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Cosmetic potentials of extracts and compounds from *Zingiber cassumunar* Roxb. rhizome

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ABSTRACT

Zingiber cassumunar Roxb. is a medically important plant with extensive traditional medicinal functions and bioactivities. It is also used in some skin-care balm by local people. Nevertheless, the research on the active components of the plant related to skin-care is not enough, which mainly involved some antioxidant and anti-inflammatory active compounds. This study investigated the extracts and components from rhizome of *Z. cassumunar* for their 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, HDFa collagen secretion promotion, tyrosinase inhibition, and NO generation inhibition activities for the purpose of identifying ingredients with potential anti-aging, skin-whitening, and anti-inflammation for use in cosmetics. Ethyl acetate (EtOAc) extract of the plant showed significant NO generation inhibition, DPPH radical scavenging, tyrosinase inhibitory activities, and weak promoting collagen secretion activity while petroleum ether (PE) extract showed obvious NO generation inhibition activity. Six active compounds, (*E*)-3-(3,4-dimethoxyphenyl)-2-propenal (**1**), *cis*-3-(3,4-dimethoxyphenyl)-4-[(*E*)-2,4,5-trimehoxystyryl]cyclohex-1-ene (**2**), 1-feruloyloxy cinnamic acid (**3**), (*1E,4E,6E*)-1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (**4**), bisdemethoxycurcumin (**5**), and curcumin (**6**), were isolated from PE and EtOAc extracts by bioassay-guided fractionation. **1**, **3**, **4**, **5**, and **6** showed significant NO generation inhibition activity with IC_{50} of 22.67 ± 2.36, 7.00 ± 0.22, 15.40 ± 0.63, 5.47 ± 0.09, and 7.16 ± 0.05 μM, respectively. **3** also showed potent DPPH radical scavenging activity with IC_{50} of 22.96 ± 0.87 μg/mL. **4** was a good tyrosinase inhibitor with the inhibitory rate of 42.56 ± 1.02% under the concentration of 100 μg/mL. **2** and **4** showed significant HDFa collagen secretion promoting activity with EC_{50} of 13.91 ± 1.56 and 8.50 ± 1.31 μg/mL, respectively. This is the first report on components from *Z. cassumunar* for their HDFa collagen secretion promotion and tyrosinase inhibition activities. These results suggest that medicinal plant *Z. cassumunar* possesses potential application value in the development of natural cosmeceutical products for anti-aging, skin-whitening, and anti-inflammation activity.

1. Introduction

The skin is the biggest organ and also as an important barrier that protects the body against injury and maintains the body temperature and water (Taofiq et al., 2016). Skin aging and skin diseases are attracting more and more attention. Skin aging is divided into intrinsic aging and extrinsic aging, in which intrinsic aging also known as natural aging results from the change of hormone occurring with age. Extrinsic aging is mainly caused by over exposure to ultraviolet (UV) radiation, which is also called photo aging. UV radiation caused the

Abbreviations: CC, column chromatography; NMR, nuclear magnetic resonance; HDFa, human dermal fibroblasts-adult; IC_{50}, sample concentration providing 50% inhibition rate; EC_{50}, sample concentration providing 50% increasing rate of collagen secretion

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formation of reactive oxygen species (ROS). ROS can cause the damage of cell tissues and also induce the expression of matrix metalloproteinases (MMPs), elastase, and hyaluronidase, which lead to the degradation of collagen, elastin, and hyaluronic acid. Skin ageing is often accompanied by collagen and elastin degradation, dryness, color spots, and wrinkles (Stierlin et al., 2017; Papakonstantinou et al., 2012; Thring et al., 2009). In addition, skin inflammation is a common skin disorder and it can cause serious skin destruction. Skin inflammation results from many factors including ultraviolet radiation, chemical irritants, and immune cells stimulated by microbial endotoxin and inflammatory mediators (Boonyuan et al., 2014; Xue et al., 2017). Hyperpigmentation is also a common skin disorder and results from the overproduction of melanin. Tyrosinase is a key enzyme controlling the production of melanin. Tyrosinase inhibitor is an available method to solve hyperpigmentation and play the role of skin whitening (Lall and Kishore, 2014). Therefore, the development of antioxidants, elastase inhibitor, hyaluronidase inhibitors, anti-inflammatory agents, tyrosinase inhibitor, and collagenase inhibitor play significant role to solve the skin aging and skin disorders. Nowadays, herbal cosmetics is gradually popular and become a trend because plant extracts and their metabolites are rich in active ingredients which can be used for treatment of skin ageing and skin disorder (De Wet et al., 2013; Mukherjee et al., 2011). Therefore, study on active extracts and individual constituents related with anti-aging, anti-inflammation, and skin-whiting are important to the development of functional cosmetics.

