



In vitro α -amylase, α -glucosidase and Lipase Inhibiting Activities of Duckweed (*Wolffia globosa*), the Possibility as Functional Food

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ABSTRACT

Background: Duckweed (*Wolffia globosa*) is a valuable source of protein, essential amino acids, dietary fiber, vitamin B12 and bioactive compounds, including antioxidants. It has potential as a functional food for managing metabolic syndrome, particularly by helping to control glycemic levels in individuals with diabetes through dietary interventions. This study aimed to determine the antioxidant properties and evaluate enzyme inhibitory activities of *W. globosa* extract (WE) against key enzymes involved in lipid and glucose metabolism.

Methods: Fresh *W. globosa* plants were dried, pulverized and extracted using ethanol by maceration. The total phenolic content (TPC) and total flavonoid content (TFC) of the extract were assessed using the Folin-Ciocalteu and aluminum chloride methods, respectively. Antioxidant activities were evaluated through several assays: DPPH radical scavenging, nitric oxide (NO) radical scavenging, ferrous iron-ferrozine complex and ferric iron-thiocyanate complex assays. *In vitro* enzyme inhibitory activities of WE were tested against pancreatic lipase, α -amylase and α -glucosidase.

Result: The extract contained phenolic compounds (TPC = 28.19 ± 0.79 mg GAE/g) but lacked detectable flavonoids. WE exhibited antioxidant activity in the DPPH and NO radical scavenging assays and inhibited lipid peroxidation (IC_{50} = 0.34 ± 0.05 , 0.23 ± 0.06 and 80.51 ± 16.02 mg/ml, respectively). However, the extract showed no metal ion chelation activity. WE strongly inhibited pancreatic lipase (IC_{50} = 1.55 ± 0.20 mg/ml) and α -amylase (IC_{50} = 0.02 ± 0.01 mg/ml), while it showed poor inhibition of α -glucosidase ($IC_{50} > 1,000$ mg/ml). These findings support the potential of WE as a functional food ingredient for managing metabolic conditions such as diabetes and hyperlipidemia.

Key words: Anti-diabetic enzymes, Anti-lipase, Functional food, Metabolic disease, *Wolffia globosa*.

INTRODUCTION

Metabolic syndrome is a cluster of disorders that increases the risk of cardiovascular disease and type 2 diabetes and it represents a growing public health concern. These conditions include obesity, insulin resistance, hypertension and dyslipidemia. Prevention strategies primarily focus on lifestyle modifications, including dietary changes and increased physical activity. Calorie-restricted diets that emphasize unsaturated fats, whole grains, fruits and vegetables (Castro-Barquero *et al.*, 2020) can effectively prevent and treat metabolic syndrome. Regular physical activity, especially aerobic exercise, helps manage conditions associated with metabolic syndrome, such as obesity and hypertension and helps preserve lean body mass during weight loss (Myers *et al.*, 2019). However, outcomes may vary due to individual responses and the need for personalized treatment strategies (Castro-Barquero *et al.*, 2020; Myers *et al.*, 2019).

Functional foods are increasingly used as dietary interventions for hyperlipidemia and hyperglycemia, as they regulate lipid and glucose metabolism through the inhibition of key enzymes, including pancreatic lipase, α -amylase and α -glucosidase and by modulating protein expression related to metabolic pathways. Recent studies have identified various natural and synthetic compounds with significant inhibitory effects on these enzymes. Functional foods contain high levels of bioactive components, such as fibers, phytosterols and antioxidants,

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which reduce oxidative stress and the risk of diabetes and metabolic syndrome-associated cardiovascular diseases (Permatasari *et al.*, 2024; Bintanah *et al.*, 2023; Permatasari *et al.*, 2023; Alkhatib *et al.*, 2021). Nevertheless, further research is required to understand the long-term effects and underlying mechanisms of individual functional foods.

