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Original Research Articles

Swordtail (*Xiphophorous helleri*) growth promoting activity and antibacterial property of pineapple (*Ananas comosus*) peel oil

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The use of phytogenics that are low-cost and highly available has the potential to address some environmental, social, and economic issues in fish culture. The present study was conducted to evaluate the effects of the dietary application of pineapple (*Ananas comosus*) peel oil (PPO) on the growth performance of Swordtail (*Xiphophorous helleri*) and the antibacterial effect against pathogenic bacteria. PPO was incorporated in the diet of Swordtail (initial average weight ± SD of 0.12±0.07g and average length± SD of1.15±0.25 cm) to analyze the effect on growth performance. Two triplicate groups (each tank stocked with 15 fish) were fed with control and experimental diet for 10 weeks. The results showed significantly enhanced growth performance of fish fed with an experimental diet. The average weight gain (282.0±35.0%), relative growth rate (2.82±0.35), specific growth rate (1.92±0.12 %day-1), and condition factor (0.42±0.02%) were all higher, and feed conversion ratio (0.0219 ±0.040) is lower in fish fed the experimental diet. The carbohydrate content (60.05±0.050 %) in the control feed was higher ($p \leqslant 0.05$), whereas moisture (11.35±0.11 %) and ash (13.77±0.03%) contents were higher ($p \leqslant 0.05$) in experimental feed. The disc-diffusion method was used to test the antibacterial activity of the crude PPO against *A. hydrophila* and *Pseudomonas* spp. The inhibition zones were 2.37±0.13 51 cm and 2.06±0.08 cm, respectively. Conclusively, the present study recommends using PPO, which has a potential antibacterial effect against bacterial pathogens, as a potential feed additive to improve the growth performance of swordtail.

INTRODUCTION

The ornamental fish industry is gaining importance in aquaculture as they have immense aesthetic and commercial demand in the export trade worldwide.[1] Due to the economic importance of ornamental fish, there is a growing interest in their artificial culture and propagation. In aquaculture, feed represents 50 – 80% of production cost, as proper and appropriate nutrition is one of the critical factors.[2] Improved feed conversion and growth can reduce feed costs.[1] Generally, feed additives are added during feed preparation to improve the feed quality, feed efficiency, health, and growth performance of the cultured fish.

Growth is generally considered a complicated process that is influenced by various metabolic processes, many of which are interconnected and which are in turn influenced by behavioral, physiological, dietary, and environmental factors. Culture species encompass behavioral and physiological elements, while management decisions primarily address nutritional and environmental factors. Nutritional, environmental, and physiological factors indirectly impact production performance by influencing feed efficiency and intake, whereas behavioral factors directly impact the rate at which food is consumed. According to Nasim Al Mahmud *et al*.,[3] fish feed holds significant importance within the value chain, necessitating rigorous oversight of raw material quality to ensure food safety. Moreover, providing efficient, high-quality feed varieties is essential to promote optimal growth across diverse fish species cultured under varying conditions. Aquaculture systems rely on a steady supply of low-cost, high nutritional-quality feeds. The feed must be nutritionally adequate and commercially viable for the fish culture system to function properly.

Many functional feed additives such as mycotoxin binders, organic acids, immune – -stimulants, probiotics,

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prebiotics, yeast products, enzymes, and phytogenics have been documented to increase culture production.[2] Among them, phytogenics stand out as a potential solution, capable of delivering essential nutrients to fish, thereby facilitating the production of high-quality and safe aquatic products. As a result, plant sources have provided significant breakthroughs over the past few years, especially in substituting with plant essential oils. Essential oils are a naturally occurring mixture of chemical compounds with a potent scent that aromatic plants produce during secondary metabolism.[4] Since essential oils often include hundreds of distinct components in varying proportions, they have the potential to emerge as a new generation of products for the nutrition and health of animals and substitutions for antibiotics and growth promoters in animal diets due to their positive response on digestion, diet acquisition, gut microbial circle, growth performance and welfare.[4]

The plant-based sources provide antibacterial, antiviral, and antifungal properties about antimicrobial properties. It is shown that the potent antimicrobial activity of plants could be attributed to many factors, such as limiting the growth of microorganisms by rendering the production of certain enzymes required for growth, preventing, and controlling infectious microbes in the culture system.

