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Original Article

EVALUATION OF THE ANTI-TYROSINASE-ANTI-AGING POTENTIAL AND METABOLITE PROFILING FROM THE BIOACTIVE FRACTION OF CORN COB (*ZEA MAYS* L.)

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ABSTRACT

Objective: Maize (*Zea mays* L.) is a crop that has been widely cultivated in Indonesia. Using corn kernels on a large scale will produce much corn cob waste, usually unused. According to the literature search, corn cobs' phytochemical studies and pharmacological activities still need to be improved. This study aims to determine the content of secondary metabolites (metabolite profiling) and their antityrosinase and anti-aging potential.

Methods: Corn cobs were macerated with methanol and fractionated with n-hexane, ethyl acetate, and butanol. The phytochemical profiling approach of the methanol extract was performed by liquid chromatography-mass spectra (LC-MS/MS). Anti-tyrosinase and anti-aging bioactivity were evaluated by thin layer chromatography (TLC)-bioautography and IC_{50} spectrophotometrically.

Results: The evaluation results show that the butanol fraction leads to a potential value (IC_{50} 99.92 µg/ml). Several compounds, especially flavonoid compounds (including catechin; kaempferol 3-arabinofuranoside 7-rhamnoside; 6,8-Di-C-beta-D-arabino pyranosyl apigenin; 5,7-Dihydroxy-8,4'-dimethoxyisoflavone) were identified by LC-MS/MS by comparing the molecular mass of MS/MS data with literature data.

Conclusion: Based on this study, it can be concluded that butanol is the fraction that most actively inhibits tyrosinase, elastase, and collagenase enzymes, which means it potentially becomes a new anti-aging candidate.

Keywords: Corn cob, Anti-tyrosinase, Anti-aging, LC-MS/MS, TLC-bioautography

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INTRODUCTION

In Indonesia, maize (*Zea mays* L.) production has reached 29.02 tons in 2020 nationwide [1]. This large-scale utilization of corn kernels as a food source produces abundant corn cob waste (fig. 1). Corn cobs are corn waste with low economic value and are usually only used as cattle feed. Corn cobs have considerable interesting chemical components. In general, identification of the chemical content of corn cob extracts that have been studied by several researchers previously showed that corn cob extracts positively contain flavonoids, polyphenols, saponins, tannins, and steroids [2]. Previous studies have also reported some activities from corn cobs as an antioxidant [3], anti-ultraviolet-B [3], singlet oxygen quenching [4].

Fig. 1: Corn cobs of *Zea mays* L.

Tyrosinase enzymes have a role in the biosynthesis of melanin, a pigment that is a photoprotective factor against skin damage by ultraviolet (UV) radiation [5]. However, increasing synthesis and accumulation of melanin causes skin damage such as melasma, sunburn, and skin cancer [5]. Inhibition of tyrosinase enzyme is one of the mechanisms to prevent melanin accumulation in the skin. Polyphenolic compounds have been reported to have tyrosinase inhibitory activity by inhibiting the oxidation reaction of L-tyrosine or L-DOPA catalyzed by tyrosinase [6-8]. Several flavonoid derivatives found in plants have been revealed to be potent tyrosinase inhibitors [9-11]. Dewi *et al.* 2021 reported that the ethyl acetate fraction of corn cob has antityrosinase activity with an IC50 value of 185.76 µg/ml (kojic acid standard IC50 150.79) [12]. This indicates that corn cobs containing polyphenols and flavonoids have tyrosinase inhibitory activity.

Elastase is a matrix-degrading enzyme found in the skin involved in elastin's degradation. Activation of the elastase enzyme is mediated by ROS or UV exposure, which causes skin wrinkles due to the enzyme attacking all connective tissue matrix proteins, including elastin and collagen [13]. Anti-aging cosmetic products with anti-wrinkle effects are associated with an increase in anti-elastase activity, where this activity reduces elastin degradation to maintain skin elasticity [14]. Several studies have investigated the interaction between elastase and its inhibitors, such as catechin [13], phenolic [14], and flavonoids [14].