Wolffia globosa, commonly known as duckweed, offers several health benefits due to its rich nutritional composition and bioactive compounds. This plant is a

valuable source of protein, essential amino acids, dietary fiber and antioxidants. In addition, it is one of the few plant-based sources of vitamin B12, which can improve serum B12 levels, especially in vegetarian and vegan diets (On-Nom *et al.*, 2023; Sela *et al.*, 2020; Monthakantirat *et al.*, 2022). The protein extract from *W. globosa* has exhibited antimicrobial and other health-promoting properties (Duangjarus *et al.*, 2022; Tipnee *et al.*, 2017). Furthermore, the rapid growth and high adaptability of *W. globosa* make it well suited for cultivation in aquaculture systems (Said *et al.*, 2022). Its protein content can reach up to 45.54% of dry weight, with consistently high yields under aquaculture conditions. These characteristics make *W. globosa* a promising raw material for nutraceutical and functional food development (Desfita *et al.*, 2025; Hu *et al.*, 2024; Bong-Hyun *et al.*, 2024).

Recently, nutritious snacks have been developed as functional food supplements, owing to their cost-effectiveness and consumer acceptability (Sharun *et al.*, 2024; Min *et al.*, 2024). Thus, *W. globosa* has potential as a functional food for the management of metabolic syndrome, particularly in controlling glycemic status through dietary intervention. In a randomized controlled trial, a shake containing *W. globosa* resulted in significantly lower postprandial glucose peaks and a faster return to baseline levels compared with a traditional yogurt shake, indicating improved glycemic responses in abdominally obese individuals (Zelicha *et al.*, 2019). The high protein and fiber content of *W. globosa* is associated with reduced fat absorption and improved satiety, which may contribute to weight management in metabolic conditions (On-Nom *et al.*, 2023; Zelicha *et al.*, 2019).

Despite these promising findings, the long-term effects and molecular mechanisms of action of *W. globosa* remain insufficiently explored. In particular, reports on its role in regulating lipid and glucose metabolism through the inhibition of key digestive enzymes are limited. Therefore, the present study aimed to evaluate the antioxidant properties of *W. globosa* using various antioxidant assays and to investigate its inhibitory effects on key metabolic enzymes involved in lipid and glucose metabolism, including pancreatic lipase, α -amylase and α -glucosidase.

MATERIALS AND METHODS

Plant collection and extraction

Five hundred grams of fresh duckweed (*Wolffia globosa*) were obtained from the Golden Banana Community Enterprise, Sang Khom District, Udon Thani Province, Thailand. Plant identification was performed based on morphological characteristics and known geographical distribution. The whole plants were shade-dried at 50°C and pulverized into a fine powder. Ten grams of the dried powder were macerated in 1 L of 95% (v/v) ethanol (RCI Labscan, Thailand) with sonication at room temperature for 72 h. The extraction was performed in triplicate (n = 3). The pooled *W. globosa* extract (WE) was concentrated to

constant weight using a rotary vacuum evaporator, followed by a water bath and stored at 4°C until further analysis.

Total phenolic content

Total phenolic content (TPC) of WE was determined using the Folin-Ciocalteu (FC) method and results were expressed as gallic acid equivalents (GAE). Briefly, 20 μ L of WE (5 mg/mL, dissolved in dimethyl sulfoxide, DMSO; RCI Labscan, Thailand) was mixed sequentially with 100 μ L of diluted FC reagent (1:10, v/v) and 80 μ L of 7.5% (w/v) Na_2CO_3 (RCI Labscan, Thailand) in distilled water. The mixture was incubated in the dark at room temperature for 30 min and absorbance was measured at 765 nm using a microplate reader. TPC was calculated from a gallic acid standard curve ($y = 77.442x + 0.0031$, $R^2 = 0.9998$) and expressed as mg GAE/g WE. All measurements were conducted in triplicate (n = 3) (Hussein *et al.*, 2010).

Total flavonoid content

Total flavonoid content (TFC) was determined using the aluminum chloride (AlCl_3) colorimetric method, with quercetin as the reference standard. Fifty microliters of WE (2.0 mg/mL in ethanol) or standard solution was added to a 96-well microplate, followed by AlCl_3 (Loba Chemie, India), ethanol and sodium acetate solution in a ratio of 10:96:10 (v/v/v). The mixture was incubated in the dark at room temperature for 40 min and absorbance was measured at 415 nm. A quercetin standard curve ($y = 3.5717x + 0.0644$, $R^2 = 0.9955$) was used for quantification. Results were expressed as mg quercetin equivalent (QE)/g WE, based on triplicate measurements (n = 3) (Biju *et al.*, 2014).