Bacteria are known to cause several fatalities, both in the wild and culture. Fish bacterial infections seriously threaten aquaculture and result in catastrophic financial loss. Among the bacteria commonly recovered from the culture systems, *Aeromonas* spp has long been identified as an important fish pathogen and associated with major disease outbreaks in cultured freshwater food fish and ornamental fish, resulting in high mortality. They cause various opportunistic infections in fresh and brackish water fish, collectively known as motile Aeromonas septicemia (MAS). Among aeromonads, the infections that *Aeromonas hydrophila* causes are considered the scourge of freshwater fish farming worldwide and have caused significant economic problems over the past decade. Further, *Pseudomonas* spp. has been identified as one of culture fish's most general infectious agents. It has been reported that *Pseudomonas* spp causes stress-related diseases in freshwater fish; especially under farming conditions, it can cause almost 100% of mortality. *Pseudomonas* sp has been identified as the etiological agent of red skin disease, affecting many freshwater fish species.

Antibiotics are widely used against opportunistic bacteria in culture systems. However, some international organizations such as the World Health Organization (WHO) and Food and Agriculture Organization (FAO) have introduced regulations to stop the excessive usage of antibiotics[5] since the widespread use of antibiotics in culture system results in antibiotic resistance, while antibiotic accumulation can be detrimental to animals, consumers, and the environment. Hence, plant-based oils have emerged as potential candidates to be recycled, reused, and brought back to the culture system by converting them to value-added products like fish feed and phytobiotics.

Pineapples (*A. comosus*) are edible tropical fruits rich in enzymes, antioxidants, and vitamins. They are considered one of the most economically important tropical fruit crops and belong to family Bromeliacea. The pineapple is also considered an exotic fruit due to its flavor and pleasant aroma. It contains water, sugars, vitamins A, C and beta-carotene, carbohydrates, protein, fat, fiber, ash, antioxidants, flavonoids, ascorbic, and citric acid. Due to the presence of such potential compounds, the incorporation of pineapple in various industries, including aquaculture, for various purposes has been increasing. According to Sriratanasart *et al*.,[6] pineapple juice is used as a hydrolyzing agent of soybean meal to replace the fishmeal in tilapia feed.

Pineapples are consumed or served cooked, fresh, juiced, and can be preserved. During consumption, the outer peel, crown, and central core are discarded as pineapple fruit waste. These account for about 50% of the total pineapple fruit weight. Among them, pineapple peels were reported as the waste generated during the canning process abundantly, which accounts for 30–42% (w/w) of the total pineapple-based waste.[7] Pineapple peel as a functional feed additive in aquaculture proves suitable. Incorporating pineapple peel extract into the basal diet of *Labeo rohita* fingerlings for aquafeed improved growth parameters, as demonstrated by Gopalraaj and Velayudhannair.[8]

The present study was designed to assess the efficacy of using pineapple peel oil as a phytogenic feed additive for the ornamental fish *X. helleri* and evaluate its effects on growth performance. This study also aimed to find more potent antibacterial agents against *A. hydrophila* and *Pseudomonas* spp. by exploring potentially beneficial active compounds from PPO. These compounds could potentially serve as a basis for synthesizing new drugs.

MATERIALS AND METHODS

COLLECTION OF PINEAPPLE PEEL

Nearly 12kg of pineapple peels were collected from local shops in Weweldeniya, Nittambuwa area in Sri Lanka. The peels, which were peeled using a sharp knife, were washed twice using tap water followed by distilled water. After removing any remaining flesh, they were sliced, sun-dried, and oven-dried at 80°C for 24 hours. The dried sample was grounded into a powder and stored at room temperature.

EXTRACTION OF PINEAPPLE PEEL OIL

PPO was obtained from the Soxhlet extraction method, followed by rotary evaporation as described by VE and Inengite.[9] For the extraction, 100g of prepared peel powder sample was soaked in 200 cm^3 n-hexane for 3 days. Following 3 days, the refluxing continued for 6 hours at 69 ±2 ºC. Following 6 hours of refluxing, the solution mixture was transferred for rotary evaporation at 69 ±2 ºC at 90 rpm for 30 minutes, and the obtained PPO was stored in the refrigerator until further usage.

