Research Article

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Phytochemical profile, *in vitro* antioxidant, and anti-protein denaturation activities of *Curcuma longa* L. rhizome and leaves

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Abstract: *Curcuma longa* L. is a famous spice cultivated in many countries with significant variations reported in its phytochemical contents and biological potential. For the first time, the present work is aimed to identify the major phytochemicals present in methanol:chloroform (MC) and petroleum ether (PE) extracts of *Curcuma longa* rhizome and leaves (by determining polyphenols and GC/MS

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analysis), and their in-vitro antioxidant and anti-protein denaturation potential. Results showed that the highest value (P < 0.05) of polyphenolic content was in MC extract of rhizome (51.46 \pm 0.46 mg GAE/g) followed by 31.20 \pm 0.53 mg GAE/g in MC leaves extract. The strong antiradical activity was evaluated in MC extract of rhizome with IC50 value of $92 \pm 0.02 \,\mu\text{g/mL}$. MC extracts of both the rhizome and leaves exerted a potent inhibitory effect against protein denaturation with IC_{50} values of 106.21 \pm 0.53 and $108.06 \pm 4.67 \,\mu\text{g/mL}$ (P > 0.5), respectively. GC/MS analysis showed that α -tumerone was the main component in the rhizome oil (32.44%), whereas in the leaf oil, palmitic acid was the prominent constituent (28.33%) and α -phellandrene recorded a comparable percentage (7.29). In conclusion, C. longa is a valuable source of natural antioxidants and anti-inflammatory constituents, as indicated by its high polyphenolic content and by its considerable in vitro antiradical and anti-protein denaturation potential.

Keywords: *Curcuma longa*, leaves, rhizome, phytochemicals, biological activities.

1 Introduction

Curcuma longa L. (C. longa) is a famous spice of the ginger family (Zingiberaceae) with the tuberous rhizome widely used as food additives, cosmetic materials, and to cure many worldwide ailments [1]. Many previous and recent studies recorded numerous therapeutic benefits of C. longa rhizome when it is used as fresh or dry material, extracts, and pure components [2]. C. longa rhizome pharmacological activities are frequently imputed to its major active molecules especially those concentrated in its yellow-orange dye (curcumin) which has antioxidant [3], antimicrobial [4], anti-inflammatory [5,6], anticancer [7,8], and wound healing [9] properties.

Despite that the utilization of *C. longa* is limited to its rhizomes, some countries such as Malaysia and India also use its leaves as a spice. Also, it was reported that C. longa leaves have good contents of macronutrients (proteins, carbohydrates, and fiber) and considerable levels of minerals (Na, Ca, K, Mg, P, and Mn), therefore leaves are recommended as food additives in Brazil [10]. Furthermore, recent studies indicated that *C. longa* leaves contain active components which can prevent premature aging and oxidative stress [10-12], relieve skin inflammation [13], and act as antitumor agents [14]. With these significant medicinal benefits of C. longa leaves, the whole aerial part of C. longa including leaves is still neglected and considered as waste material and discarded post rhizome harvesting. There are limited reports on C. longa leaves compared to that carried out on rhizomes. Some previous reports studied the chemical composition of leaves and rhizomes of C. longa from different regions like Reunion Island in France [15], the lower Himalayan region [16], Plains of Northern India [17], southwest Nigeria [18], and south of Brazil [19]. Their results showed significant variations in the phytochemical contents of C. longa cultivated in different countries, and these differences were reflected in its quality and biological aspects. It is well-known that ecological conditions and geographical divergence are important factors directly affecting the plant growth, synthesis, and storage of plant products. It is worth noticing that there is no report on the chemical composition and biological activities of C. longa grown in Sudan. Therefore, the present work was designed to investigate phytochemicals, in vitro antioxidants against DPPH free radical activity, and in vitro anti-protein denaturation activity of C. longa rhizome and leaves cultivated in Sudan.

2 Materials and methods

2.1 Plant materials and preparation of extracts

Fresh leaves and rhizomes of *Curcuma longa* were collected from seven months old *C. longa* cultivated in the botanical garden, Al Neelain University, Khartoum, Sudan. The samples were cleaned, dried at room temperature, and powdered for extraction. Ten grams of the dried powder of *C. longa* rhizome and leaves were soaked separately in 200 mL of petroleum ether (PE) and methanol:chloroform (MC) (1:1, v/v) for 72 h, after which the

extracts were filtered and left for evaporation. All processes were conducted at room temperature.

