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SHORT COMMUNICATION

Chemical compositions and repellent activity of *Clerodendrum bungei* Steud. essential oil against three stored product insects

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Abstract

Background Several species of Verbenaceae have been widely used in medicine, and some species of Verbenaceae have been observed good insecticidal activity, such as *Lantana camara* and *Vitex negundo*. There is no report about repellent activity of *Clerodendrum bungei* Steud. (*C. bungei*) against stored product insects. The chemical composition of *C. bungei* essential oil (EO) were identified, repellent activity of methanol extract, EO of *C. bungei* and two main components of EO against *T. castaneum*, *L. serricorne* and *L. bostrychophila* were evaluated for the first time.

Results EO of *C. bungei* was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC–MS) and GC. A total of 25 components of the *C. bungei* EO were identified. The principal compounds in the EO were myristicin (75.0%), 2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one (4.1%) and linalool (3.4%). Results of bioassays indicated that *C. bungei* EO exerted strong repellent activity against three target insects. As main constituents, myristicin and linalool also had certain repellency.

Conclusion This work suggests that the EO of *C. bungei* has promising potential to develop into botanical repellents for the control of pest damage in warehouses and grain stores.

Keywords *Clerodendrum bungei* Steud. · Essential oil · Repellent activity · *Tribolium castaneum* · *Lasioderma serricorne* · *Liposcelis bostrychophila*

Introduction

Insect infestation, mainly by beetles and moths, are major causes of the loss of grains and their products during storage and transportation [1]. Stored cereal and gains products are frequently infested by more than 600 species of coleopteran pests [2]. There are many kinds of storage pests among which the dominant species with the most serious harm are *Tribolium castaneum*, *Lasioderma Serricorne*, *Liposcelis bostrychophila*, *Stegobium paniceum*, *Araecetus fasciculatus*, and so on. They may directly or indirectly contribute to varying degrees of food infestation, which finally endangers human health [3]. Chemical insecticides have been considered of efficient tools. At present, the recurrent and extensive application of chemical-based methods to combat the pests were popular [4], but overuse of chemical insecticides has led to physical or behavioral resistance in many insect species. The toxic substance of chemical insecticide can be left in the grain, and then do harm to human health [5]. As food safety issue has become a public concern in many countries, people's demand of life quality is generally growing [6], botanical insecticides have been thriving as alternatives to synthetic insecticides for pest control, because they are easy degradation in the environment and less resistance to pests [7, 8]. Therefore, environment friendly insecticides must be developed to manage stored product insects [9].

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As the natural source of medicine, plants have great research potential in many areas. It is found that plant extracts have good antibacterial effects and insect-resistant effect [10]. *C. bungei* of Verbenaceae family is widely distributed in southeast, south and southwest of China. Various kinds of *C. bungei* extracts have been widely used in clinical practice. The *C. bungei* has certain antimicrobial activity and improves the immune function of human body [11]; The medicinal serum of *C. bungei* can effectively inhibit the proliferation of MHCC97-H hepatoma cells and promote its apoptosis [12]. In the aspect of pest control, Li found that the growth and development of cotton bollworm larvae could be significantly inhibited by *C. bungei*. In the Leigong Mountain minority area of China, there is an ethnologic insect control application to reduce the breeding of mosquitoes and flies by using the rotten roots of *C. bungei.* While there is still a gap in the research on the repellent activity of *C. bungei* against storage pests. In this work, *C. bungei* was chosen as the research object to identify the chemical composition of *C. bungei* EO and evaluate repellent activity of methanol extract, EO of *C. bungei* and two main components of EO against *T. castaneum*, *L. serricorne* and *L. bostrychophila* for the first time.

