Active Components and Skin Care Properties of Tea Seed Oil from *Camellia sinensis*

Jianjun Guo,[a,b] Jun Luo,[c] Yi Zhou,[b] Huanhuan Liu,[b] Daochao Jin,[a,*] and Jianjun Guo [a,*]

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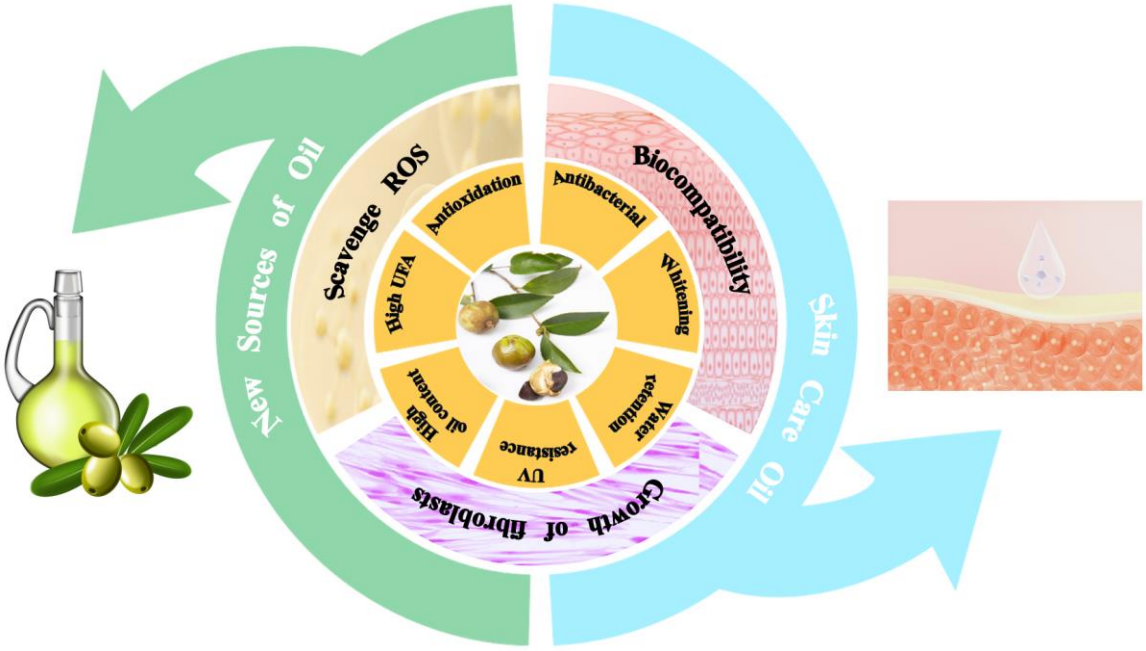
Statement of Novelty

The ripe seeds of the tea plant (*Camellia sinensis* (L.) O. Kuntze) known as tea seeds are lost as agricultural waste in China, and there is not much fundamental research on the use of tea seed oil (TSO). In this study, the chemical composition and activity characteristics of TSO in different areas of Guizhou, China were investigated. This research will help to alleviate the problem of low oil self-sufficiency in China, and also offers a scientific basis for the in-depth development of TSO, especially in the field of skin care.

GRAPHICAL ABSTRACT



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In China, many tea seeds, the ripe seeds of the tea plant (*Camellia sinensis* (L.) O. Kuntze), are discarded as agricultural waste. Therefore, the tea seed oil that could have been obtained from tea seeds has also been wasted. To fully understand the content and efficacy of the main active ingredients in tea seed oil (TSO), the chemical composition and activity characteristics of TSO in different areas of Guizhou, China were investigated. The results demonstrated that TSO had high content of unsaturated fatty acids (UFA) and good skincare properties, such as anti-oxidation, anti-ultraviolet, moisturizing, whitening, and bacteriostasis. Furthermore, TSO showed a scavenging effect on reactive oxygen species in mouse fibroblasts cells (L929) and rat cardiomyocytes cells (H9C2). TSO exhibited high biocompatibility and promoted the proliferation and migration of L929. Southwest Guizhou (T4) and southern Guizhou (T6) might be used as high-quality producing areas for cosmetic oil by the weight analysis of each indicator. In summary, as the main producing area of tea in the world, this study helps to alleviate the problem of low oil self-sufficiency in China. The work offers a scientific basis for the in-depth development of TSO, especially in the field of skin care.

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Keywords: Tea seed oil; Agricultural waste; Chemical composition; Skin-care properties

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INTRODUCTION

China is a significant oil importer, and the self-sufficiency percentage of edible vegetable oil is less than 35% (Bai *et al*. 2021). China urgently needs to improve its supply capacity of edible oil and seek new sources of vegetable oil. As the world's largest tea producer, China's tea production is about 2.6 million tons, accounting for about 45% of the world's total tea production. Tea planting area reached about 2.9 million hectares, which is about 61% of the global area. However, many tea seeds in Chinese tea gardens are often ignored as agricultural waste. In recent years, tea seed oil (TSO) has attracted attention as a new source of vegetable oil supplement (Scicchitano *et al*. 2014).

Tea seed oil is an edible vegetable oil contained in the mature seeds of tea plants (*Camellia sinensis* O. Kuntze) (Kim *et al*. 2010). The beneficial effects of green TSO are already well known. The oil content of tea seeds is 20% to 30%, and the fatty acids are mainly oleic, linoleic, palmitic, and stearic acids, accounting for more than 90% of the total fatty acids (Wang *et al*. 2017). The content of unsaturated fatty acids (UFA) in TSO is as high as 75% to 85% (Sarmah and Das 2018).

The essential linoleic acid content is about 3 to 6 times that of olive oil, and the vitamin E content is about 5 to 10 times that of olive oil. The content of tea polyphenol and tocopherol is much higher than that of other oils, so it is called 'oil gold' (Elagizi *et al*. 2021; Riangjanapatee *et al*. 2022). At present, the common tea plant planted for seeds in China is *Camellia oleifera* Abel (*C. oleifera*). The seeds of *C. oleifera* are used to produce TSO, which is an edible oil and can also be used in the cosmetic and pharmaceutical industries (Wang *et al*. 2017). Most of the studies were based on camellia seed oil (*C. oleifera*) as the research sample, while the study of TSO (*Camellia sinensis*) is very rare, including only its volatile components and antioxidant and antibacterial activity analyses. In particular, the skin-care activity of TSO is rarely reported.