2.2 Determination of total polyphenol content (TPC)

The TPC was determined by adopting the method described by Wolfe et al. [20]. TPC was expressed as Gallic acid equivalents (GAE, mg/g dry weight). The extract (1 mg/mL) was taken in a 10 mL glass tube and made up to a volume of 3 mL with ethanol of 0.5 mL, Folin–ciocalteau reagent (1:1 with water), and 4 mL of sodium carbonate (7.5%) added sequentially in each tube. The test solution was kept in dark for 30 min, cooled, and absorbance was measured at 765 nm. TPC was expressed as GAE mg/g dry weight using the following equation based on the calibration curve: Y = 0.0076x - 0.0785, R = 0.999.

2.3 Gas chromatography/mass spectrometry (GC/MS) analysis

PE extracts of rhizome and leaves were analyzed by GC/MS (Model GC-MS-QP2010 Plus, Shimadzu, Japan). Separation was performed using Rtx-5MS capillary column (5% of diphenyl-95% of dimethylsilicone, $30~\mathrm{m}~\times~0.25~\mathrm{mm}~\times~0.25~\mathrm{m}$) and a temperature program of 50°C (1 min) ramped to 300°C (3 min) at 5°C/min. Identification of compounds was based on the comparison of the mass spectra with the GC/MS system data bank (NIST 08 library), published data, and retention indices. The relative amount of each compound was expressed as the percent peak area relative to the total peak area of the GC chromatogram.

2.4 Determination of antiradical activity

Anti-DPPH free radical scavenging activity was determined using the method described by Mensor et al. [21] with a modification. Extracts were prepared separately to get test solution of 1 mg/mL. Series of extract solutions of different concentrations (100–1,000 μ g/mL) were prepared by diluting with methanol. Assays were performed in 96-well microtiter plates. 140 mL of 0.6 \times 10⁻⁶ mol/L DPPH were added to each well containing 70 mL of sample.

The mixture was shaken gently and left to stand for 30 min in dark at room temperature. The absorbance was

measured spectrophotometrically at 517 nm using a microtiter plate reader (Synergy HT Biotek, logiciel GEN5). Blank was done in the same way using methanol and sample without DPPH and control was done in the same way but using DPPH and methanol without sample. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%)

=
$$100 - [Abs_{sample} - Abs_{blank}]/(Abs_{control}) \times 100$$
,

where Abs_{sample} is the absorbance of DPPH radical + sample; Abs_{blank} is the absorbance of sample + methanol; and Abs_{control} is the absorbance of DPPH radical + methanol.

The IC_{50} value was calculated from the linear regression plots of concentration of the test sample against the mean percentage of the antioxidant activity.

2.5 In vitro anti-protein denaturation activity

In vitro anti-inflammatory activity was assessed by determination of inhibition of albumin denaturation as described by Shallangwa et al. [22]. PE and MC extracts of C. longa rhizome and leaves were dissolved in 0.2 mL of dimethylsulphoxide (DMSO) and diluted with 2.6 mL of phosphate buffer (0.2 M, pH 7.4). The mixture was made up to 5 mL with 0.2 mL of egg albumin (from fresh hen's egg) and 2 mL of varying concentrations (100, 200, 400, 600, 800, and 1,000 µg/mL) of each extract. The reaction mixtures were incubated at 37°C for 15 min. Then, the denaturation was induced by keeping the mixture at 60°C in the water bath for 10 min. After cooling at room temperature, the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium at the same concentrations as that of the extract was used as a reference drug. The inhibition percentage of albumin denaturation was calculated by the following equation and results were recorded as IC₅₀ values:

% Inhibition =
$$100 \times [A_{\text{sample}}/A_{\text{control}}-1]$$
,

where A_{sample} = absorbance of sample or standard and A_{control} = absorbance of negative control (DMSO).

2.6 Statistical analysis

All experiments (except GC/MS) were performed in triplicate and the obtained results were expressed as mean values \pm standard deviation. One-way ANOVA was performed for determining significant differences between the four extracts and their antiradical activity.