Fig.1 Bioassays of repellent toxicity against *T. castaneum* at the third concentration after 2 h

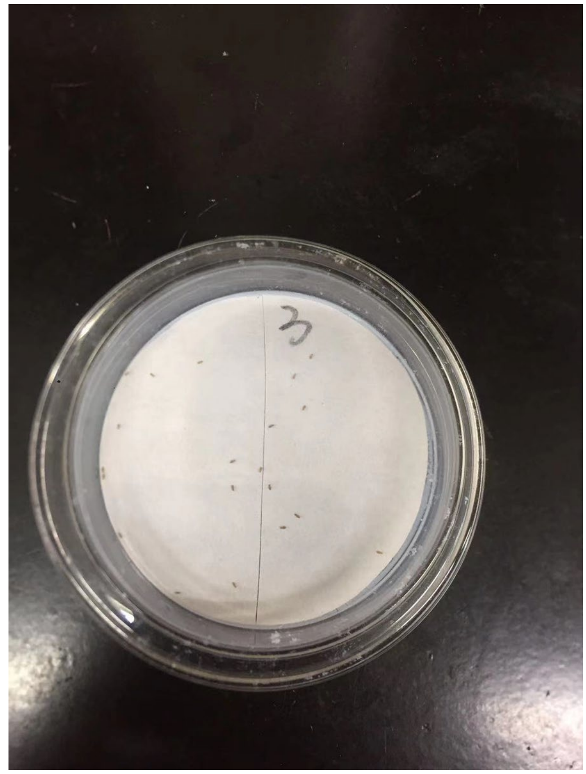

Fig.2 Bioassays of repellent toxicity against *L. bostrychophila* at the third concentration

Materials and methods

Plant material and the extraction of essential oil

The aerial parts of samples used in the experiment were collected from Guiyang city, Guizhou Province in November 2018 and were stored in a cool and ventilated place, the sample was identified by Dr QR. Liu (College of Life Sciences, Beijing Normal University, Beijing 100,875, China).

The samples were air-dried in the shade at room temperature, 1.81 kg of air-dried samples of *C. bungei* were weighed and crushed then were put into a modified extracter. Water was added in accordance with 1:10 ratio of material to liquid, and samples were extracted by hydrodistillation for 6 h. Anhydrous sodium sulfate took responsibility for the dehydration of EO. Finally, 0.2 ml of EO was obtained and the EO yield was calculated as 0.011%. The dehydrated EOs were finally stored in sealed glass bottles for refrigeration at 4 ℃.

Methanol extract

The ground parts of the *C. bungei* were crushed in a high-speed pulverizer. 25 g of coarse powder was weighed then soaked in methanol and extracted with ultrasonic instruments for half an hour for 3 times, and the ratio of material to liquid was 1:10, 1:10 and 1:8 respectively. Combined the three solutions and filtered to a clear liquid, connected vacuum instrument with rotary evaporator to reduce pressure and accelerated concentration of the solution, and volatilized the solvent then got the methanol extract, weighed it and calculated the extract rate.

Insects

Tribolium castaneum, *Lasioderma Serricorne*, *Liposcelis bostrychophil*a were identified by Professor Zhi-Long Liu (Department of Entomology, China Agricultural University, Beijing 100,875, China). Three target insects were incubated in a duck incubator kept under laboratory conditions. The incubator kept a temperature between 28 to 30 ℃ and the humidity within 70–80%. *T. castaneum* and *L. serricorne* lived in glass bottle with the mixture of wheat flour and yeast, and the ratio of wheat to yeast was 10:1 (*w/w*). While, *L. bostrychophila* lived in a glass conical flask with the mixture of wheat flour, yeast and milk powder mixed at 10:1:1(*w/w/w*). Insect adults about 1–2 weeks old regardless of gender were adopted for bioassays.

GC–MS analysis

Chromatographic conditions: A quartz capillary column (DB-5MS, 30 m × 0.25 mm × 0.25 m) was used to analyzed the EO, the carrier gas was high pure helium, the flow rate was constant 1.0 mL/min, the injection volume was 1 μL, and the fractional ratio was 1:20 injection. The inlet temperature was 250 ℃, and the initial temperature of the column was 60 ℃. After 5 min of constant temperature, the temperature rose to 290 ℃at 7 ℃/min and remained for another 5 min. The temperature of the four-stage rod was 150 ℃.

Mass spectrometry conditions: EI (electron bombardment) ionization source was selected, the electron energy was 70 eV, the ion source temperature was 200℃, the transmission line temperature was 250℃, the mass scanning range was 45–800 m/z, the full scanning mode was 0.4 s each time.