Hyaluronic acid (HA) is one of the extracellular matrixes (ECM) constituents widely distributed in the skin. HA is involved in cell proliferation and differentiation, wound healing, inflammation, cell migration, and tissue turnover [15]. HA is highly hydrating and acts as an extracellular reservoir to store a large amount of water [16]. HA is degraded by the enzyme hyaluronidase, causing reduced viscosity of body fluids and increased permeability of connective tissues. Hyaluronidase inhibitors are involved in the balance between anabolism and catabolism of HA [16]. Hyaluronidase inhibitors have different chemical forms, such as polyphenols, flavonoids, antioxidants, polysaccharides, fatty acids, proteins, and glycosaminoglycans [16]. According to previous studies, polyphenols and flavonoids act as contributing factors eliciting hyaluronidase inhibition [17].

In line with ongoing research on Sumatran medicinal plants and the

search for alternative medicines [18-20], corn cobs are reported to have the potential for anti-tyrosinase and antiaging (through inhibition of elastase and hyaluronidase enzymes). The evaluation method is based on *in vitro* inhibiting enzyme action (tyrosinase, elastase, and hyaluronidase). In addition, the study of secondary metabolism profiles is of interest using liquid chromatography-mass spectra (LC-MS/MS) approaches. Thus, the economic worth of corn cobs that have been discarded will rise as a result of this research.

MATERIALS AND METHODS

Corn cob material

The material used is Paragon F1 hybrid sweet corn, which was sourced from the corn fields of local corn farmers in the Tikalak hamlet, Nagari Tanjung Baringin, sub-district of Lubuk Sikaping, Pasaman Regency, West Sumatera (fig. 1).

Preparation of corn cob extract and fraction

Corn cobs were sorted, washed, chopped, dried in the oven at 60 °C for two days, and then pulverized. Corn cob powder was macerated using methanol for three days. The macerate was filtered and evaporated with a rotary evaporator at 55 °C. Furthermore, the concentrated corn cob extract was dissolved in aquadest and partitioned with hexane, ethyl acetate, n-butanol, and residue (water).

LC-MS/MS analysis of corn cob extract

The LC-MS/MS test was conducted using High-Performance Liquid Chromatography (HPLC) and mass spectrometry. This test employed a C18 column at a temperature of 50 °C (column) and 25 °C (room). The LC analysis used a mobile phase consisting of water+0.05% formic acid (A) and acetonitrile+0.05% formic acid (B), with a flow rate of 0.2 ml/min running for 23 min and an injection volume of 5 µl. Mass spectrometry (MS) analysis was performed using electrospray ionization (ESI) in positive mode with a scanning range of 50-1200 m/z and desolvation temperatures set at 100 and 350 °C, respectively. Masslynx software version 4.1 was used for data analysis.

Anti-tyrosinase profiling by TLC-bioautography assay

Five microliters of extract with a concentration of 5000 µg/ml were photographed on a TLC plate. Subsequently, the plates were eluted with toluene: ethyl acetate: formic acid (70:25:5). The TLC plate was dried after elution and then dipped in tyrosinase enzyme solution (500 mU/ml) and immediately dipped in 1 mM L-Tyrosine solution. Tyrosinase enzyme inhibitor activity is observed from the white/clear color on the spot stain with a brown background [21].

Extract inhibitory concentration 50% (IC50) value determination

Tyrosinase inhibition

The tyrosinase inhibition assay method used in this study refers to the method developed by Momtez *et al.*, with some modifications [22]. Each fraction was prepared with 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.9063, and 1.9531 µg/ml concentrations. For comparison, kojic acid was used. Fifty microliters of each sample solution with a certain concentration were added 20 µl of tyrosinase (250 Units/ml in phosphate buffer pH 6.5), then increase the volume to 100 µl by adding 30 µl of 50 mM phosphate buffer pH 6.5, then incubated at room temperature for 5 min, after that add 100 µl of 5.07 mM L-DOPA substrate for each hole. The microplate was incubated for 20 min at room temperature. Readings were taken at a wavelength of 492 nm. The percentage inhibition of tyrosinase was calculated as follows:

$$\%\text{Inhibition} = 1\text{-}B/A \times 100\%$$

A is the absorbance change of blank solution-blank control, and B is the absorbance change of test solution-test solution control. The IC_{50} value (inhibitor concentration that produces 50% inhibition of enzyme activity) is measured by linear regression analysis between the concentration of the sample and the %inhibition of enzyme activity with the equation y=a+bx. The IC_{50} value is obtained from the x value after replacing y=50.