The iodine value of TSO is below 100 (George *et al*. 2013). It is a typical non-drying oil with good skin care and moisturizing effects that is suitable for the skin. Tea seed component determination has shown that tea seed oil is composed of oleic, linoleic, palmitic, and stearic acids. According to Guynot *et al*. (2003), the UFA, just like polyphenols, have exhibited antibacterial and antifungal activities. Among them, oleic acid has the effect of promoting penetration and skin absorption. Linoleic acid is widely used as a cosmetic nutritional additive that can improve skin moisture retention, promote skin metabolism, and soften the stratum corneum. It is suitable as a base oil for cosmetics. Sunlight affects the skin and hair through ultraviolet light. The maintenance method of essential oils to improve the scalp has a positive effect on improving hair density, hair thickness, and scalp state (Park and Lee 2012; Choi *et al*. 2013). Min *et al*. (2013) found that green tea seed oil prevents reinjury from heat and UV rays for colored and decolored hairs (Min *et al*. 2013). The tea seed oil from *C. sinensis* has also been shown to contain polyphenols (Njuguna *et al*. 2014).

The main mechanisms of these polyphenols are that they have anti-oxidative activity and can scavenge reactive nitrogen and oxygen species by chelating redox-active transition metal ions. Tea seed oil was found to significantly increase cell viability and reactive oxygen species (ROS) scavenging activity (Kim *et al*. 2018). The oils from two tea varieties, *C. oleifera* and *C. sinensis* cultivars, were evaluated for their antimicrobial activity against five microbes, *viz*. *Escherichia coli*, *Staphylococcus aureus, Candida albicans, Cryptococcus neoformans,* and *Trichophyton. mentagrophytes*. The results obtained confirmed that TSO indeed has antimicrobial properties. At an oil concentration of 100%, *S. aureus*, a gram-positive bacterium, was the most susceptible microbe to the oils (Ruto *et al*. 2022).

Various studies have outlined the beneficial effects of TSO, such as hepatoprotection (Lee *et al*. 2007) and a reduction in weight gain (Kim *et al*. 2008). In a study by Zhang *et al*. (2014), the ability to clear lipid peroxidation in rat livers was detected, hence alleviating liver disease. In summary, TSO has the potential to be a high-quality edible oil and skin care oil but has been studied less. It has great development value.

Guizhou Province is the largest tea-producing province in China. The oil content and fatty acid composition of tea seeds in different regions are different (Liu *et al*. 2016), resulting in different biological activities of TSO as a skin care oil (Ruto *et al*. 2022). Therefore, it is very important for the secondary development of tea seed oil to screen out TSO producing areas with high oil content, rich UFA, and suitable skin care biological activity.

EXPERIMENTAL

Materials

The tea seeds used in November 2021 were from 7 producing areas in Guizhou Province, China (Table 1). They were stored according to a unified standard after harvest, and the samples were mature tea fruit (Fig. 1). The samples were identified by Sun Sisheng, Associate Professor, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, as the dried mature seeds of *Camellia sinensis* O. Kuntze. Voucher samples are preserved in the Herbarium of Traditional Chinese Medicines of Anshun University. Seeds of each variety were randomly selected, and three biological replicates were performed. The fatty acid standard was chromatographically pure, purchased from Sigma, USA. Isooctane, potassium hydroxide, sodium bisulfate, and petroleum ether were all analytically pure and purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Rat cardiomyocytes cells (H9C2) and mouse fibroblasts cells (L929) were purchased from Wuhan Servicebio Technology Co., Ltd. (Wuhan, China).

The milliQ ultrapure water meter was acquired from Millipore Co., Ltd. (Burlington, USA). The electronic balance was acquired from Mettler Toledo instruments Co., Ltd. (Zurich, Switzerland). The high-speed cryogenic centrifuge was procured from Beckman Coulter Inc. (Brea, CA, USA). The constant temperature water bath was procured from Jintan Medical Instrument Co., Ltd. (Changzhou, China). The infinite 200 PRO multi-function microplate reader was procured from Tecan Trading AG. (Männedorf, Switzerland). The gas chromatograph (Agilent 7890A) and mass spectrometer (Agilent 5975C) were from Agilent Technologies Co., Ltd. (Santa Clara, CA, USA).

Table 1. Source Information of Tea Seeds in Different Regions of Guizhou

No.	Region	Collection Location
T1	Northern Guizhou	Zunyi City
T2	Western Guizhou	Bijie City
T3	Southeastern Guizhou	Kaili City
T4	Southwestern Guizhou	Xingyi City
T5	Eastern Guizhou	Tongren City
T6	Southern Guizhou	Duyun City
T7	Middle Guizhou	Anshun City

Chemical Composition of TSO

Kernel and oil content of tea seed

Twenty tea seeds were evenly and randomly selected, and the total mass of tea seeds (m) was determined. After shelling, the total mass of tea seed kernels (n) was weighed.

$$\text{Tea seed kernel rate} = n/m \times 100\% \quad (1)$$

After the tea seed was peeled, it was dried in an oven at 40 °C for 8 h and ground to 30 mesh. The crude fat was extracted by Soxhlet extraction method, and the oil content was determined according to GB/T 14488.1 (2008).

Fatty acid content in TSO

The fatty acid content in TSO was determined by methyl esterification gas chromatography (GB 5009.168 2016). Conditions used for gas chromatography: Agilent HP-5MS chromatographic column (30 m×0.25 mm×0.25 μm), the injection volume was 1 μL, the carrier gas was He, the inlet temperature was 260 ℃, the split ratio was 60:1, the flow rate was 1.0 mL/min. Temperature programming conditions: column temperature was 40 ℃. It was heated to 200 ℃ at a rate of 20 ℃/min for 5 min, then heated to 220 ℃ at a rate of 5 ℃/min, and finally heated to 250 ℃ at a rate of 10 ℃/min for 5 min.

Activity Characterization of TSO

TSO samples of 1.0, 5, 10, 15, and 20 mg/mL were prepared. These samples were then tested for the optimal concentration of TSO for tyrosinase inhibition, water retention capacity, transmittance capacity, antibacterial, and antioxidant activities.