By computer retrieving and matching with WILEY 275 and NIST 05 standard spectrum database data were conducted, and spectrogram analysis was combined with relevant literatures to identify the types of compounds, and the relative percentage content of each component was obtained by gas chromatography peak area normalization method.

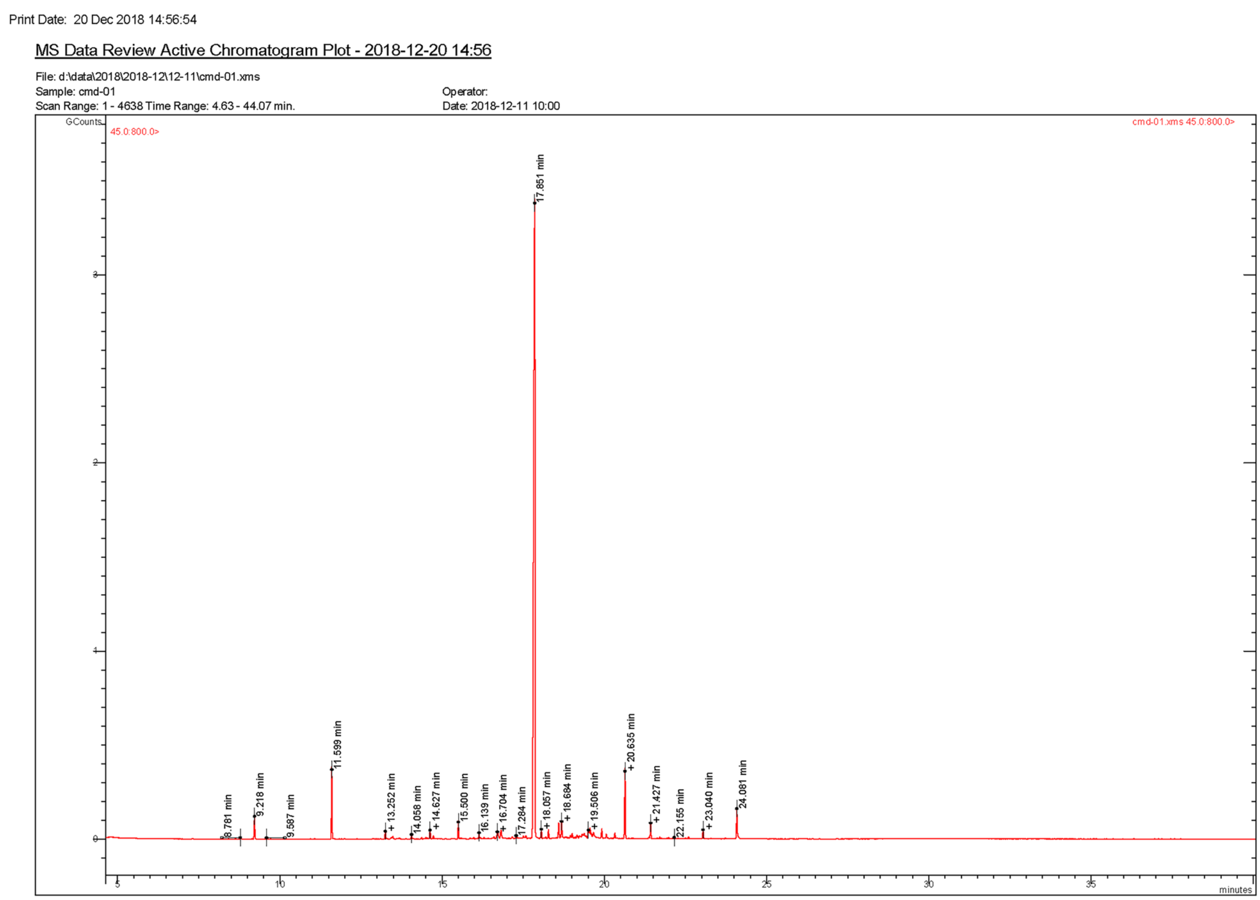

Fig. 3 GC–MS total ion flow chromatogram of *C. bungei* EO

Bioassays of repellent toxicity

The repellent effects of the *C. bungei* EO, methanol extract and two major components (myristicin and linalool) against *T. castaneum* and *L.serricorne* adults were assessed using the area preference method [13]. The EO and two major components were prepared in *n*-hexane to obtained five testing concentrations, while the methanol extract were prepared in anhydrous methanol. The testing concentrations of the four samples are78.63, 15.73, 3.15, 0.63 and 0.13 nL/cm^2. Cut the filter paper into a circle with a diameter of 9 cm and cut it in half along the diameter. One half was uniformly treated with 500 μL of a testing solution and the other half was treated with an equal volume of n-hexane as negative control. DEET was set as the positive control. Glue two halves of filter paper onto the petri dish. The 20 test insects were released into the center of the petri dish, each text in five concentrations were repeated five times. The number of insects presented on the negative control should be observed and recorded after 2 h and 4 h respectively, and the percent repellency (PR) was calculated. As shown in Fig. 1, there was the distribution of the *T. castaneum* species under the third concentration after two hours.

For *L. bostrychophila* adults, some modifications have been made to the above methods. The filter paper and the Petri dish used in the test had a diameter of 5.5 cm. The testing concentrations of the samples are 63.17, 12.63, 2.53, 2.53, 0.51 and 0.10 nL/cm2, and the application volume of the testing solution was150 μL. Cut the filter paper into a circle with a diameter of 5.5 cm and cut it in half along the