Elastase inhibition

The method of elastase inhibition test used in this study refers to the method developed by Moon *et al.*, with some modifications [23]. The elastase activity of corn cob fractions was examined using N-Suc-(Ala)3-nitroanilide as substrate. Thirty microliters of sample were put into a 96-well microplate. Then 200 mmol Tris-HCl buffer (pH 8.0) was added, followed by 30 ul of elastase enzyme solution (0.038 Units/ml). The plate was incubated for 20 min at room temperature. Next, 40 µl of substrate (1 mmol in buffer) was added and incubated for 20 min. The absorbance was measured at 410 nm wavelength. The percentage inhibition of elastase was calculated as follows:

$$\%\text{Inhibition} = 1\text{-}B/A \times 100\%$$

The A is the change in absorbance of blank solution-blank control, and B is the change in absorbance of test solution-test solution control. Critical IC_{50} value can be determined by linear regression analysis between the concentration of the sample and the % inhibition of enzyme activity with the equation y=a+bx.

Hyaluronidase inhibition

The hyaluronidase inhibitory activity of corn cob fractions was measured as Sahasrabudhe *et al.* (2010) previously described with few modifications. Thirty microliters of sample were put into a 96-well microplate. Then, 50 µl phosphate buffer (pH 7) was added, followed by 50 µul hyaluronidase enzyme solution (0.038 Units/ml). The plate was incubated for 10 min at room temperature. Then, 50 µl of substrate hyaluronic acid (0.3 mg/ml in acetate buffer) was added and incubated for 45 min [24].

The percentage inhibition of hyaluronidase was calculated as follows:

$$\%\text{Inhibition} = 1\text{-}B/A \times 100\%$$

Where A represents the change in absorbance of the blank solution-blank control, and B is the change in absorbance of test solution-test solution control. The value of IC_{50} is determined by linear regression analysis between concentrations of samples and % inhibition of enzyme activity under the equation y=a+bx.

RESULTS

Sample examination

The yield of extract and fractions of corn cob of *Zea mays* L. was found to be 2.003%, 0.1989%, 0.3551%, 0.4125% (w/w) for methanol extract, *n*-hexane, ethyl acetate, butanol fraction respectively (table 1).

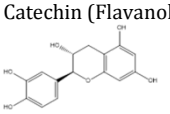
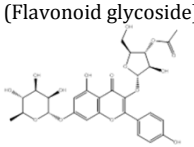
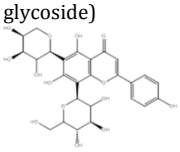
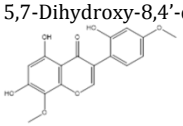
LC-MS/MS analysis of corn cob extract

LC-MS/MS analysis identified 13 phytochemicals, including four flavonoids (table 2).

Table 1: Yield of corn cob extract

Extract	Extracted simplicia weight (g)	Extract Weight (g)	Yield (%)
methanol extract	5000	100.015	2.003
Fraction		**Fraction weight**	**Yield (%)**
n-hexane fraction		9.948	0.1989
Ethyl acetate fraction		17.756	0.3551
Butanol fraction		20.628	0.4125

Table 2: Compounds identified by LC-MS/MS in extract methanol from corn cob

Known compounds				
Rt (min)	**[M+H]⁺, *m/z***	**Formula**	**Matched metabolite**	**References**
3.96	291.0865	$C_{15}H_{14}O_6$	Catechin (Flavanol) 	[25, 26]
4.466	565.1551	$C_{26}H_{28}O_{14}$	Kaempferol 3-arabinofuranoside 7-rhamnoside (Flavonoid glycoside) 	[27]
4.726	535.1450	$C_{25}H_{26}O_{13}$	6,8-Di-C-beta-D-arabino pyranosyl apigenin (Flavonoid-C-glycoside) 	[27]
7.911	331.0800	$C_{17}H_{14}O_7$	5,7-Dihydroxy-8,4'-dimethoxy isoflavone (Isoflavone) 	[26]
Unknown compounds				
Rt (min)	**[M+H]⁺, *m/z***	**Formula**	**Matched metabolite**	**References**
5.58	207.1017	$C_{12}H_{14}O_3$	Unknown	-
6.77	441.2009	$C_{18}H_{32}O_{12}$	Unknown	-
9.02	541.1717	$C_{28}H_{28}O_{11}$	Unknown	-
11.58	328.1544	$C_{16}H_{23}O_7$	Unknown	-
12.09	295.2270	$C_{18}H_{30}O_3$	Unknown	-
12.57	520.3407	$C_{30}H_{47}O_7$	Unknown	-
13.18	496.3399	$C_{28}H_{47}O_7$	Unknown	-
16.77	427.3569	$C_{29}H_{46}O_2$	Unknown	-