Tyrosinase inhibition

Tyrosinase inhibition was evaluated according to the method described by Zhong *et al.* (2008). In brief, the reaction mixture was incubated at 37 ℃ in a water bath for 10 min, and then 0.40 mL of 0.35 mg/mL of tyrosinase was added. After 5 min, the complete reaction solution was measured at 475 nm wavelength for obtaining OD values of A_{C1}, A_{C2}, A_{T1}, and A_{T2}. The tyrosinase inhibition rate was calculated using Eq. 2:

$$\text{Inhibition rate} = [1 - (A_{T2} - A_{T1}) / (A_{C2} - A_{C1})] \times 100\% \quad (2)$$

Water retention capacity

Different concentrations of TSO (1.0 mL) in a 35-mm culture dish were placed at 25 ℃ for 4 h, 8 h, and 12 h, respectively. Water loss (N) was calculated as the water retention capacity using Eq. 3 as given below:

$$\text{Water loss } (N) = (A_0 - A_1) / A_0 \quad (3)$$

In the equation, A_0 indicates initial weight, and A_1 indicates final weight after incubation at 25 ℃.

Transmittance capacity

Different concentrations of TSO were scanned at a 280 to 400 nm wavelength using a spectrophotometer (UV-2450, Shimadzu, Japan). Petroleum ether was used as a blank control. UV transmittance at different wavelengths was indexed as transmittance capacity.

Antioxidant activity

Antioxidant activity was represented by the scavenging rate of superoxide radicals assayed according to the method described by Wu *et al*. (2012). Briefly, each sample at different concentrations was mixed with a solution of 0.04 mg/mL DPPH in ethanol. The mixture was shaken vigorously and centrifuged at 3000 r for 10 min at room temperature. The absorbance of the supernatant was measured at 517 nm. DPPH radical scavenging capacity was calculated using Eq. 4,

$$\text{Scavenging capacity} = [1 - (A_1 - A_2) / A_0] \times 100\% \quad (4)$$

where A_0 is the absorbance of the control (ethanol instead of sample), A_1 is the absorbance of the sample, and A_2 is the absorbance of the blank (obtained by replacing the DPPH ethanol solution with ethanol).

Antibacterial assay

Using *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) as test organisms, the antibacterial characteristics of the TSO were determined. Both *E. coli* and *S. aureus* were provided by Sericultural Research Institute, Chinese Academy of Agricultural Sciences. The spread plate technique was utilized to seed 100 μL of diluted bacterial culture (containing approximately 106 colony-forming units per milliliter) onto the solidified nutrient agar medium, followed by the careful placing of the TSO on the plate's surface to allow the bacteria to colonize. Imaging of the plates was done after they had been incubated for 12 h at 37 ℃, and the diameters of the blocks were quantified utilizing Image-J software to determine their size.

Antioxidant Activity Mechanism of TSO

Cell cytotoxicity assay

Rat cardiomyocytes cells (H9C2) and mouse fibroblasts cells (L929) were seeded in a 96-well cell culture plate and cultured for a certain period of time (24 h). The TSO (1000 μg/mL) solutions were diluted to 1/10 of original concentration with Dulbecco's Modified Eagle Medium (DMEM). Each diluted solution was added to 96-well plate (90 μL/well) for certain amount of time (2D culture model for 24 h, 3D culture model for 72 h). Finally, 10 μL of CellTiter-Blue reagent was added to each well for 3 h incubation. The plates were then read to collect the fluorescence absorbance of 560 nm by a SynergyTM H1 microplate reader (Bio Tek Inc., USA). At the same time, the growth of cells was observed by cell imaging system.

$$\text{Relative survival rate of cells (\%)} = (OD_{\text{samples}} / OD_{\text{control}}) \times 100 \quad (5)$$

Scavenging capacity of ROS

The ROS clearance rate of the sample in the cell was detected by Biyuntian ROS detection kit. The specific experimental steps were in accordance with the instructions of the kit, and the aseptic operation was ensured.

Cell scratch assay

L929 cells were seeded in a 12-well cell orifice plate with 100,000 cells per well. After 24 h of culture, the growth status of the cells was observed. After the cells were overgrown, the cells were scratched with a disposable 10-μL pipette tip. All wells were washed twice with 1 mL of DMEM medium to remove cell debris. Then, the diluted sample was added, and the distance between the scratches was observed by fluorescence inverted microscope, which was recorded as d_0. After incubation in the incubator for 24 h, the distance between the scratches was observed and recorded as d_1. The relative migration distance is calculated using Eq. 6:

$$\text{Relative migration distance} = [(d_0 - d_1) / d_0] \times 100\% \quad (6)$$

Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation. Statistical evaluation was made using analysis of variance (ANOVA) using SPSS 18.0 software, with $p < 0.05$ being considered as significant.

RESULTS AND DISCUSSION

Kernel and Oil Content of TSO from Different Regions

The appearance of tea seed shell and kernel from different producing areas was observed, and the results were shown in Fig. 1. The shell of tea seed is brown, and the seed kernel is pale yellow. The average diameter of tea seed from Guizhou was 9.49 mm, and the average weight of tea seed kernels was 0.89 g (Table 2). As shown in Table 1, there were significant differences in the kernel content and oil content of tea seeds from different regions. The kernel content of tea seeds was 57% to 69%, with an average of 63.8%. The kernel content of tea seeds in western Guizhou (T2), southwestern Guizhou (T4), and southern Guizhou (T6) was higher than the average, and the highest in southern Guizhou (T6) was 68.4%. The average oil content of tea seed kernels was 24.7%. The oil content of samples in western Guizhou (T2), southwestern Guizhou (T4), and southern Guizhou (T6) were higher than the average value, and the highest content in western Guizhou (T2) was 26.9%. Based on the difference in kernel content and oil content of tea seeds in different regions, it can be concluded that the kernel content and oil content of tea seeds in western Guizhou (T2), southwestern Guizhou (T4), and southern Guizhou (T6) were higher than those in other tea seed-producing areas in Guizhou.