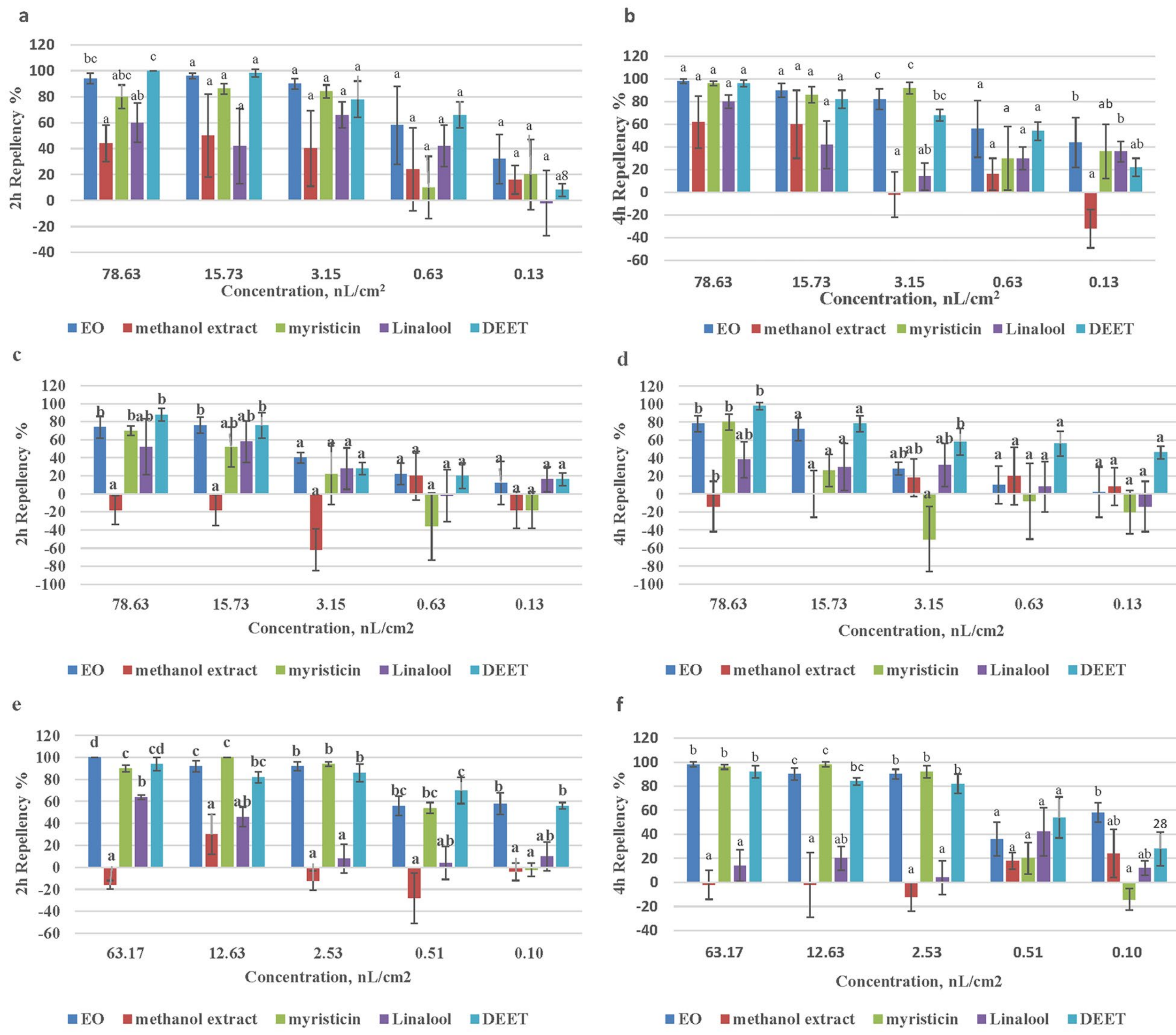

Figure 4 **a**, **c**, **e** Percentage repellency of *C. bungei* EO, major components of EO and methanol extract against *T. castaneum*, *L. serricorne*, and *L. bostrychophila* adults after 2 h of exposure, respectively. **b**, **d**, **f** Percentage repellency of *C. bungei* EO, major components of EO and methanol extract against *T. castaneum*, *L. serricorne* and *L. bostrychophila after 4 h*

diameter. One half was uniformly treated with 150 µL of a testing solution and the other half was treated with an equal volume of n-hexane as negative control, as shown in Fig. 2.

In repellent activity bioassay, the percent repellency was calculated by the following formula:

$$PR\ (\%) = \frac{N_c - N_t}{N_c + N_t} \times 100 \quad (1)$$

where Nc was the number of insects present in the negative control half and Nt was the number of insects present in the treated half. PR values were subjected to arcsine square root transformation before Analysis of variance (ANOVA) and Tukey's test. The results showed significant differences at $P < 0.05$ levels. The mean values were assigned to different classes (0 to V) using the scales described by Liu and Ho [14]: PR < 0.1, Class 0; PR = 0.1–20.0, Class I; PR = 20.1–40.0, Class II; PR = 40.1–60.0, Class III; PR = 60.1–80.0, Class IV; PR = 80.1–100.0, Class V.

Results

The chemical components of the essential oil

The yield of the EO was 0.01% (*V/W*), according to the above GC–MS conditions, the chemical composition of the *C. bungei* EO was identified. Figures 3, 4 shows the GC–MS total ion flow chromatogram of the EO. Finally, 25 compounds were identified, accounting for 96.3% of the total EO, as shown in Table 1. The result shows that the main constituents of the EO were 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)- generally (myristicin), 2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one and 1,6-Octadien-3-ol, 3,7-dimethyl- (linalool). Their content ranged from high to low was myristicin (75.0%), 2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one (4.1%) and linalool (3.4%) respectively.

Repellent toxicity

The results of repellent activity for the EO, myristicin, linalool and methanol extract on three target insects at different time points were shown in the Fig. 4. For *T. castaneum*, at the tested concentration above 3.15 nL/cm^2, the PR value of *C. bungei* EO and myristicin showed the best level of repellency and there was no significant difference with the positive control. At the concentration above 15.73 nL/cm^2, linalool and methanol extract also had repellent activity, and the PR value at 78.63 nL/cm^2 increased slightly when the exposure time was extended to 4 h. For *L. serricorne*, at the concentration of 78.63 nL/cm2, the *C. bungei* EO and myristicin had good repellent activity, but with the decrease of concentration, the PR values showed a significant decrease, linalool showed repellent activity only at the concentration above 15.73nL/cm^2 and 2 h post-exposure. Methanol extract had no repellent activity, but showed weak attractive effect at 2 h. For *L. bostrychophila*, at the tested concentration above 2.53 nL/cm^2, the PR values of EO and myristicin were more than 90%, and the repellent activity was significant, and the EO still has a certain repellent activity at the lowest concentration. At the concentration of 63.17 nL/cm^2 and 2 h post-exposure, linalool showed good repellent effect, but with the decrease of concentration and the extension of time, its PR values decreased significantly. Methanol extract showed no repellent activity but weak attractive effect.