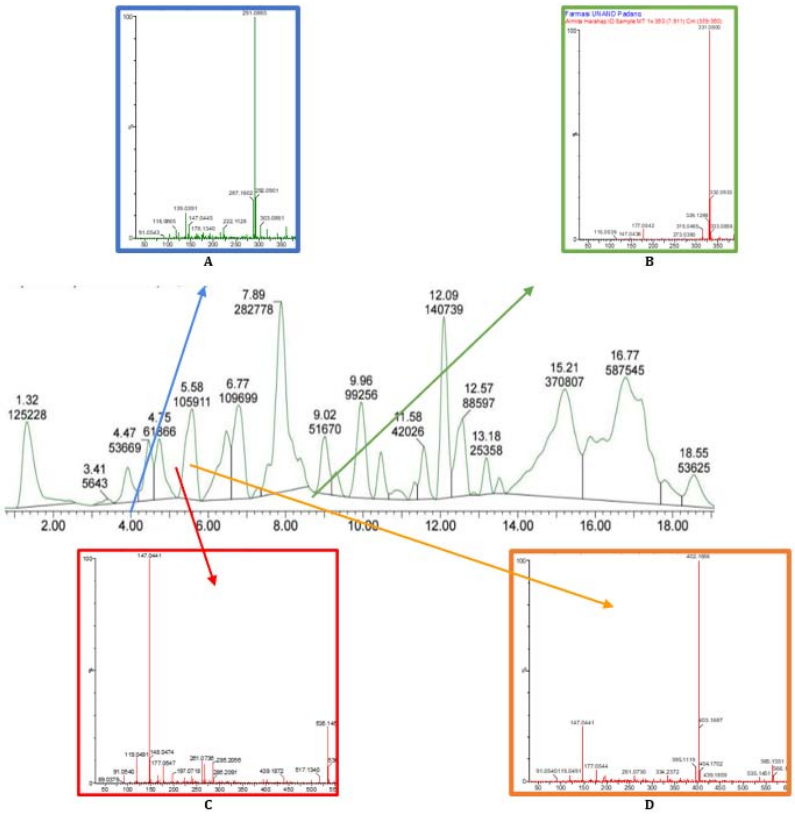


Fig. 2: LC chromatogram and MS/MS corn cob methanol extract spectrum. Catechin (A); 5,7-Dihydroxy-8,4'-dimethoxy isoflavone (B); Kaempferol 3-arabinofuranoside 7-rhamnoside (C); 6,8-Di-C-beta-D-arabinofuranosyl apigenin (D)

Inhibitory activity of tyrosinase enzyme by TLC-bioautography method

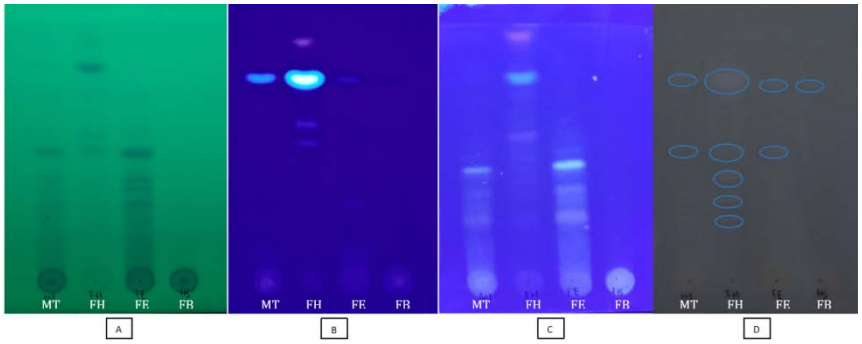


Fig. 3: TLC bioautogram from corn cob extract and fraction with mobile phase toluene: ethyl acetate: formic acid (70:25:5). MT=Methanol, FH= *n*-hexane fraction, FE= Ethyl acetate fraction, and FB= butanol fraction. (A) chromatogram under UV254 nm (B) UV365 nm (C) UV365 nm after spraying with citroborate reagent (D) TLC bioautogram of the tyrosinase inhibitory spots