CHEMICAL CHARACTERIZATION OF PINEAPPLE PEEL OIL

The chemical characterization of extracted PPO was carried out using GC-MS analysis. A weight of about 0.05 g of extracted oil was mixed with 4.00 ml of ethanol. Into that solution mixture, 200 µL of 2 M methanolic KOH was added and shacked vigorously for 30 seconds. Around 1 g of sodium dihydrogen orthophosphate was added and vortexed until cloudiness disappeared. Then, the upper layer was decanted, dried with anhydrous sodium sulfate, filtered to a GC glass vial using a 0.22 m syringe filter, and subjected to the GC-MS analysis. The GC temperature program was set up as mentioned by Teai *et al*.[10]

PREPARATION OF FISH FEED USING PINEAPPLE PEEL OIL

The fish diet was prepared following a modified method by Wagde *et al*.[1] The experimental feeds were prepared with basic ingredients, as shown in Table 3. While preparing the control feed, olive oil was used, and PPO was added instead of olive oil to the experimental feed. The dry ingredients were combined with water to prepare the dough. The dough was steamed for 30 minutes in a water bath. Oil (olive oil for the control diet and PPO for the experimental diet) was added after heating. After thorough mixing, the dough was pelleted and allowed to air dry. Both diets were stored in air-tight containers at room temperature. This was extruded through a pelletizer, air-dried, and stored in an airtight container at room temperature. PPO incorporated feed was produced using the same protocol, replacing olive oil with the same amount of extracted PPO.

DETERMINATION OF PROXIMATE COMPOSITION

The moisture content of the prepared feed was measured using a moisture analyzer at 105 ^{0}C for 20 minutes. Ash, protein, and lipid contents were analyzed using AOAC methods.[11] Ash content was determined by incineration in a muffle furnace at 550 ^{0}C for 24 hours. We employed the Kjeldahl method to analyze crude protein, involving acid digestion at 420 °C for 2 hours, followed by distillation and titration using a 0.02M standard HCl solution. The Crude lipids (CL) were analyzed using the Soxhlet extraction method with petroleum ether as a solvent. According to Romano *et al*.,[12] the carbohydrate content was determined by subtracting crude protein percentage, crude lipid percentage and ash percentage from 100%.

EXPERIMENTAL DESIGN, TANK MANAGEMENT, AND FISH STOCKING

Experimental fish were obtained from the local ornamental fish shop established in Dehiwala, Sri Lanka, and acclimated to lab conditions for one week. The fish were divided into six, 8 L aquaria with 15 fish per aquaria. The initial average weight was 0.118±0.07182g, and the initial average standard length was 1.1544±0.2532 cm. The fish were fed twice daily at a rate of 2% body weight for ten weeks. Feed intake was recorded for FCR. The fish were weighed every week, and the ration of the respective diets was readjusted accordingly. Standard lengths were recorded every 5 weeks. Mortality was monitored and recorded daily. Water conditions were monitored and maintained at appropriate levels.

DETERMINATION OF FISH GROWTH PERFORMANCE AND FEED UTILIZATION EFFICIENCY

At the end of the rearing period, the fish were starved for 24 hours, and three fish from each tank were randomly collected for further analysis.

The growth parameters were calculated by using the following formulas.

Absolute growth (ABG) = Final mean body weight (g)– Initial mean body weight (g)

Relative Growth Rate (RGR; %)

= Final Mean weight (g) – Initial mean weight(g) / Initial mean weight (g) × 100%Specific growth rate (SGR; %day^{-1})

= ln final body weight – ln initial body weight / Rearing period (days) × 100%

Feed conversion ratio (FCR) = Dry feed intake (g) / Weight gain (g)

Condition factor (CF; %) = Body weight (g) / Body length (cm) × 100%

Survival rate (%)

= Total number of fish at the end of the experiment - Total number of fish at the start of the experiment × 100%

Additionally, the randomly selected fish weights were measured and dissected, and the reproductive organs (Testis / Ovaries) and livers were separated. Their weight was measured using the following equations to calculate the gonado-somatic index (GSI) and hepatosomatic index (HSI)

GSI = Weight of reproductive organs (g) / Total body weight (g) × 100%

HSI= Weight of the liver (g) / Total body weight (g) × 100%

COLLECTION OF BACTERIAL FISH PATHOGENS

Bacterial strains of *Aeromonas hydrophila* and *Pseudomonas* spp were obtained from the Center for Aquatic Animal Disease Diagnosis and Research (CAADDR), Faculty of Veterinary Medicine and Animal Science, University of Peradeniya. The obtained bacterial strains were subcultured using the streak plate method using nutrient agar as medium and stored at -80 °C until used.