Table 1 Chemical constituents of *C. bungei* EO

Peak no	Compounds	RI $_{Lit}$	RI $_{Exp}$	Relative content (%)
1	1,2,4-Trioxolane, 3,5-diphenyl-	-	976	0.1
2	1-Octen-3-ol	981	997	1.3
3	3-Octanol	-	1018	0.1
4	Linalool	1090	1144	3.4
5	α-Terpineol	1174	1268	0.5
6	Geraniol	1241	1334	0.3
7	Bornyl acetate	1272	1382	0.5
8	Dihydroedulan IIA	-	1391	0.2
9	α-Terpinyl acetate	1360	1461	0.9
10	β-Elemen	1388	1521	0.3
11	Nerolidyl acetate	1687	1587	0.8
12	β-Guaiene	1494	1664	0.3
13	Myristicin	1489	1692	75.0
14	Elemicin	-	1714	0.5
15	Nerolidol	1540	1738	0.5
16	Spathulenol	1563	1772	1.0
17	Caryophyllene oxide	1566	1782	1.1
18	Cubenol	1601	1835	1.4
19	1,4-*trans*-1,7-*cis*-Acorenone	-	1924	0.6
20	2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	-	2009	4.1
21	6,10,14-Trimethyl-2-pentadecanone	-	2109	0.8
22	Z,E-2,13-Octadecadien-1-ol	-	2122	0.1
23	Farnesol (E), methyl ether	-	2204	0.1
24	(E)-Nuciferol	-	2324	0.6
25	Phytol	2128	2376	2.0
	Total			96.3

RI $_{Exp}$.: Experimentally determined linear retention indices using homologous series of C7-C30 alkanes

RI $_{Lit}$.: Linear retention indices taken from literature [15]

Discussions

According to the results of repellent activity for the EO, myristicin, linalool and methanol extract on three target insects at two time points, it can be analyzed that in high tested concentration, the EO of *C. bungei* has significant repellent activity on the three storage pests, with good persistence and long effective time. The myristicin and linalool also had considerable repellent activity. The repellent activity of EO is better than that of the single components, which indicates that there may be synergistic effect among the components. No obvious repellent activity was observed in methanol extract, which may be due to the presence of non-volatile substances in methanol extract, and the antagonistic effect with myristicin and other components. It is also possible that methanol extract shows poor repellent activity due to its low content of EO and low concentration of effective constituent. Methanol extract showed weak attraction effect, which may be due to the presence of non-volatile substances with strong attraction effect, obscuring its repellent components, and thus showing an attraction effect on target insects. At the same time, there are some errors and randomness in biological activity test, which may lead to deviations in the results within a certain range.

Conclusion

In this experiment, the EO of *C. bungei* extracted by hydrodistillation was analyzed by GC–MS and GC, all components were identified, the principal compounds were myristicin (75.0%), 2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one (4.1%) and linalool (3.4%). The bioassay results showed that the EO had significant repellent activity against *T. castaneum, L. serricorne* and *L. bostrychophila*, the main components myristicin and linalool also had certain repellent activity. Therefore, it is feasible to develop the botanical repellents based on the EO and its main components of *C. bungei.* But security issues whether botanical pesticides are truly safe to humans and environment still needs a lot of toxicological tests to investigate and verify.

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Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Xin-Xin Lu and Na-Na Hu. The first draft of the manuscript was written by Xin-Xin Lu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and material All data are fully available without restriction.

Declarations

Consent for publication All data generated or analyzed during this study are included in this published article.

Conflicts of interest The authors declare that they have no competing interests.

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