Table 1: Total polyphenolic contents in MC and PE extracts of rhizome and leaves of *C. longa*

Extract	TPC mg GAE/g mean value \pm SD			
MC rhizome	51.46 ± 0.46^{a}			
MC leaves	31.20 ± 0.53^{b}			
PE rhizome	13.70 ± 0.15^{c}			
PE leaves	14.53 ± 0.34^{c}			

Mean values followed by a common letter are not significantly different by the Duncan-test at the 95% level of significance.

3 Results

3.1 TPC

The TPC values were expressed as mg GAE/g. Results are presented in Table 1. The highest TPC was significantly (P < 0.05) amounted to MC extract of rhizome 51.46 \pm 0.46 mg GAE/g followed by 31.20 \pm 0.53 mg GAE/g in MC extract of leaves. PE extracts of rhizome and leaves had a small amount of TPC which was 13.7 \pm 0.15 mg GAE/g in PE extract of rhizome and 14.53 \pm 0.34 mg GAE/g in PE extract of leaves.

3.2 DPPH free radical scavenging activity

The anti-DPPH free radical scavenging activity of the MC and PE extracts of C. longa rhizome and leaves was determined and compared with standard Propyl Gallate. The inhibition percentage of the highest concentration used (1 mg/mL) against DPPH free radical is depicted in Figure 1 and IC₅₀ values are calculated (Table 2). MC extract of rhizome revealed the highest antiradical activity with

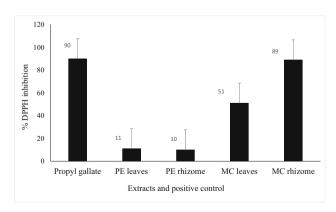


Figure 1: Inhibition percentage against DPPH free radical of MC and PE extracts of *C. longa* rhizome and leaves and standard propyl gallate at 1 mg/mL.

Table 2: IC₅₀ values of *in vitro* antioxidant and anti-protein denaturation activities of *C. longa* rhizome and leaves extracts

Extract	Inhibition of DPPH activity IC ₅₀ ± SD µg/mL	Inhibition of protein denaturation $IC_{50} \pm SD$ $\mu g/mL$		
MC rhizome	92.15 ± 0.02 ^b	106.21 ± 0.53 ^b		
MC leaves	436.21 ± 0.30^{c}	108.06 ± 4.67^{b}		
PE rhizome	NA	212.52 ± 2.22 ^c		
PE leaves	NA	246.42 ± 3.83^{d}		
Positive control*	77.02 ± 0.01^{a}	53.18 ± 0.29^a		

^{*}Propyl gallate (as anti-DPPH standard) and diclofenac sodium (as anti-protein denaturation reference drug); Mean values followed by a common letter are not significantly different by the Duncan-test at the 95% level of significance, lower value of IC₅₀ means higher activity. NA; not active.

DPPH inhibition percentage of 89 \pm 0.01% and IC $_{50}$ value of 92 \pm 0.02 µg/mL which were comparable to that obtained from the positive control (% DPPH inhibition of 90 \pm 0.01 with IC $_{50}$ value of 77 \pm 0.01 µg/mL), followed by MC leaves extract with inhibition percentage of 51.7 \pm 0.00% and IC $_{50}$ value of 436 \pm 0.30 µg/mL, whereas PE extracts of rhizome and leaves showed weak antioxidant activity with inhibition percentage of 10 \pm 0.02 and 11 \pm 0.03%, respectively.

3.3 In vitro protein denaturation inhibition

In vitro anti-inflammatory activity of rhizome and leaf extracts of *C. longa* and Diclofenac Sodium (reference drug) was evaluated using protein denaturation assay. The results are shown in Table 2. MC extracts of both the rhizome and leaves showed good inhibitory effect against protein denaturation with IC₅₀ values of 106.21 \pm 0.53 and 108.06 \pm 4.67 μg/mL, respectively, (P > 0.5) compared to that recorded by reference drug (IC₅₀ value of 53.18 \pm 0.29 μg/mL). PE rhizome and leaves extracts revealed weak protein denaturation inhibitory effect with IC₅₀ values of 212.52 \pm 2.22 and 246.42 \pm 3.83 μg/mL, respectively.