Zingiber cassumunar Roxb. (Family *Zingiberaceae*) commonly known as “Bulei” by Dai people in Xishuangbanna of Yunnan province of China, is a common raw materials of Dai medicine which is one of China’s four major ethnic medicines. The rhizome of this plant has many traditional medicinal functions, such as treating joint inflammations, muscle pain, sprains, contusions, menstrual disorders, abscesses, and skin diseases (Koontongkaew et al., 2014; Pongprayoon et al., 1997). *Z. cassumunar* Roxb. is also called as “plai” in Thailand and plai cream showed obvious effects for pain reduction and acne vulgaris treatment (Chongmelaxme et al., 2017). The essential oil (EO) of *Z. cassumunar* Roxb. could be used for acne control and the treatment of skin infections (Lertsatitthanakorn et al., 2006; Pithayanukul et al., 2007). Antioxidant activities of cassumunarins A, B, C, and curcumin isolated from *Z. cassumunar* had been confirmed (Masuda et al., 1995; Okazaki et al., 1997). The EO shows anti-inflammatory activity in the topical rat inflammation models and antioxidant activity (Jeenapongsa et al., 2003; Leelapornpisid et al., 2008; Leelarungrayub et al., 2017; Pongprayoon et al., 1997). The study on the anti-inflammatory activity of *Z. cassumunar* mainly focuses on two phenylbutenoids, (*E*)-1-(3,4-dimethoxyphenyl)butadiene (DMPBD) and (*E*)-4-(3′,4′-dimethoxyphenyl) but-3-en-1-ol, and cassumunins A, B, C and curcumin (Han et al., 2005; Jeenapongsa et al., 2003; Masuda et al., 1995; Panthong et al., 1997; Priprem et al., 2016; Suksaeree et al., 2015). Cis-3-(2′,4′,5′-trimethoxyphenyl)-4-{(*E*)-*2′″,4′″,5′″*-trimethoxystyryl}cyclohex-1-ene and (*E*)-4-(3′,4′-dimethoxyphenyl)but-3-en-1-ol (compound D) isolated from *Z. cassumunar* prossess chondroprotective activity, which suggested it had the potential in the treatment of degenerative joint diseases (Rujirek et al., 2016). Compound D also could enhance Melanogenesis (Park et al., 2015). Moreover, anti-tyrosinase and antioxidant activity of crude extract from *Z. cassumunar* has also been reported (Sungthong and Phadungkit, 2015). To sum up, *Z. cassumunar* has extensive bioactivities involving its volatile oil, extracts, and active compounds. However, the active components of some reported cosmetic related bioactivities are still unclear except for the reports of antioxidant and anti-inflammatory active ingredients. Therefore, the aim of the present study is to comprehensively evaluate cosmetic potentials of extracts and compounds from *Z. cassumunar* Roxb. rhizome for its anti-aging, skin-whitening, and anti-inflammation. The anti-aging activity was evaluated by designing DPPH radical scavenging and promoting HDFa collagen secretion assay. The skin-whitening and the anti-inflammation activities were evaluated by the tyrosinase inhibition assay and NO generation inhibition assay, respectively.

2. Materials and methods

2.1. Chemicals and regents

The mouse mononuclear macrophage cell (RAW 264.7) was obtained from Shanghai cell bank of Chinese academy of sciences (Shanghai, China). The human dermal fibroblasts-adult (HDFa) cell was purchased from Invitrogen (California, U.S.A.). Trypsin (including EDTA) was obtained from Gibico (New York, U.S.A.). Lipopolysaccharide (LPS), 2,2-diphenyl-1-picrylhydroazyl (DPPH), Trolox, Griess reagent, L-NMMA, L-Dopa, kojic acid, α-arbutin, and mushroom tyrosinase was obtained from Sigma (Shanghai, China). DMEM (high sugar) medium, PBS, Hank's balanced salt solution (HBSS), streptomycin and fetal bovine serum (FBS) were purchased from Thermo Fisher (Massachusetts, U.S.A.). The transforming growth factor beta (TGF-β) was obtained from PeproTech (New Jersey, U.S.A.). Collagen ELISA kit was purchased from TaKaRa Bio. MTS reagent was obtained from Promega (Wisconsin, U.S.A.).