Antioxidant assays

WE was dissolved in absolute ethanol to obtain concentrations of 0.001, 0.01, 0.1, 1.0 and 10 mg/mL. Antioxidant activity was evaluated using four established assays: DPPH radical scavenging, nitric oxide (NO) radical scavenging, ferrous ion-ferrozine metal chelation and ferric thiocyanate lipid peroxidation inhibition assays. These assays measured DPPH radical reduction, NO radical reduction using Griess reagent (Sigma-Aldrich, USA), metal ion chelation capacity and inhibition of lipid peroxidation, respectively. Absorbance was recorded at assay-specific maximum wavelengths (λ_{max}) using a microtiter plate reader (BIO-RAD, USA). All assays were performed in triplicate (n = 3). Antioxidant activity was expressed as IC_{50} values (mg/mL), calculated from dose-response curves. Vitamin C (ascorbic acid; Sigma-Aldrich, USA), vitamin E (α -tocopherol; Sigma-Aldrich, Germany) and EDTA (Sigma-Aldrich, USA) were used as positive controls, as appropriate (Yen and Duh, 1994; Manosroi *et al.*, 2012; Aktas *et al.*, 2013).

Assays of metabolic enzymatic inhibition

Inhibition of pancreatic lipase

WE was dissolved in 10% (v/v) DMSO to obtain concentrations ranging from 0.001 to 10 mg/mL. Orlistat

(0.0005-5 mg/mL in 10% DMSO) served as the positive control. Lipase inhibitory activity was determined by measuring the release of p-nitrophenol at 415 nm, following enzymatic hydrolysis of p-nitrophenyl butyrate (Sigma-Aldrich, USA) by pancreatic lipase (Sigma, USA). All measurements were performed in triplicate ($n = 3$) (Bustanji *et al.*, 2010).

Inhibition of α -glucosidase

α -Glucosidase inhibitory activity was assessed by monitoring the release of p-nitrophenol from p-nitrophenyl- α -D-glucopyranose (Sigma, Switzerland). WE (0.001-10 mg/mL in 10% DMSO) was tested, while acarbose (0.0005-5 mg/mL in 30 mM phosphate buffer, pH 6.5) was used as the positive control. Absorbance was measured at 415 nm and assays were conducted in triplicate ($n = 3$) (Alam *et al.*, 2017).

Inhibition of α -amylase

This assay was determined the reducing sugar, the product cleaved from starch, catalyzed by α -amylase present in WE or control. Each concentration of WE dissolved in 10% DMSO (0.001 to 10.0 mg/ml) and starch in phosphate buffer (pH 6.9) was mixed. The reaction was started with pancreatic α -amylase (Fluka, Germany) and incubated at 37°C. The reaction mixture was prepared with dinitrosalicylic acid (DNSA) solution (1% w/v DNSA, 30% w/v sodium potassium tartarate and 20%v/v 2N NaOH in distilled water) and kept in boiling water for stopping reaction. The mixture was diluted with distilled water and absorbance was measured at 540 nm by spectrophotometer (T80, Oasis Scientific, USA). Positive control was acarbose (Sigma-Aldrich, Germany) dissolved in 30 mM phosphate buffer, pH 6.5 (0.00025, 0.0025, 0.0025, 0.025, 0.25, 2.5 mg/ml) (Keerthana *et al.*, 2013). The calibration curve between the inhibition of enzyme and WE concentration was compared with calibration curve between the inhibition of enzyme and positive control. The results are represented as IC_{50} .

Data analysis

Dose-response curves were constructed for WE and positive controls and IC_{50} values were calculated by linear regression analysis. Results are presented as mean $\pm$ standard deviation (SD). Comparisons were based on

calibration curves generated from enzyme inhibition percentages versus sample concentration.

RESULTS AND DISCUSSION

The *W. globosa* extract (WE) was semi-solid and brownish colored and the yield of extraction was about 5-10%. The WE contained only phenolic compounds (TPC = 28.19 ± 0.79 mg GAE/g), whereas no detectable flavonoids were observed. DPPH and NO radical scavenging activity was observed and lipid peroxidation was inhibited (IC_{50} = 0.34 ± 0.05 , 0.23 ± 0.06 and 80.51 ± 16.02 mg/ml, respectively).