3.4 GC/MS analysis of *C. longa* rhizome and leaves oils

PE extracts of both rhizome and leaves showed a lack in their polyphenol content by total phenol determination test. So it raised our attention to determine the main bioactive components responsible of their evaluated activities. GC/MS profiles of rhizome and leaves oil were determined, and their results are presented in Table 3. GC/MS chromatogram of *C. longa* rhizome oil revealed the presence of 45 identified components comprising 99.54% of the total oil. The oil was dominated by the presence of oxygenated sesquiterpene (56.76%) followed by alcohols (13.16%), amides (12.3%), sesquiterpenes (10.86%), and oxygenated monoterpenes (2.29%). α -tumerone was the major component found in highest concentration (32.44%), followed by curlone (13.76%), 2-amino-3-phenyl propanamide (12.3%), 3-(1-methyl-2-phenylethoxy)-2-butanol (10.65%), tumerone (7.09%), α -Curcumene (4.09%), and β -curcumene (1.43%).

Twenty-four components were characterized from the leaf oil. The oil was dominated by a high percentage of fatty acids (57.55%) followed by alcohol (16.26%), oxygenated monoterpenes (10.04%), and triterpenoids (7.18%). Palmitic acid (28.33%) was the prominent component in leaves oil, followed by octadecenoic acid (9.62%), *trans*phytol (8.36%), stearic acid (7.39%), α-phellandrene (7.29%), squalene (7.18%), linoleic acid (5.06%) and other components were found in small concentrations (Figures 2 and 3).

4 Discussion

Natural products especially those derived from plants represent the safest, effective, and alternative source for chemical drugs. With the high demand for plant products, scientists' interest tends to detect these products in each part of the plant rather than the main part used. In the current study, the chemical profile, *in vitro* antiradical scavenging activity, and anti-protein denaturation effect of PE and MC extracts of *C. longa* leaves and rhizomes were investigated.

The result of the chemical analysis showed that the highest TPC (51.46 \pm 0.46 mg GAE/g) was determined in MC rhizome extract. This TPC is higher by 6.9-fold than what was reported in the ethanolic extract 80% of *C. longa* rhizome (7.45 mg GAE/g) [23], and by 75-fold than what was detected in the juice extract (0.68 mg GAE/g) of *C. longa* rhizome [24]. In contrast, Choi [25] results showed higher values of TPC, 228.7 \pm 2.3 mg GAE/g in ethyl acetate and 140.7 \pm 10.6 mg GAE/g in chloroform extracts of *C. longa* rhizome from Korea. Also, *C. longa* leaves in the present work had good levels (31.20 \pm 0.53 mg GAE/g) of TPC in their MC extract, which was 2-fold more than that measured in *C. longa* leaves from Malaysia 15.33 mg GAE/g [11], and higher by 7.57-fold than that determined in fresh