ANTIBACTERIAL PROPERTY

Preliminary screening for the antibacterial activity of PPO was carried out using the disc diffusion method. Initially, inocula suspensions were prepared by the broth culture method. After 48 hours of incubation, the bacterial suspensions were standardized using a spectrophotometer for 0.35 absorbance value at 595nm wavelength, and the concentration of bacterial suspensions was adjusted accordingly. From each prepared test bacterial suspension, 0.1 ml suspension was poured into three nutrient agar plates, and they were then thoroughly spread using a spreader. In each

Table 1. Important fatty acid compounds and their relative percentage in PPO

	Fatty Acid Composition			
	Common name	**Chemical Name**	**Symbol**	**Percentage (%)**
Saturated Fatty Acids	Palmitic acid	Hexadecanoic acid	(C16:0)	9.779
	Stearic acid	Octadecanoic acid	(C18:0)	3.123
	Arachidic acid	Eicosanoic acid	(C20:0)	2.266
Unsaturated Fatty Acids	Linoleic acid	(Z,Z)-9,12-octadecadienoic acid	(C18:2) (n-6)	6.128
	Oleic acid	(Z)-9-octadecenoic acid	(C18:1) (n-9)	13.463

plate, four sterilized discs were placed. From these four discs, two discs were impregnated with 10 µl of crude PPO. One well was impregnated among the remaining two wells with 10 µl of solvent (n-hexane). The remaining well had no plant extract or solvent as the negative control. The prepared agar plates were incubated overnight at 37^{o}C. The inhibition zone formed was observed on the plate on the next day. The inhibition zone diameter around each disc was measured and recorded accordingly. Subsequently, the average mean diameter of the inhibition zone was indicated for each bacterial test strain.

STATISTICAL ANALYSIS

All data are presented as mean ± standard error. Statistical analysis for growth performances and proximate analysis were performed for one-way ANOVA using Minitab version 21. All tests used a significance level of $p<0.05$. Before statistical analysis, the data were tested for normality and heterogeneity of variances, where necessary data transformation was done.

RESULTS

CHEMICAL COMPOSITION OF PINEAPPLE PEEL OIL

The important fatty acid compounds and their relative percentages in the extracted PPO are presented in **Table 1**. From GC analysis, five major fatty acid compounds were detected in PPO. The most abundant chemical constituent was oleic acid (13.463 %), followed by palmitic acid (9.779%) and linoleic acid (6.218), respectively. Other PPO constituents obtained from GC-MS are provided in **Table 2**, along with their relative percentage.

PROXIMATE COMPOSITION OF PREPARED FEEDS

Table 3 shows the results of proximate analysis of both prepared fish feeds. Both feeds formulated in this study have a proximate composition: the experimental feed has higher crude lipid, ash, and moisture contents than the control feed, whereas higher crude protein and carbohydrate values were reported in the control feed. The statistical analysis of proximate composition for both feeds showed no significant difference in crude lipid and crude protein content. However, the carbohydrate, ash, and moisture contents differed significantly between the fish feeds.

GROWTH PERFORMANCE

The results of the statistical growth performance analysis are shown in **Table 4**. According to statistical analysis, all other tested parameters except GSI and HSI differed significantly ($P < 0.05$) between both groups of fish reared.

ANTIBACTERIAL ACTIVITY OF PINEAPPLE PEEL OIL AGAINST TESTED BACTERIAL PATHOGENS

Results of the antibacterial activity of pineapple peel oil against tested pathogenic bacteria by the disc diffusion method are presented in **Table 5**. The pineapple peel oil produced a maximum zone of inhibition of 2.371±0.127cm against *A. hydrophila,* whereas 2.0571±0.0751 cm of inhibition zone was obtained against *Pseudomonas* sp.

DISCUSSION

The global trade of ornamental fish has naturally flourished as interest in aquarium fish grows. Despite the industry's economic importance, there is little information accessible on the nutritional requirements of ornamental fish. In this context, the present study represents the first attempt to determine the growth performance of swordtail fed with PPO as a supplement in its diets. The present study documented that the fish fed with PPO showed better growth performance than fish fed with the control diet after 10 weeks of feeding trials despite not having much difference between the proximate composition. This confirms that the fish had effectively utilized the dietary supplementation of PPO. The findings of our current study align with previous research conducted by Yuangsoi *et al.*,[13] who demonstrated that the aqueous extract of pineapple peel residues promoted the growth of Nile tilapia (*Oreochromis niloticus*). Similarly, Van Doan *et al.*[14] found that supplementing with pineapple peel improved the growth and feed efficiency of Nile tilapia, while Sukri *et al.*[15] reported that the inclusion of pineapple waste enhanced the growth rate of Nile tilapia. However, little information is available regarding the effect of different plant-based oil on fish growth. Still, the present study's finding agrees with the results of Mohamed *et al.*[16] in which the plant-based oils enhanced the growth and health performances of cultured species.