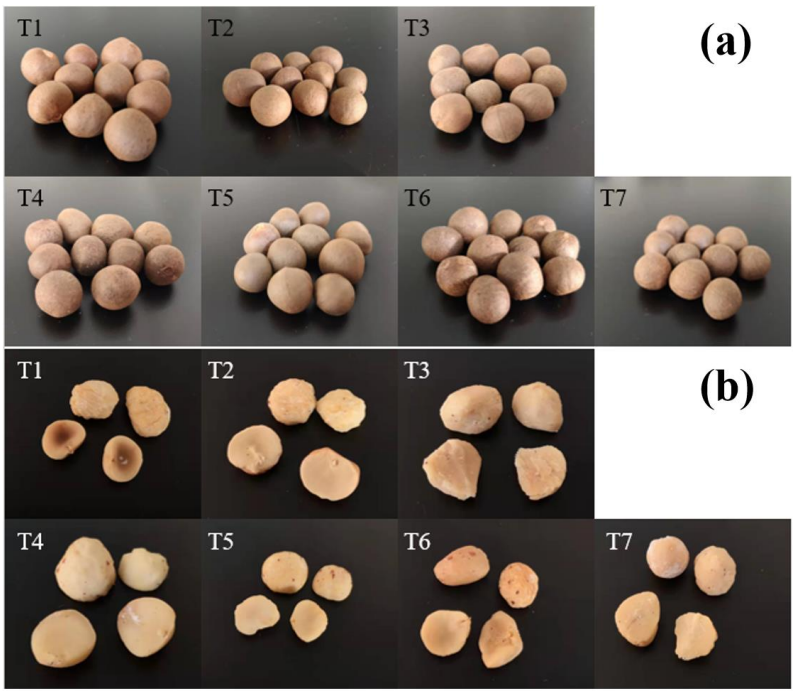

Fig. 1. The physical map of tea seeds from different producing areas in Guizhou: (a) Appearance of tea seeds; (b) Kernel of tea seeds

Table 2. Kernel Content, Oil Content, and Appearance of Tea Seeds

No.	Kernel Content (%)	Oil Content (%)	Diameter (mm)	Kernel Weight (g)
T1	57.2 ± 0.04	24.21 ± 0.04	11.71 ± 0.23	1.21 ± 0.06
T2	66.8 ± 0.03	26.88 ± 0.05	10.53 ± 0.11	0.95 ± 0.02
T3	63.6 ± 0.05	24.69 ± 0.02	8.10 ± 0.14	0.91 ± 0.03
T4	68.2 ± 0.04	26.38 ± 0.06	8.56 ± 0.17	0.74 ± 0.05
T5	59.4 ± 0.03	21.39 ± 0.05	7.78 ± 0.15	0.71 ± 0.04
T6	68.4 ± 0.05	26.28 ± 0.07	9.62 ± 0.16	0.82 ± 0.04
T7	63.2 ± 0.06	23.12 ± 0.04	10.14 ± 0.21	0.91 ± 0.07
Mean Values	63.8	24.71	9.49	0.89

Chemical Components of TSO from Different Regions

Using GC-MS technology, this method can separate the peaks of TSO in different regions with good resolution. The relative content of fatty acid components in TSO was calculated by the area normalization method (Liu *et al*. 2016). There were significant differences in the composition and content of fatty acids in TSO from different regions, and 16 fatty acid components were detected (Table 3). Among them, the tea seed in western Guizhou (T2) had the most abundant fatty acid species, with 15 kinds; the tea seed in southern Guizhou (T6) had the least fatty acid species, with 9 kinds. *Cis*-13-docosaenoic acid was only detected in tea seed samples in eastern Guizhou, and the content was only 0.11%. 10-undecylenic acid was only detected in tea seeds in western and eastern Guizhou.

Table 3. Contents of Fatty Acid Components in Tea Seed Oil from Different Regions

No.	Fatty Acids	Content of Different Fatty Acids (%)						
		T1	T2	T3	T4	T5	T6	T7
1	a	0.10	0.14	—	0.14	0.13	0.15	—
2	b	0.72	0.81	0.76	0.93	0.58	0.86	0.52
3	c	0.05	0.11	0.06	—	0.07	—	0.06
4	d	0.16	0.13	0.16	0.17	0.14	—	0.14
5	e	16.03	16.51	17.49	16.59	17.57	16.19	16.60
6	f	0.15	0.13	0.09	0.12	0.08	0.12	0.11
7	g	0.25	0.18	0.15	0.21	0.15	0.21	0.18
8	h	17.91	20.28	23.20	22.23	23.47	23.05	21.49
9	i	60.89	66.84	54.19	68.05	53.70	68.51	56.69
10	j	2.70	3.03	3.32	3.37	3.04	3.44	3.47
11	k	0.53	0.41	0.14	—	—	—	—
12	l	—	0.17	—	—	0.12	—	—
13	m	0.21	0.23	0.10	—	0.15	—	0.09
14	n	0.92	1.10	0.98	1.23	1.13	1.14	1.05
15	o	0.11	0.13	0.12	0.13	0.13	0.13	0.12
16	p	—	—	—	—	0.11	—	—

Note：‘—’ means that the substance was not detected.

a. 2-Hexenedioic acid; b. α-Linolenic acid; c. 12-methyl-Tridecanoic acid; d. 9-Hexadecenoic acid; e. Palmitic acid; f. 2-hexyl-Cyclopropaneoctanoic acid; g. Heptadecanoic acid; h. Linoleic acid; i. Oleic acid; j. Stearic acid; k. 10-Octadecenoic acid; l. 10-Undecynoic acid; m. 3-octyl-Oxiraneoctanoic acid; n. cis-11-Eicosenoic acid; o. Eicosanoic acid; p. 13-Docosenoic acid

There were four kinds of fatty acids with high relative content in TSO: oleic acid, linoleic acid, palmitic acid, and stearic acid (Table 4). Oleic acid and linoleic acid are unsaturated fatty acids that can regulate blood lipid levels, reduce cholesterol, and effectively reduce the incidence of hypercholesterolemia and cardiovascular disease (Li *et al*. 2023). Among them, linoleic acid is an essential fatty acid in human and animal nutrition. The average content of linoleic acid in Guizhou TSO is 21.66%, which is much higher than that in olive oil (Moghaddam *et al*. 2012). TSO in southwestern Guizhou (T4) and southern Guizhou (T6) contains more unsaturated fatty acids, which can reduce blood lipids and prevent the formation of atherosclerotic plaques (Yang *et al*. 2022).