Therefore, this extract was unable to chelate metal ions (Table 1). WE strongly inhibited pancreatic lipase (IC_{50} = 1.55 ± 0.20 mg/ml) and α -amylase (IC_{50} = 0.02 ± 0.01 mg/ml), while it poorly inhibited α -glucosidase (IC_{50} > 1,000 mg/ml) (Table 2). The weak α -glucosidase inhibition may be due to the selective enzyme specificity and low levels of flavonoids, which are typically effective α -glucosidase inhibitors.

The TPC and TFC were calculated and applied from standard curves of gallic acid and quercetin, respectively. The relationship between antioxidant activities and sample concentration is represented as graphs with linear formulae and coefficients of determination, R^2 (Fig 1). The relationship of inhibition of key metabolic enzymes, including lipase, α -glucosidase and α -amylase and sample concentration was also represented as graphs with linear formulae and coefficients of determination, R^2 (Fig 2).

The nutritional composition of *W. globosa* contains approximately 45.54% protein in dry weight, which is higher than many traditional foods, along with high dietary fiber content and essential amino acids. This duckweed species is a valuable food source with potential applications in human nutrition and aquaculture. It has been successfully integrated into snack products, enhancing their nutritional value and sensory appeal (Appenroth *et al.*, 2018; Said *et al.*, 2022; On-Nom *et al.*, 2023). High-protein diets have been found to improve glucose tolerance and lipid profiles in mouse models by reduction of fatty acid synthesis and inflammatory markers in the liver and colon. In addition, high protein and dietary fiber diet significantly improved glycemic control and insulin resistance in diabetic mice that demonstrated protective effect against diabetes-related

Table 1: The phenolic and flavonoid contents and antioxidant activities of *W. globosa* extract (WE)*.

Sample/ assay (Units)	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH (mg/ml)	NO (mg/ml)	MC (mg/ml)	LPI (mg/ml)
WE	28.19 ± 0.79	ND	0.34 ± 0.05	0.23 ± 0.06	ND	80.51 ± 16.02
Vitamin C	-	-	0.01 ± 0.00	0.04 ± 0.01	-	-
Vitamin E	-	-	-	-	-	0.01 ± 0.00
EDTA	-	-	-	-	0.04 ± 0.00	-

*Antioxidant activities are represented as IC_{50} (mg/ml). TPC= Total phenolic content, TFC= Total flavonoid content, DPPH= 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, NO= Nitric oxide radical scavenging activity, MC= Metal chelating activity, LPI= Lipid peroxidation inhibition activity, GAE= Gallic acid equivalent, QE= Quercetin equivalent, EDTA= Ethylenediaminetetraacetic acid, ND= Not detectable.

metabolic syndromes (Ni *et al.*, 2022; Xu *et al.*, 2024). High-fiber diets have been linked to improved insulin sensitivity and satiety, which is beneficial for the management of glucolipid metabolic disorders. The metabolites from high fiber diets can also alleviate neurodegenerative symptoms in obese individuals with diabetes (Dahiya *et al.*, 2017; Luo *et al.*, 2023). Moreover, high-protein and fiber-based supplements have led to weight loss and improved metabolic markers in clinical trial (Glynn *et al.*, 2022).

Table 2: *In vitro* inhibition of metabolic key metabolic enzymes of *W. globosa* extract (WE)*.

Sample /assay (Units)	Lipase inhibition (mg/ml)	α -glucosidase inhibition* (mg/ml)	α -amylase inhibition* (mg/ml)
WE	1.55 $\pm$ 0.20	>1,000	0.02 $\pm$ 0.01
Orlistat	0.10 $\pm$ 0.01	-	-
Acarbose	-	0.12 $\pm$ 0.03	0.007 $\pm$ 0.0002

*Enzymatic inhibitions were represented as IC₅₀ (mg/ml).