The GC-MS analysis result of the present study is in agreement with the findings of Morais *et al.*,[17] in which the extract of pineapple peel contained major fatty acids such

Table 2. Chemical compounds and their percentage in PPO

Compound	Percentage (%)
Methyl (7-hydroxy-1H-benzimidazol-2-yl) carbamate	0.804
1,2-Bis(trimethylsilyl)benzene	1.050
3,4-Dihydroxybenzyl alcohol tris(trimethylsilyi)	2.403
7-Chloro-4-methoxy-3-methylquinoline	0.810
Benzo(h)quinoline, 2,4-dimethyl	0.595
3-phenyl-2H-chromene	6.385
1,2-Benzisothiazol-3-amine	5.553
1,3,5-Triethyl-1-(ethylbutoxysiloxy)cyclotrisiloxane	1.761
Dibenzo(b,E)-1,4-diazabicyclo(2.2.2)octadiene	2.332
Ergost-7-en-3-ol, (3. beta.)	6.188
1b-tetrahydro-4H-benzo(a)quinolizine-3-yl), methyl ester	5.435
Stigmasta-4,22-dien-3. beta-ol	10.415
Beta-Sitosterol	14.929
Bis(trimethylsilyl)estradiol	6.492

Table 3. Formulation of Control and experimental Diets (Ingredients in g/100g) and the proximate composition mean (±SE) of both control and experiment feed in percentage (%)

Formulation of Diets			
Ingredients	Weight/ 100g		
	Control Feed	Experimental Feed	
Rice bran	33	33	
Fish meal	32	32	
Flour	32	32	
Cod-liver oil	1	1	
Olive oil	1	-	
PPO	-	1	
Vitamin E	0.5	0.5	
Salt	0.5	0.5	
Proximate Composition			
			P value
Crude Protein (%)	17.767±0.426	17.067±0.353	0.274
Crude Lipid (%)	8.997±0.468	10.531±0.448	0.077
Ash (%)	13.187±0.0808	13.771±0.0322	0.003*
Moisture (%)	10.947±0.0240	11.353±0.113	0.024*
Carbohydrate (%)	60.049±0.0504	58.5223±0.0836	0.05*

*Indicates significant differences ($P < 0.05$) (one-way ANOVA test)

as palmitoleic, oleic, linoleic, stearic, arachidic, behenic and lignoceric acids; among which, linoleic acid (18:2n-6) is known to be the most vital fatty acid required by species that have omnivorous feeding habits.[18] Moreover, in general, freshwater fish typically require either linoleic acid (18:2 n-6), linolenic acid (18:3 n-3), or both, as their essential fatty acid (EFA) requirements predominantly center around linolenic acid (18:3n-3). This is because they possess the ability to convert α-linolenic acid (ALA) into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[19] These fatty acids are crucial for maintaining fish health, as they contribute significantly to growth, survival, stress resistance, and disease immunity.

The feeding behavior of fish is complicated, yet acceptance or rejection of diet is regulated by the physiological process that relies on chemoreception inputs.[20] The unique aromatic flavor of plant-based oils makes it a potent appetizer, boosting voluntary feed intake.[21] Such oils affect fish's feeding behavior by influencing eating patterns, digestive fluid production, and feed intake.[22] Flavonoids are the secondary metabolites that are responsible for flavor and fragrance. Due to the presence of such compounds in

Table 4. The growth parameters of *X. helleri* reared in the control and experimental tanks and results are expressed as mean ± SE.