9-ol,syn-

Curlone

Myristic acid

cis-Nuciferol

41

42

43

13.47

13.58

13.62

Table 3. Continued

No.	RT	Compound name	Area (%)		No.	RT	Compound name	Area (%)	
			Rhizome	Leaves				Rhizome	Leaves
1	3.359	Hexanoic acid		0.37	44	13.89	Di(decahydro-1-naphthyl)	0.48	
2	3.615	Neoheptanol		0.9			methane		
3	4.650	2-Ethylhexanol		2.2	45	13.96	Cyclopentanecarboxylic	0.94	
4	4.76	Eucalyptol	0.09	1.47			acid,3-isopropylidene-bornyl		
5	6.326	3-Oxabicyclo[4.3.0]non-8-en- 2-one, <i>cis-</i>		1.47	46	14.17	ester 3-(1-Methyl-2-phenylethoxy)-	10.65	
6	6.905	α-Phellandrene		7.29			2-butanol		
7	6.989	L-α-Terpineol		1.28	47	14.27	Spiro[bicyclo[3.3.0]octan-	1.53	
8	8.12	O-Cymene	0.02	1,20			6-one-3-cyclopropane]		
9	9.55	4-Methy-4-pheny-2-	0.48		48	14.53	2-Amino-3-	12.3	
	7.33	pentanone	01.10				phenylpropanamide		
10	9.84	Zingiberene	0.05		49	14.68	2-Decylfuran		4.8
11	9.89	<i>P</i> -Toluene, <i>N</i> -methyl-	0.18		50	14.87	6,10,14-Trimethylpentadecan-		1.93
		N-nitroso-					2-one		
12	10.15	Caryophyllene	0. 10		51	14.96	Octadecenal	2.6	
13	10.26	α-Bergamotene	0.03		52	15.67	Palmitic acid		28.33
14	10.43	<i>p</i> -Cymene	0.38		53	15.67	Hexadecanoic acid	0.3	
15	10.45	β-Farnesene		0.99	54	16.53	FW 306 4,8,13-		1.69
16	10.62	D-Guaiene	0.03				Cyclotetradecatriene-1,3-diol,		
17	10.70	Acoradiene	0.06				1,5,9-trimethyl-12-(1-		
18	10.84	α-Curcumene	4.09				methylethyl)-		
19	10.99	β-Curcumene	1.43		55	17.32	Linoleic acid	0.28	5.06
20	11.16	β-Bisabolene	0.61		56	17.37	Octadecenoic acid		9.62
21	11.27	Dodecanoic acid		0.85	57	17.37	Oleic acid	0.13	
22	11.36	β-Sesquiphellandrene	2.8		58	17.40	Linolenic acid	0.07	
23	11.42	α-Bergamotenol	0.05		59	17.50	<i>Trans</i> -phytol		8.36
24	11.46	α-Bisabolene	0.09		60	17.58	Stearic acid	0.1	7.39
25	11.73	cis-Sesquisabinene hydrate	0.19		61	19.35	Arachidic acid	0.04	1.28
26	11.78	Caryophyllene oxide	0.15		62	19.65	Δ -Octadecalactone		0.94
27	11.80	Nerolidol		0.59	63	20.26	Antioxidant 2246		2.29
28	11.85	2,5,9-Trimethylcycloundeca-	0.11		64	20.97	Docosanoic acid		1.95
		4,8-dienone			65	23.21	Squalene		7.18
29	12.04	<i>p</i> -Menthane,2,3-dibromo- 8-phenyl-	8.0		_		Total	99.54	99.99
30	12.17	<i>cis-</i> Myrtanol	0.19				of each compound were expres		
31	12.21	1,4-Cadinadiene	0.46		area	relative	to the total peak area of the GC	cnromatogi	ram.
32	12.35	1-(3-Cyclopentylpropyl)-2,4- dimethylbenzene	1.11		C. le	onga lea	aves from Brazil 4.12 \pm 5.72 m	g GAE/g [1	0]. These
33	12.44	α-Bisabolol	0.56			_	in TPC may be due to the diff		
34	12.59	10-(Tetrahydro-pyran-2-	0.6				-		
		yloxy)-tricyclo[4.2.1.1(2,5)] dec-7-en-9-one					conditions and cultivation fr se differences were detected a		
35	12.70	α-Bergamotol	0.66		in T	PC of s	trawberry and potato peel cu	ltivated in	different
36	12.74	4,2,8-Ethanylylidene-2 <i>H</i> -	0.34		Eur	opean r	egions [26].		
		1-benzopyron,octahydro-				_	analysis of the oil of rhiz	ome reve	aled that
		2-methyl-			tha		s characterized with high p		
37	12.89	Isolongifolol	0.41					•	
38	13.06	α-Tumerone	32.44		oxygenated sesquiterpene and α -tumerone represented				
39	13.10	Tumerone	7.09		32.44% of the total oil. Previously α -tumerone was reported				
40	13.33	Bicyclo[3.3.1]non-2-en-	0.38		as the major compound of rhizome oil found in higher				her levels
		0 1 .				0/ [4/]	25 22/ [42] 1 1 1	24 /0/ 54	E1 42 CC/

13.67

0.6

1.76

e revealed that ent (56.76%) of ne represented ne was reported as the major compound of rhizome oil found in higher levels 44.1% [16], 35.9% [18], or in lower levels, 21.4% [15], 12.9% [17], and 8.52% [27] compared to that obtained in our study. GC/MS chromatogram of the leaves oil showed the absence of the main constituents detected in rhizome oil, instead,