Inhibition concentration 50 (IC_{50}) value of corncob fractions

The results of the tyrosinase, elastase, and hyaluronidase enzyme inhibitor assays of the methanol extract and corn cob fractions showed that the butanol fraction is the most active fraction in inhibiting the three enzymes (anti-tyrosinase IC_{50} 99.92 ug/ml; anti-elastase IC_{50} 6.05 µg/ml; anti-hyaluronidase IC_{50} 38.88 ug/ml) (table 3).

Table 3: IC_{50} Value of corn cob extract and fractions

Sample	Tyrosinase (µg/ml)		Elastase (µg/ml)		Hyaluronidase (µg/ml)	
	IC_{50}	RIC_{50}	IC_{50}	RIC_{50}	IC_{50}	RIC_{50}
Methanol Extract	>250*	-	13.89±0.003	1.21	33.03±0.041	0.95
N-hexane fraction	>250*	-	44.39±0.035	0.38	22.65±0.021	1.40
Ethyl acetate fraction	>250*	-	54.59±0.005	0.31	>125*	-
Butanol fraction	99.92±0.210	0.07	6.05±0.234	2.78	38.88±0.034	0.81
Positive control**	7.33±0.002		16.81±0.012		31.51±0.003	

*IC_{50} value shows the extract/fraction is not active, **This fig. represents the positive control of each assay. Positive control for tyrosinase inhibition assay is kojic acid, meanwhile for elastase and hyaluronidase inhibition activity is ascorbic acid.

DISCUSSION

This study evaluated the inhibition of tyrosinase, elastase, and hyaluronidase enzymes from corn cobs. The assay was performed using fractions from corn cobs to investigate which fraction is more active in inhibiting these enzymes. This study showed that the butanol fraction is the active fraction of corn cob that is more capable of inhibiting tyrosinase, elastase, and hyaluronidase enzymes than other fractions.

The maceration method performed Corn cob extraction, where the sample was soaked with methanol solvent for three days. Subsequently, fractionation (liquid-liquid partitioning) with n-hexane, ethyl acetate, and butanol was carried out, respectively. These three solvents were chosen due to their different polarities. The purpose of the partition is to separate the components in the total methanol extract based on their polarity. The yield of extract and fractions are shown in table 1.

LC-MS/MS investigated the methanol extract of corn cob to obtain initial information about the secondary metabolite components of the extract with anti-tyrosinase and anti-aging activities. Analysis of LC-MS/MS revealed the presence of 13 phytochemicals belonging to various subclasses, such as phenolics and flavonoids (table 2). They are identified based on the m/z of $(M+H)^+$molecular ions and comparison interpretation of MS and MS/MS spectra with MassLynx V4.1 library.

The LC-MS/MS analysis of a methanol extract of Corn cobs (*Zea mays* L.) indicated the presence of bioactive flavonoids (catechin; kaempferol 3-arabinofuranoside 7-rhamnoside; 6,8-Di-C-beta-D-arabino pyranosyl apigenin; 5,7-Dihydroxy-8,4'-methoxyisoflavone) (table 2). Catechins have been widely isolated from several plants [28-31], and it has been reported that catechin has antioxidant [32] and anti-skin aging activities [33]. The kaempferol group and its glycoside derivatives have several activities, such as antioxidants [34, 35] and anti-aging [36]. The apigenins group and their glycoside derivatives have also been previously reported to have antioxidants [37-39] and photoprotective activities against ultraviolet A (UVA) and ultraviolet B (UVB) rays [39]. Moreover, isoflavone groups also possess activities as antioxidants [40, 41]. This indicates that the four compounds in the corn cob extract contributed significantly to the inhibition of tyrosinase, elastase, and hyaluronidase enzymes in this study.