Through the analysis of the chemical components of TSO in different regions of Guizhou, it was concluded that the kernel and oil content of tea seeds in western Guizhou (T2), southwestern Guizhou (T4), and southern Guizhou (T6) were higher than those in other tea seed-producing areas in Guizhou and contained more and richer UFA. Therefore, western Guizhou (T2), southwestern Guizhou (T4), and southern Guizhou (T6) can be used as potential high-quality producing areas for TSO with high oil content and rich fatty acids.

Table 4. Proportion of Main Fatty Acids in Total Fatty Acids (%)

No.	Oleic Acid	Linoleic Acid	α-Linolenic Acid	Total	Palmitic Acid	Stearic Acid
T1	60.89	17.91	0.72	79.52	16.03	2.70
T2	66.84	20.28	0.81	87.93	16.51	3.03
T3	54.19	23.20	0.76	78.15	17.49	3.32
T4	68.05	22.23	0.93	91.21	16.59	3.37
T5	53.70	23.47	0.58	77.75	17.57	3.04
T6	68.51	23.05	0.86	92.42	16.19	3.44
T7	56.69	21.49	0.53	78.71	16.60	3.47
Mean Values	61.27	21.66	0.74	83.67	16.71	3.20

Activity Characterization of TSO

To understand the skin care activity characteristics of TSO, properties of tyrosinase activity, water retention capacity, transmittance capacity, antibacterial activity, and antioxidant activity were evaluated.

The transmittance of different concentrations of TSO was measured using a spectrophotometer with incident UV light at 280 to 400 nm, which is the most harmful to human skin (Vayalil *et al*. 2004). With the increasing concentration of TSO, the UV transmittance decreased. With increasing UV wavelengths, transmittance rapidly increased from 280 to 320 nm. Among the evaluated regions, 10 mg/mL TSO in southeastern Guizhou (T3) showed the lowest UV transmittance at 280 nm (Figs. 2a,b). The inhibition value of tyrosinase effectively reflects the whitening performance of a substance. As shown in Fig. 2c, 10 mg/mL of TSO in western Guizhou (T2) effectively inhibited the tyrosinase activity by 96.47%. When the concentration of TSO was 1.0 to 10 mg/mL, the inhibition value of tyrosinase activity increased significantly with the increase in concentration. When the concentration of TSO was 10 to 20 mg/mL, the inhibition of tyrosinase showed no significant change.

Water retention capacity was determined by the amount of water evaporation evaluated at 25 ℃ in a windless incubator for 4 h. The water evaporation gradually decreased with the increase in TSO concentration in different regions, but the amount

decreased slowly after 10 mg/mL of TSO (Fig. 2d). Among the evaluated regions, the water evaporation of 10 mg/mL TSO in western Guizhou (T2), southern Guizhou (T6), southeastern Guizhou (T3), and northern Guizhou (T1) was less than 1%. The scavenging of superoxide radicals was determined to evaluate the antioxidant activity of SFO. As shown in Fig. 2d, with the increase in TSO concentration in different regions, the scavenging of superoxide radicals increased. Among the evaluated regions, the DPPH scavenging of 10 mg/mL TSO in southwestern Guizhou (T4) could reach 89.46% (Fig. 2e).