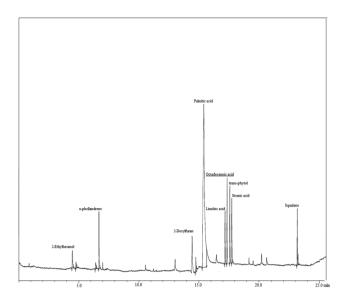


Figure 2: GC-MS profile for PE extract of Curcuma longa leaves.

leaves oil was rich in fatty acids (57.55%), especially unsaturated fatty acids, palmitic acid represented 28.33% of the total leaves oil content. Also, α -phellandrene, which was previously described as a prominent component of the oil of *C. longa* leaves was found in a comparable amount (7.28%) to that recorded earlier as 2.8% [15], 9.1% [17], and 41.99% [19] of *C. longa* leaves essential oil.

Regarding antioxidant activity results, a strong DPPH scavenging activity (IC₅₀ = 92 \pm 0.02 μ g/mL) was evaluated in MC extract of the rhizome. This result was significantly higher than that reported recently through the evaluation of the antioxidant activity of four *Curcuma* species from Thailand and their isolated components [28]. They revealed that *C. longa* crude extract had an anti-DPPH activity with IC₅₀ = 134.9 \pm 1.5 μ g/mL. Moreover, curcumin, the main pigment in *C. longa*, was evaluated in their study which had the strongest antioxidant activity with IC₅₀ = 68.9 \pm 0.6 μ g/mL compared to the other constituents estimated. However, it was much lower in activity in comparison to the ethanolic extract activity of *C. longa* rhizome with IC₅₀ value of 27.2 \pm 1.1 μ g/mL [29].

MC extract of leaves displayed moderate antioxidant activity with a DPPH inhibition percentage of 51.7%. This result is comparable to that reported in aqueous extract of *C. longa* leaves from Korea with an inhibition percentage of 51.10% against DPPH activity [30].

The *in vitro* anti-inflammatory activity results showed that MC extracts of both rhizome and leaves recorded potent *in vitro* anti-inflammatory activity by suppressing albumin denaturation with IC₅₀ = 106.21 \pm 0.53 and IC₅₀ = 108.06 \pm 4.67 µg/mg (P > 0.5), respectively.

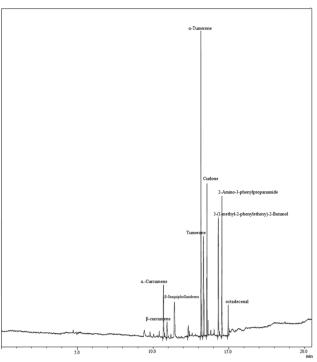


Figure 3: GC-MS profile for PE extract of Curcuma longa rhizome.

Protein denaturation is a process that happens when proteins lose their structure and biological function due to inflammation and could be induced in vitro by factors such as heat, stress, or some chemical compounds. Therefore, the denaturation of tissue proteins is recognized as a marker of inflammation [31]. It is worthwhile that searching for natural agents can prevent protein denaturation to develop a new anti-inflammatory drug. C. longa anti-inflammatory activity is well-documented. Many studies have been proven the potent anti-inflammatory properties of C. longa rhizome and its main constituent curcumin [28,32-34]. Also, the antiinflammatory activity of C. longa leaves is recently reported, as the inhibitory effect of their essential oil on skin inflammation [13]. Many studies ascribed the pharmacological activities of C. longa to its polyphenol molecule contents, especially curcumin as a primary polyphenol [35]. In our study, high positive correlations were observed between anti-DPPH and TPC (r = 0.99), but also between TPC and anti-protein denaturation activity (r = 0.86). This indicated that *C. longa* phenolic molecules are the main contributor to antioxidant and anti-inflammatory activity in this plant.

5 Conclusion

Based on the findings obtained from this work, *C. longa* could be suggested as a promising source of natural

antioxidants and anti-inflammatory, as indicated by its strong antiradical scavenging activity of rhizome MC extract, potent anti-protein denaturation activity of MC rhizome and leaves extracts, and by its high content of polyphenols especially via MC extraction. Also GC/MS analysis showed considerable contents of the major components in the rhizome and leaf oils compared to that cultivated in other countries.

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The authors confirm that the data supporting the findings of this study are available within the article.

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