2.2. General experimental procedures

Nuclear magnetic resonance (NMR) spectra was recorded on a Bruker AV-500 instrument and Bruker AV-600 instrument (Bruker, Bremen, Germany) at 500 MHz and 600 MHz for the ^{1}H (500 MHz and 600 MHz) and ^{13}C (125 MHz and 150 MHz) NMR spectra. Chemical shifts (δ) were given in parts per million (ppm) with reference to the solvent signals and coupling constants were expressed in hertz. The absorbance was measured by a Microplate Spectrophotometer (BioTek, Vermont, US). Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Marine Chemical Co. Ltd., China), silica gel H (10–40 μm, Qingdao Puke Parting Materials Co. Ltd., China) and Sephadex gel LH-20 (GE healthcare bio-sciences AB, Sweden). All solvents used for column chromatography were distilled prior to use. Semipreparation HPLC with a DAD detector was performed with a Hanbon semi-preparation liquid chromatography system (Hanbon Sci. and Tech. Co., Ltd., Jiangsu, China) equipped with a column YMC-Pack ODS-AQ (250 mm × 10 mm, 5 μm, YMC Co., Japan). Fractions were analyzed by thin layer chromatography (TLC) on silica gel GF254 (Qingdao Marine Chemical Co. Ltd., China), and spots were visualized by heating silica gel plates sprayed with 1% vanillinand and 10% H_2SO_4 in ethanol.

2.3. Plant materials

Fresh rhizomes of *Z. cassumunar* Roxb. were collected from the Dai hospital in Dai Autonomous Prefecture of Xishuangbanna, Yunnan Province of China and identified by Professor ShengJi Pei. A voucher specimen (KUN 1415653) was deposited in Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

2.4. Extraction and isolation of chemical compounds

The air-dried and powdered rhizomes (4.5 kg) of *Z. cassumunar* were extracted three times with 95% ethanol (4 × 6 L) at the room temperature. The combined filtrate was concentrated by rotary evaporator under reduced pressure to obtain ethanol extract. Then, the ethanol extract was suspended in 1 L water and sequentially extracted with 1 L petroleum ether (PE), 1 L chloroform ($CHCl_3$) and 1 L ethyl acetate (EtOAc) for times. Finally, PE extract (55.85 g), $CHCl_3$ extract (310.10 g) and EtOAc extract (10.90 g) were obtained.

The PE extract (43.5 g) was performed with a silica gel column with the elution of PE-EtOAc solvent system (100:0, 20:1, 15:1, 9:1, 4:1, 3:1, 2:1) to yield nineteen fractions (P1–P19). P17 was chromatographed with a gradient elution of PE−$CHCl_3$ (3:1, 2:1, 1:1, 0:100) on a silica gel

column to afford three fractions P17a, P17b and P17c. P17b (186 mg) was purified by a silica gel column, eluted with PE−$CHCl_3$ (2:3, 2:4, 1:4) to give **1** (60 mg). P18 (1.619 g) was chromatographed with elution of acetone on a Sephadex LH-20 column to obtained three fractions P18a, P18b and P18c. P18b was chromatographed on a silica gel column with a gradient elution of PE−$CHCl_3$ (1:2, 1:3, 1:4, 1:5, 1:8) to afford four fractions P18b1–P18b4. P18b2 (550 mg) was separated by a silica gel column with a gradient elution of PE−$CHCl_3$ (1:4, 1:5, 1:7, 1:9) to furnish two fractions P18b2a and P18b2b. P18b2b (90 mg) was further subjected to semipreparative HPLC on ODS-AQ column (250 mm × 10 mm I.D., 3.0 mL/min, 78% MeOH-H_2O) to afforded **2** (45 mg).