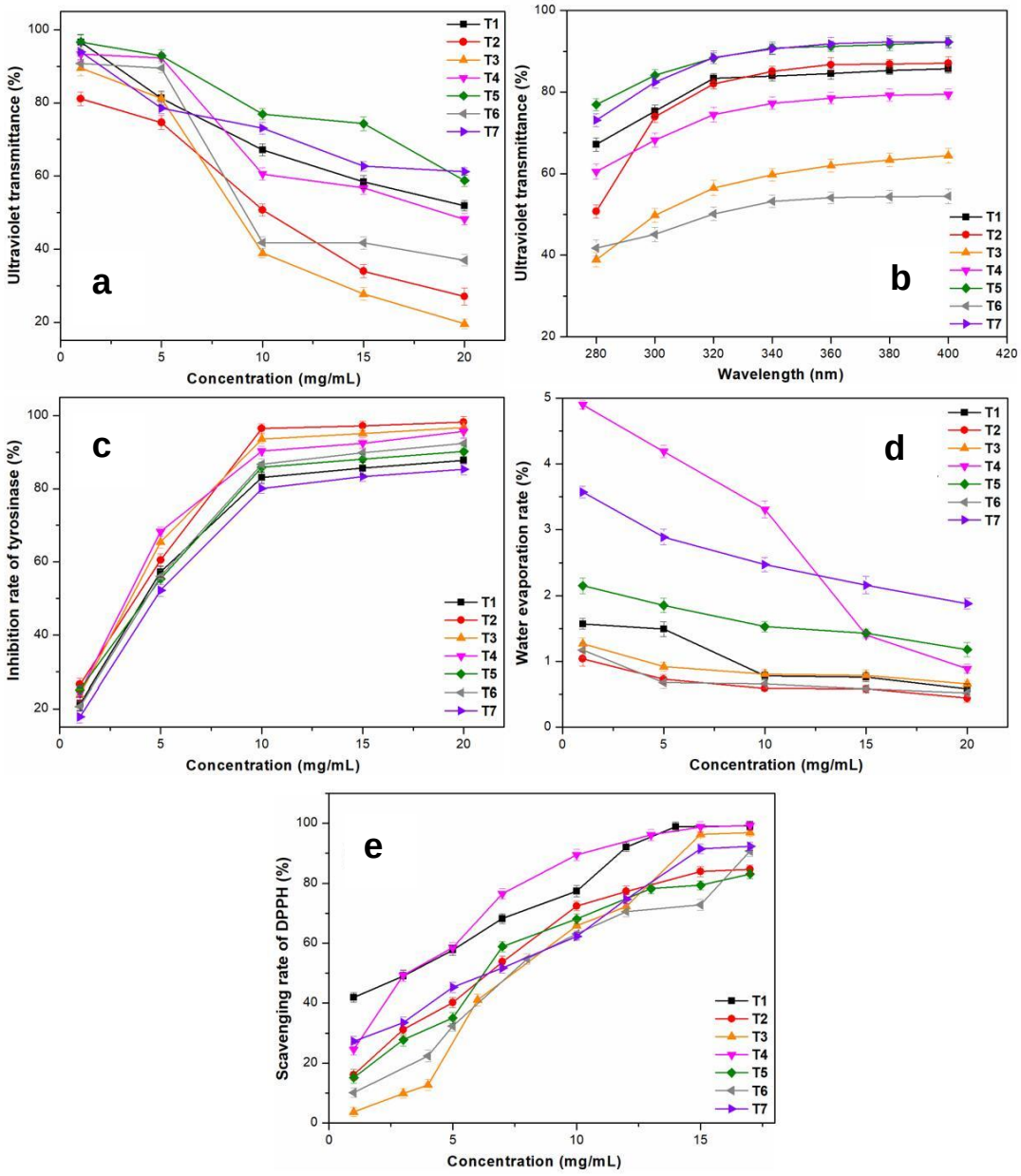

Fig. 2. Activity characteristics of TSO: (a) Transmittance of UV at 1 to 20 mg/mL; (b) Transmittance of UV at 280 to 400 nm; (c) Tyrosinase inhibition rate; (d) Water evaporation rate at 25 °C; (e) Scavenging rate of DPPH. The experiments were repeated three times, and the results are shown as mean ± SE.

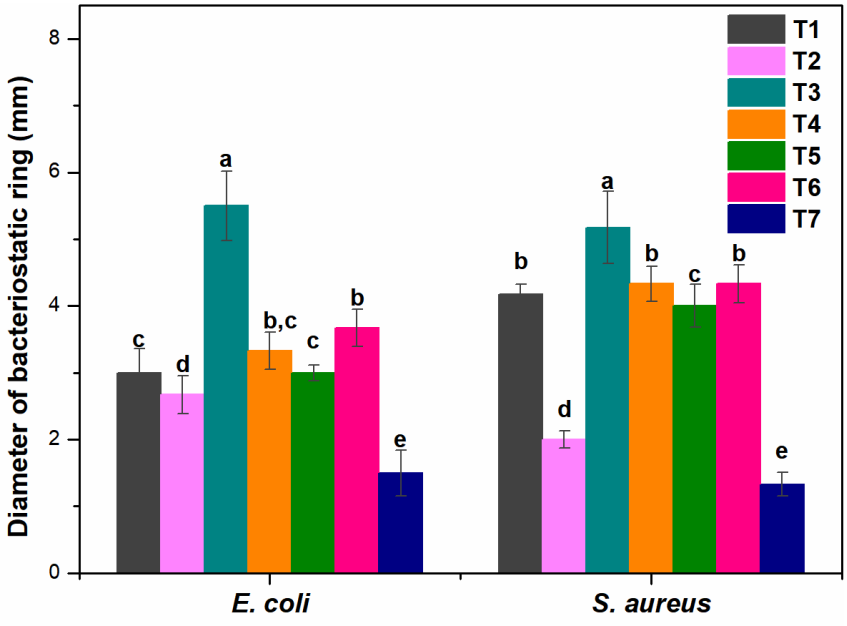

Fig. 3. The diameter of bacteriostatic ring of TSO in different regions of Guizhou; Bars without common superscript letters differed statistically ($p < 0.05$). The experiments were repeated three times, and the results are shown as mean ± SE.

The antibacterial diameter of TSO was determined by the filter paper method. As shown in Fig. 3, TSO from different regions had antibacterial activity on *E. coli* and *S. aureus*, and the antimicrobial effect was more obvious with the increase of concentration. Among them, the most significant antimicrobial effect was TSO in southeastern Guizhou (T3), and the maximum antibacterial diameter could reach 5.50 mm. The least obvious antimicrobial effect was TSO in middle Guizhou (T7). In addition, the antibacterial effect of TSO from different regions on *S. aureus* was better than that of *E. coli*. Based on the above data, the TSO from Guizhou had good skin care properties such as anti-oxidation, anti-ultraviolet, moisturizing, whitening, and bacteriostats. It is a high-quality and potential raw material for cosmetics.

Comprehensive Evaluation of TSO

Taking the above five detection activities as indicators, the weight coefficients of each indicator were given according to the difference in importance between the indicators, and the comprehensive score was carried out (Zhao *et al*. 2020). The weighted coefficients of different skin care indicators (anti-oxidation, anti-ultraviolet, moisturizing, whitening, and bacteriostats) all were set to 0.2. The comprehensive score of the weight analysis can be seen in Table 5. There were significant differences in the comprehensive scores of skin care characteristics of TSO in different regions. Among them, the comprehensive scores of skin care characteristics of TSO in southeastern Guizhou (T3), southwestern Guizhou (T4), and southern Guizhou (T6) were the highest, so the skin care characteristics of TSO in these areas were better and more balanced. Combined with the kernel content, oil content (3.1), fatty acid composition (3.2), and activity characterization (3.3) of tea seeds in various regions, it was recommended that southwestern Guizhou (T4) and southern Guizhou (T6) could be used as potential high-quality producing areas of tea seed oil in Guizhou Province, China.

Table 5. Comprehensive Scores of TSO in Different Regions of Guizhou in Weight Analysis

No.	DPPH Scavenging Rate (%)	Water Retention Capacity (%)	UV Transmittance (%)	Tyrosinase Inhibition Rate (%)	Antibacterial Diameter (%)	Score
T1	77.40	99.22	32.86	83.07	67.20	71.95
T2	72.40	99.41	49.30	96.47	43.77	72.27
T3	65.90	99.19	61.10	93.62	100.00	83.96
T4	89.46	96.69	39.47	90.28	71.79	77.54
T5	68.10	98.47	23.09	85.87	65.60	68.23
T6	63.12	99.34	58.22	86.67	74.98	76.47
T7	62.29	97.53	26.89	80.10	26.52	58.67

The ROS Scavenging Ability of TSO in Cells

Based on the comprehensive evaluation of TSO in various regions, TSO in southwestern Guizhou (T4) was selected, and its related biological activity at the cellular level was observed, to better understand the skin care characteristics of TSO in Guizhou. The ability of 0.5 mg/mL T4 to scavenge ROS in H9C2 cells and L929 cells was observed. As shown in Fig. 4, NC and PC were the ROS contents in cells after negative control and oxidative induction, respectively. The content of ROS in H9C2 cells and L929 cells increased significantly after oxidative induction. After the addition of TSO in southwestern Guizhou (T4), the content of ROS in H9C2 cells and L929 cells decreased, and the difference was statistically significant. In addition, the ability of TSO in southwestern Guizhou (T4) to scavenge ROS in H9C2 cells was more significant than that in L929 cells. The results showed that TSO had a scavenging effect on ROS in H9C2 cells and L929 cells, which was also consistent with the *in vitro* antioxidant results.