W. globosa exhibits significant antioxidant activity due to the richness of bioactive compounds. Previous studies have indicated that various extraction methods, such as boiling, freeze-thawing and mechanical crushing, yield varying antioxidant activities. The boiling method had resulted in the highest total phenolic content, which may be a candidate for nutraceutical applications (Yadav *et al.*, 2024). Therefore, the ethanolic extract has shown significant DPPH inhibition (75.77%) and contained the notable levels of carotenoids, flavonoids and phenolic compounds (Monthakantirat *et al.*, 2022). In our study, we were extracted *W. globosa* by the simple macerated with ethanol as WE. Compared with the previous study, the extract had a lower content of phenolic compounds and lacked flavonoids. The finding indicated the importance of the extraction methods and conditions on the yield of bioactive compounds. However, this extract exhibited preferable antioxidant activities, including DPPH and NO radical scavenging activity and the inhibition of lipid peroxidation. It was implied that the antioxidant activities of

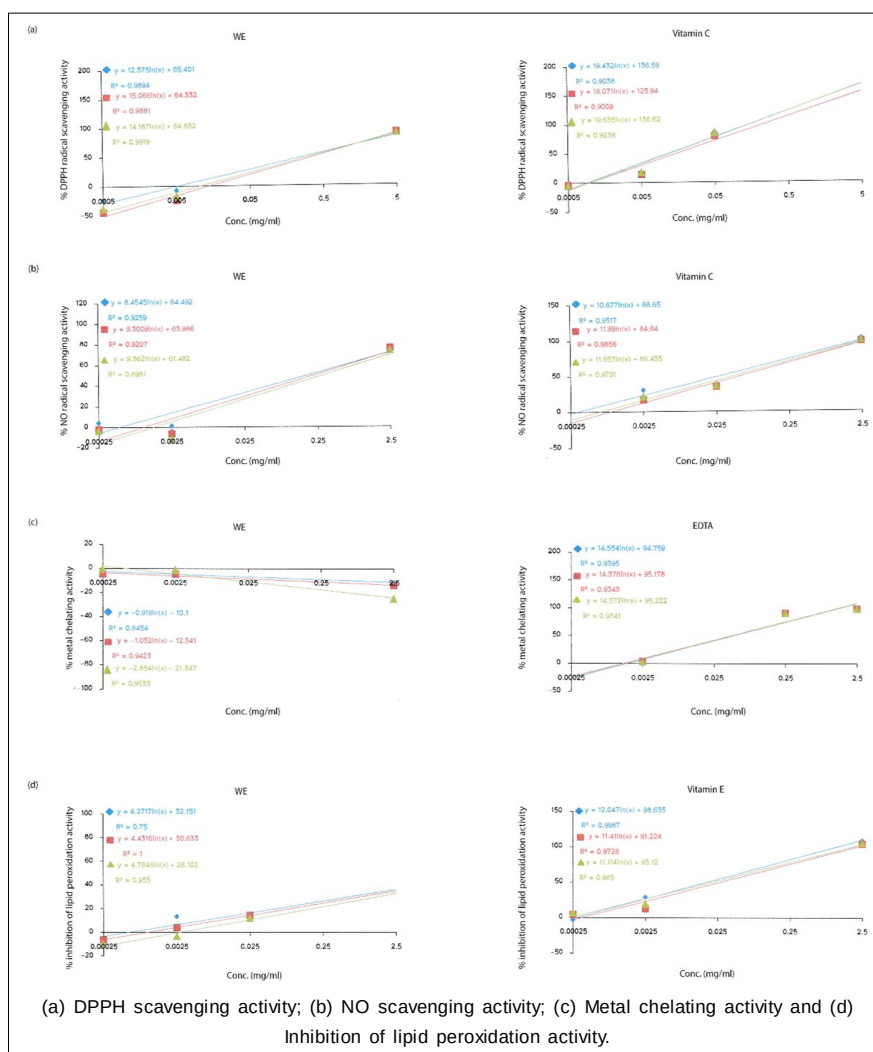


Fig 1: The relation of antioxidant activities and sample concentration.