EtOAc extract (10 g) was chromatographed on a silica gel with a gradient elution of $CHCl_3$-acetone (70:1, 50:1, 30:1, 15:1, 4:1, 1:1) to yield ten fractions E1–E10. E3 (170 mg) was separated on a silica gel column using PE-acetone(9:1, 5:1, 3:1, 1:1 v/v) as the eluent to afford five fractions E3a–E3e. E3e (20 mg) was purified by semipreparative HPLC on ODS-AQ column (250 mm × 10 mm I.D., 3.0 mL/min, 75% MeOH−H_2O) to give **6** (6 mg). E4 was separated on a silica gel column using PE-acetone (5:1 to 4:1) as the eluent to yield four fractions E4a–E4d. E4c(20 mg) was subjected to semipreparative HPLC on ODS-AQ column (250 mm × 10 mm I.D., 3.0 mL/min, 80% MeOH-H_2O) to afford E4c1. E4c1 (12 mg) was purified by a vacuum silica gel column (silica gel H) using PE-acetone (7:1) as the eluent to give **3** (7 mg). E6 (435 mg) was separated on a Sephadex LH-20 column with the elution of MeOH to obtained four fractions E6a-E6d, in which E6d was the pure compound **5** (127 mg). E6b (25 mg) was subjected to semipreparative HPLC on ODS-AQ column (250 mm × 10 mm I.D., 3.0 mL/min, 80% MeOH−H_2O) to afford E6b1. E6b1 (12 mg) was purified by a vacuum silica gel column, eluted with PE-acetone (6:1) to give **4** (4 mg).

2.5. Anti-inflammatory activity

Anti-inflammatory activity was evaluated by the inhibitory effect of NO Production induced by LPS in mouse peritoneal macrophages cell (RAW264.7). RAW264.7 was cultured in DMEM medium at 37 °C and 5% CO_2. Then, cells were inoculated into 96-well plates and induced with LPS (1 μg/ml). The sample solution which was diluted from 50 μg/ml or 50 μM with two times dilution to give five concentration gradients was added into the 96-well plates to react. A blank control which didn't contain sample and a positive control which used L-NMMA to replace sample were set. After overnight culture, the production of NO was detected from the culture supernatant according to the Griess method by measuring the absorbance at 540 nm (Guevara et al., 1998). MTS were added to the remaining culture medium to detect the cell survival and exclude the toxic effects of the testing sample. The inhibition rate of NO production was calculated by following formula: Inhibition rate (%) = $(1 - A_{S/AB}) \times 100\%$, where A_B and A_S were the absorbance of the blank control and the sample solution, respectively. Finally, the calculation of IC_{50} (sample concentration providing 50% inhibition rate) performed by the Reed and Muench method (Reed and Muench, 1937).

2.6. In vitroantioxidant capacity

Antioxidant activity was conducted by DPPH radicals scavenging method. The free radicals 2,2-diphenyl-1-picrylhydroazyl (DPPH) were used to evaluated antioxidant activity of *Z. cassumunar* Roxb. The testing sample and DPPH (Final concentration = 90 μM) were mixed. A blank control which didn't contain testing sample and a positive control Trolox were set. After mixing, it was maintained for 1 h at 30 °C. Then, absorbance was measured by microplate reader at 515 nm. DPPH radical scavenging rate which was equal to the percentage of DPPH discolouration was calculated by following formula: Scavenging rate (%) = $(1 - A_{S/ADPPH}) \times 100\%$, where A_s was the absorbance of the solution containing the sample at 515 nm, and A_{DPPH} was the absorbance of the DPPH solution.

2.7. Collagen production by human dermal fibroblasts adult (HDFa)

HDFa and samples solution were mixed in 12-well plates and cultured for three days at 37 °C and 5% CO_2. A blank control which didn't contain sample and a positive control TGF-β were set. Then, the supernatant of cell culture were collected and stored in −80 °C. Cell viability was evaluated by using MTS colorimetry to detect the Absorbance of 490 nm. The secretion of collagen was detected following the method provided by collagen ELISA kit. The supernatant was diluted at 1:100 times. Absorbance was determined by microplate reader at 450 nm. All experiments were conducted in triplicate. The cell viability (%) and increase rate (%) of collagen secretion were calculated by following formulas: Cell viability (%) = $A_{s\text{-}490\ nm}/A_{b\text{-}490\ nm} \times 100\%$; Increase rate (%) = $[(A_{s\text{-}450\ nm}/CV/A_{b\text{-}450\ nm}) - 1] \times 100\%$, where $A_{s\text{-}490\ nm}$ was the absorbance of test groups containing sample solution at 490 nm; $A_{b\text{-}490\ nm}$ was the absorbance of the blank group at 490 nm; $A_{s\text{-}450\ nm}$ was the absorbance of test groups containing sample solution at 450 nm; CV was the cell viability of the HDFa; $A_{b\text{-}450\ nm}$ was the absorbance of the blank group at 490 nm.