Parameters	Control tank	Experimental tank	P values
Relative Growth Rate	1.180 ±0.025	2.820 ±0.350	0.010*
Specific Growth Rate	1.098 ±0.014	1.922 ±0.115	0.002*
Feed Conversion Rate	0.03851 ±0.16	0.02187 ±0.04	0.001*
Survival rate (%)	86.67 ±0.000	91.11 ±2.22	0.114
Coefficient Factor (%)	0.3026 ± 0.015	0.4180 ± 0.018	0.008*
GSI (%)	3.89 ± 1.0	3.90 ± 1.2	0.994
HSI (%)	2.21 ± 0.6	2.26 ±0.7	0.958

*Indicates significant differences (P < 0.05) (one-way ANOVA test)

Table 5. The mean diameter (±SE) of the inhibition zone for two bacteria species tested

	A. hydrophila	*Pseudomonas* sp.
Inhibition Zone (cm)	2.371±0.127	2.0571±0.0751

extracted oil, the gustatory system of fish might have activated towards more food acquisition and ingestion, ultimately resulting in improved swordtail growth performances.

The antibacterial assay against *A. hydrophila* and *Pseudomonas* spp revealed that pineapple peel extract exhibited antibacterial properties. This finding aligns with previous studies, such as that by Zharfan *et al*.,[23] who documented the antimicrobial effectiveness of pineapple (*A. comosus* L. Merr) extract against multidrug-resistant *P. aeruginosa*. Additionally, Kabir *et al*.[24] reported the antimicrobial activities of ethanolic pineapple extracts against *P. aeruginosa*, while Mapanao *et al*.[25] demonstrated antibacterial activity of pineapple waste extracts, including peel, against *A. hydrophila*. Furthermore, Gunwantrao *et al*.[26] conducted studies indicating that pineapple peel extract displayed antimicrobial activity against various pathogenic microbes.

The antibacterial action mechanism of plant-derived oils is not fully understood. However, the mechanism of different plant-based oils acting against bacteria depends on their chemical composition.[27] Consequently, the effects of such oils may vary with the chemotype.[5] Essential compounds like fatty acids are suggested to have significant antimicrobial activities. Oleic, linoleic, and linolenic acids can inhibit the growth of bacteria as antimicrobial agents.[28] The antibacterial activities of the microalgal extracts are attributable to myristic, lauric, stearic, palmitic, oleic, linoleic, and linolenic acids.[29] Öntaş *et al*.[28] have stated that the Aragan (*Araganis spinosa*) oil contained oleic, linoleic, palmitic, and stearic acids and showed antibacterial effects against tested bacterial pathogens. The present study undoubtedly confirms the presence of those fatty acids that have antibacterial properties for potential applications.

The phenolic compounds present within plant oils are responsible for their antimicrobial effects[5]; mainly Beta-sitosterol has been recorded to have antibacterial activity with a comparable zone of inhibition to other standard antimicrobial agents.[30] The antibacterial action of phenolic compounds is mediated by their capacity to function as non-ionic surface-active agents, which eventually leads to disruption of the lipid-protein interface or denaturation of proteins and inactivating enzymes in pathogens. Phenols also affect membrane permeability, which may result in uncoupling of oxidative phosphorylation, suppression of active transport, and metabolite loss owing to membrane damage.[31] Behera *et al*.[32] documented that quinoline and its derivatives also possess antibacterial activity. In this study, the extracted oil has these compounds that might contribute to its antibacterial activity against tested pathogenic bacteria. Thereby, PPO can be used as an alternate antibiotic source. It can be used against antibiotic-resistant bacteria emerging in aquaculture systems as it has potential compounds that can be attributed to its antibacterial properties.

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AUTHORS' CONTRIBUTION

Data curation: Thanushanthahi Loganathan (Lead). Formal Analysis: Thanushanthahi Loganathan (Lead). Investigation: Thanushanthahi Loganathan (Lead). Methodology: Thanushanthahi Loganathan (Lead). Resources: Thanushanthahi Loganathan (Equal), Fathima Sumaiya Idroos (Equal). Software: Thanushanthahi Loganathan (Lead). Validation: Thanushanthahi Loganathan (Lead). Vi-

sualization: Thanushanthahi Loganathan (Lead). Writing – original draft: Thanushanthahi Loganathan (Lead). Writing – review & editing: Thanushanthahi Loganathan (Equal), Liyanage Gayani Yasodara (Equal), Pathmalal Manage (Equal), Fathima Sumaiya Idroos (Equal). Supervision: Liyanage Gayani Yasodara (Equal), Pathmalal Manage (Equal), Fathima Sumaiya Idroos (Equal). Conceptualization: Fathima Sumaiya Idroos (Lead). Funding acquisition: Fathima Sumaiya Idroos (Lead). Project administration: Fathima Sumaiya Idroos (Lead).

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The present study's data are available from the authors upon reasonable request.

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