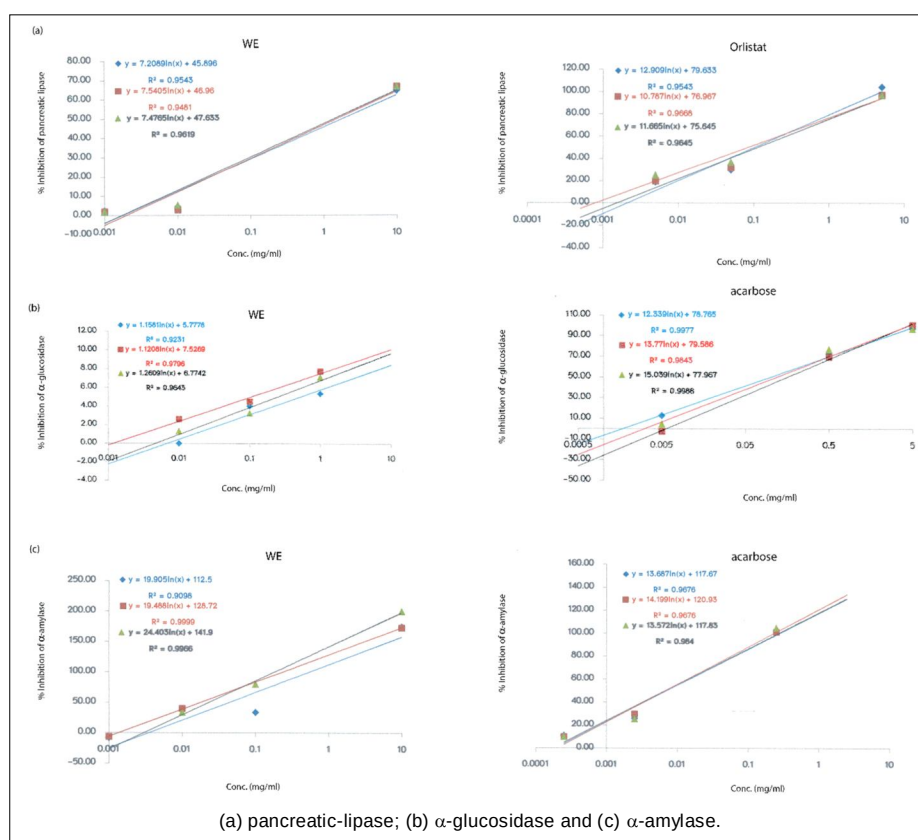


Fig 2: The relation of inhibition of key metabolic enzymes and sample concentration.

WE were independent of levels of bioactive compounds. In addition, these antioxidant activities may relate to other bioactive compounds such as, phytosterols rather than phenolics and flavonoids in some antioxidant assays.

The *W. globosa* plants contain high levels of antioxidants, such as phenolic compounds, which may support metabolic health by modulating key metabolic enzymes. The extracts of *W. globosa* have demonstrated significant radical scavenging activity, which has indicated the potential of protective effects against oxidative stress and the association of glucose and lipid metabolism (Tipnee *et al.*, 2017; Monthakantirat *et al.*, 2022). As our results, WE strongly inhibited pancreatic lipase and α -amylase, while there was poorly inhibited α -glucosidase. The finding corresponded to the previous study, which had reported that *W. globosa* Mankai had significantly reduced postprandial glucose peaks compared to traditional dairy shakes (Zelicha *et al.*, 2019). In addition, *W. globosa* extract has demonstrated significant radical scavenging activity, indicating potential protective effects against oxidative stress related to lipid metabolism. There were contained phytosterols, such as β -sitosterol and stigmasterol, which have shown anti-inflammatory effects that may indirectly influence lipase activity and improve insulin sensitivity (Tipnee *et al.*, 2017; Monthakantirat *et al.*, 2022). This study supported that *W. globosa* extract can become the main ingredient of functional food, had potential for the

management of metabolic conditions, such as anti-diabetic and anti-lipemic properties. Further study is necessary to conduct clinical trial of *W. globosa* snack products within overweight or obese people who have metabolic conditions.

CONCLUSION

Duckweed (*Wolffia globosa*) has emerged as a promising functional food ingredient for the management of metabolic disorders. Its value is attributed to its dense nutritional composition, characterized by high protein and dietary fiber levels, along with a diverse array of bioactive compounds, including phenolics, flavonoids and phytosterol. Our study supported that *W. globosa* ethanolic extract exhibited DPPH and NO radical scavenging activity, inhibition of lipid peroxidation and inhibition of key metabolic enzymes, including pancreatic lipase and α -amylase.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Use of artificial intelligence

Not applicable.

Declarations

Authors declare that all works are original and this manuscript has not been published in any other journal.

Conflict of interest

Authors declare that they have no conflict of interest.

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