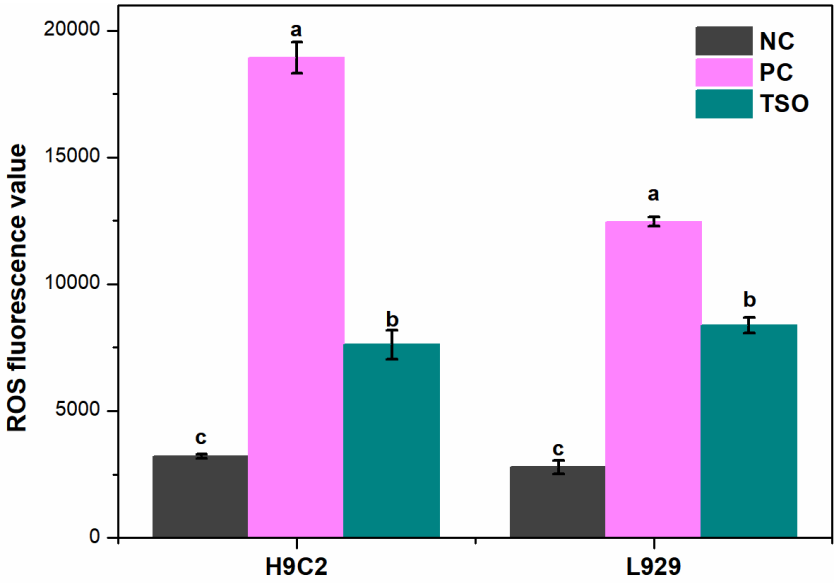

Fig. 4. Effects of TSO on ROS content in H9C2 and L929 cells under oxidative induction; Bars without common superscript letters differed statistically ($p < 0.05$). The experiments were repeated three times, and the results are shown as mean ± S.E.

Cell Cytotoxicity of TSO

Biocompatibility is an important characteristic of cosmetic raw materials. The biocompatibility of rat cardiomyocytes cells and mouse fibroblasts cells also indirectly indicated that TSO could be used as a cosmetic raw material. The growth of H9C2 cells and L929 cells on TSO in southwestern Guizhou (T4) was detected by CTB reagent, and the biocompatibility of TSO was evaluated (Liu *et al*. 2010). Compared with the NC group, there was no significant difference in the survival rate of H9C2 cells and L929 cells after TSO treatment (Fig. 5). According to the fact that the survival rate of H9C2 cells and L929 cells in TSO was greater than 95%, it could be seen that TSO had good biocompatibility for H9C2 cells and L929 cells. This might be related to the fact that TSO contained bioactive factors such as UFA, which provided a stable proliferation environment for H9C2 cells and L929 cells (Yoon *et al*. 2015; Lhomme *et al*. 2021).

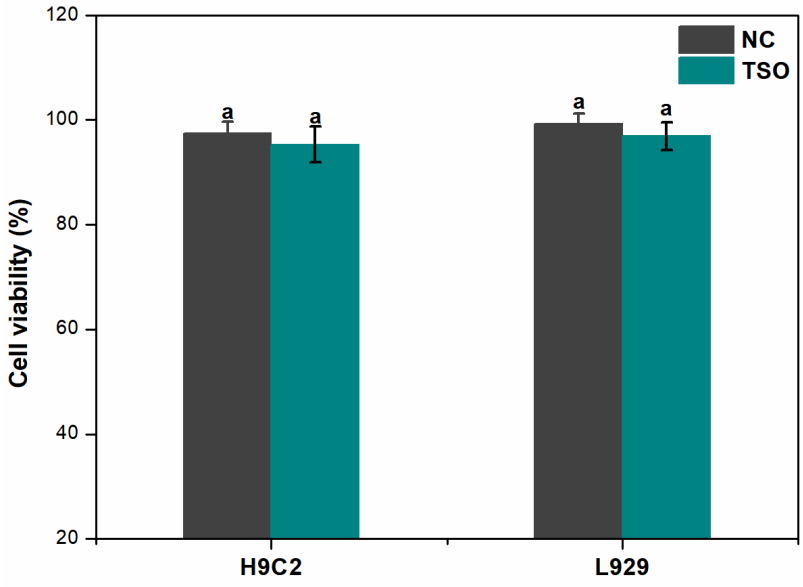

Fig. 5. Toxicity of TSO to H9C2 and L929 cells *in vitro*; Bars without common superscript letters differed statistically ($p < 0.05$). The experiments were repeated three times, and the results are shown as mean ± SE

The Performance of TSO to Promote the Growth of Fibroblasts

Cells would produce directional movement, angiogenesis, biological stress, and other processes after being stimulated by external signals, and these processes were related to cell migration. Through the behavior of cell migration, it could provide guidance for the application of TSO as a high-quality cosmetic raw material in the field of skin care. Figure 6 shows the results of mouse fibroblasts cells (L929) migration after 24 h of incubation. After 24 h, L929 cells migrated to the center, and the cells in the TSO group migrated significantly. The relative migration distance of cells was quantified by Image J. As shown in Fig. 7, the relative migration distance of the cells in the NC group and the TSO group was 8.85% and 28.21%, respectively. There were significant differences. It showed that TSO was helpful to cell migration and plays an active role in cell growth, migration, differentiation, and other biological processes.