TLC bioautography is a method that combines the separation of extract on a thin layer with its biological analysis [42]. TLC bioautography described herein is rapid and simple use. It shows good background-spot contrast. Methanol extract, n-hexane fraction, ethyl acetate fraction, and n-butanol fraction were separated using TLC with eluent toluen: ethyl acetate: formic acid (70:25:5). L-tyrosine as a substrate oxidizes by tyrosinase enzyme as a catalyst to become dopachrome, indicated by the brown color. The TLC result showed that many constituents in n-hexane fraction inhibited tyrosinase, seen from the clear zone on a dark background as some in methanol extract, ethyl acetate fraction and n-butanol fraction but not as much as in n-hexane fraction (fig. 3). In the n-hexane fraction, one big clear zone has the biggest inhibition zone. This constituent is also positive with citroborate. Citroborate is a specific reagent for flavonoid groups, which react with ortho-dihydroxy groups in flavonoids. The positive reaction showed a yellow color under UV lamp 365 nm. So, it can be concluded that the biggest inhibition zone is the flavonoid group compound. From the phytochemical study in methanol extract, we identify the flavonoid compounds such as 5,7-Dihydroxy-8,4'-dimethoxy isoflavone with the highest peak in the chromatogram. Some isoflavone reported inhibit tyrosinase activity such as Glabridin; Neobavaisoflavone; 6aR,11aR)-3,8-dihydroxy-9-methoxy pterocarpan; Puerarin; Calycosin; Lupinalbin A; and 20-hydroxygenistein-7-O-gentibioside [43].

The calculation of 50% inhibition of the tyrosinase enzyme is seen from the absorbance data at a wavelength of 517 nm, which is the λ max of dopachrome (the result of the oxidation of L-tyrosine and L-DOPA by the tyrosinase enzyme). Then, the RIC_{50} value is also calculated, namely the relative ratio of the IC_{50} of the reference compound to sample expressed in µg of equivalent reference compound per µg of test sample [44]. The tyrosinase inhibitor result showed that the butanol fraction inhibited the enzyme with IC_{50} 99.92 µg/ml (table 3). Methanol extracts inhibited elastase enzyme more active than ascorbic acid as the standard. It is shown on the RIC50 1.21, which means 1.21 µg of ascorbic acid is equivalent to 1 µg of methanol extract in inhibiting 50% of elastase enzyme. Butanol fraction is the most active in inhibiting elastase enzyme with IC50 6.05, which 2.78 µg of ascorbic acid is equivalent to 1 ug of the butanol fraction (RIC50 2.78). N-hexane fraction is active as inhibitory hyaluronidase activity more active than the standard (ascorbic acid) with IC50 22.65, which 1.4 µg ascorbic acid is equivalent with one µg of fraction (RIC50 1.4). The butanol fraction is the most active fraction in inhibiting the three enzymes, which means it has the potential to be a new antiaging candidate. At the same time, ethyl acetate is the fraction that has no potential as an antiaging candidate.

CONCLUSION

In this study, the butanol fraction showed higher activity of tyrosinase inhibition (IC_{50} 99.92 ug/ml) and antiaging (anti-elastase IC_{50} 6.05 ug/ml; anti-hyaluronidase IC_{50} 38.88 ug/ml) in comparison to the total extract, hexane fraction and ethyl acetate fraction. This study shows that the butanol fraction of corn cob has the potential to become a new anti-aging candidate through its anti-tyrosinase, anti-elastase, and anti-hyaluronidase activities.

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AUTHORS CONTRIBUTIONS

Armita Harahap designed the experiment, performed the experiment, provided some resources, analyzed and interpreted of result, and drafted the article. Suci Triamarta conducted the experiment, supplied some resources, and did the editing. Dinda charisma undertook the experiment, assigned some resources, and writing-review. Wiwik Hanifah ran the experiment, prepared manuscript, and collected data. Muhammad Iqbal set up the experiment, provided some resources, and did the data collection. Nurwahidatul Arifa conducted the experiment, analyzed and interpreted of result, drafted the article, and supervised the investigation. Friardi Ismed designed study conception and experiment, analyzed the data, supervised the investigation, writing-review, and published the manuscript. All authors contributed to the manuscript, arranged, read, revised, and approved the submitted version.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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