Humayan Kabir, Ivvala Anand Shaker, Md. Abul Kashem Tang; Visualization: Md. Humayan Kabir; Supervision: Ivvala Anand Shaker, Md. Abul Kashem Tang.

ETHICAL APPROVAL

This research was approved by the Ethical Review Committee of the Faculty of Biological Sciences, Islamic University, Kushtia, Bangladesh (Ref. No: FBS/ERC/IU/2023/03, Date: 19.10.2023).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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