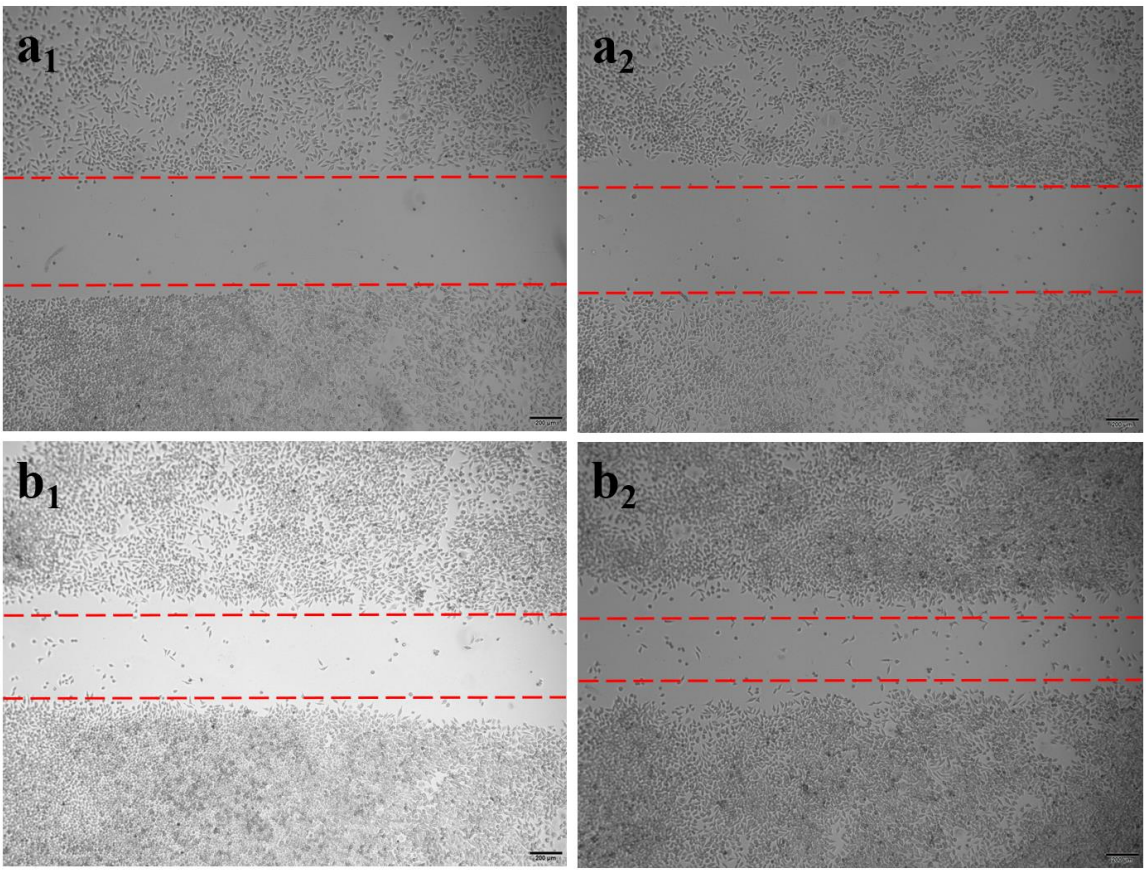

Fig. 6. L929 cells scratch experiment: (a) cell images after incubation for 0 h, (a_1) NC, (a_2) TSO; (b) cell images after incubation for 24 h, (b_1) NC, (b_2) TSO

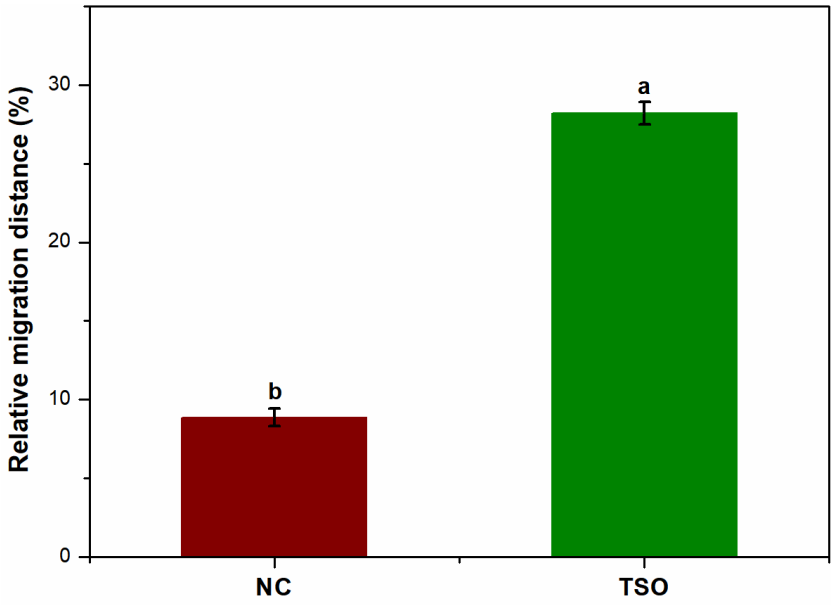

Fig. 7. The relative migration distance of L929 cells after TSO treatment; Bars without common superscript letters differed statistically ($p < 0.05$). The experiments were repeated three times, and the results are shown as mean ± SE.

CONCLUSIONS

1. The chemical composition and activity characteristics of tea seed oil (TSO) in different regions of Guizhou, China were comprehensively analyzed. This study showed that TSO had a high content of UFA and good skin-care properties, such as moisturizing, whitening, bacteriostats, anti-oxidation, and anti-ultraviolet.
2. *In vitro* studies on cells revealed that TSO exerts a scavenging effect on reactive oxygen species (ROS) in both H9C2 and L929 cells. The TSO contributed to the growth and migration of mouse fibroblasts cells (L929), and TSO had good biocompatibility.
3. Furthermore, the kernel content, oil content, fatty acid composition, and biological activity of tea seeds in various regions were comprehensively evaluated by weight analysis. The results indicated that southwestern Guizhou (T4) and southern Guizhou (T6) could be used as potential high-quality producing areas for cosmetic oils.
4. This study found that in China, TSO, as an agricultural waste, has high content of UFA and good skin-care properties, which has important application value in the field of skin care.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Author Contributions

JJG: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing-original draft; JL: Investigation, Resources; YZ: Validation, Visualization, Resources; HHL: Resources; DCJ: Project administration, Funding acquisition; JJG: Writing-review & editing, Supervision. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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