2.8. Tyrosinase in vitroinhibition

After sample solution and L-Dopa were mixed in 96 well plate, tyrosinase (final concentration 25 U/ml) was added to start the reaction. The reaction kept 5 min at room temperature. Furthermore, a blank control which didn't contain sample and a positive control α-arbutin were set. All experiments were conducted in triplicate. Absorbance was measured by microplate reader at 490 nm. Tyrosinase inhibitory rate was calculated by following formula: Inhibitory rate (%) = $(1 - A_{s}/A_{b}) \times 100\%$, where A_s is the absorbance of the solution containing the sample at 490 nm. A_b was the absorbance of the blank group.

3. Results and discussion

3.1. Activities of the extracts from the rhizomes of Z. Cassumunar Roxb

Crude ethanol extract of rhizomes *of Z. cassumunar* was further extracted by petroleum ether (PE), chloroform ($CHCl_3$), and ethyl acetate (EtOAc) successively to give three different solvent extracts. Cosmetic activities of the above three extracts were evaluated by NO generation inhibition, DPPH radical scavenging, tyrosinase inhibitory, and promoting HDFa collagen secretion bioassay and results were summarized in Table 1. PE extract showed potent anti-inflammatory activity and EtOAc extract showed significant anti-inflammatory, antioxidant, tyrosinase inhibitory activities, and weak promoting collagen secretion activity. $CHCl_3$ extract only showed moderate activity in antioxidant bioassay, as well as weak activity in the anti-inflammatory and tyrosinase inhibitory bioassay. Therefore, PE and EtOAc extracts were regarded as the active fractions and subjected to further systematically fractionation to discover their active constituents.

3.2. Isolation and structure elucidation of compounds from the rhizomes of Z. cassumunar Roxb

In total, six active compounds were isolated by repeated column chromatography and semipreparative HPLC from PE and EtOAc extracts of *Z. cassumunar* Roxb rhizome, in which two compounds were afforded from PE extract and four compounds were obtained from EtOAc extract. Structures of the six isolated compounds were elucidated by spectroscopic methods and comparing the experimental spectroscopic date with the data previously reported. They are (*E*)-3-(3,4-dimethoxyphenyl)-2-propenal (**1**), *cis*-3-(3,4-dimethoxyphenyl)-4-[(*E*)-2,4,5-trimehoxystyryl]cyclohex-1-ene (**2**), 1-feruloyloxy cinnamic acid (**3**), (*1E,4E,6E*)-1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (**4**), bisdemethoxycurcumin (**5**), and curcumin (**6**). The structures of these

Table 1
Anti-inflammatory and cosmetic activity of extracts from rhizome of *Z. cassumunar* Roxb.

Extracts	NO generation inhibition (%)[a]	DPPH radical scavenger (%)[b]	Promoting HDFa collagen secretion (%)[c]	Tyrosinase Inhibition (%)[d]
PE extract	73.33 ± 1.46	−1.52 ± 0.22	2.31 ± 0.26	11.36 ± 0.01
$CHCl_3$ extract	27.84 ± 2.95	62.69 ± 0.18	−6.66 ± 0.021	9.24 ± 0.04
EtOAc extract	88.46 ± 0.94	86.23 ± 0.08	13.11 ± 0.03	36.99 ± 0.62

[a] Anti-inflammatory activity was evaluated by the inhibitory effect of NO Production induced by LPS in Mouse Peritoneal Macrophages (RAW264.7) and conducted at the concentration of 50 μg/mL.
[b] DPPH radical scavenging were measured at the concentration of 100 μg/mL.
[c] Promoting HDFa (human dermal fibroblasts-adult) collagen secretion assay was conducted at the concentration of 10 μg/mL.
[d] tyrosinase inhibition assay were measured at the concentration of 100 μg/mL.

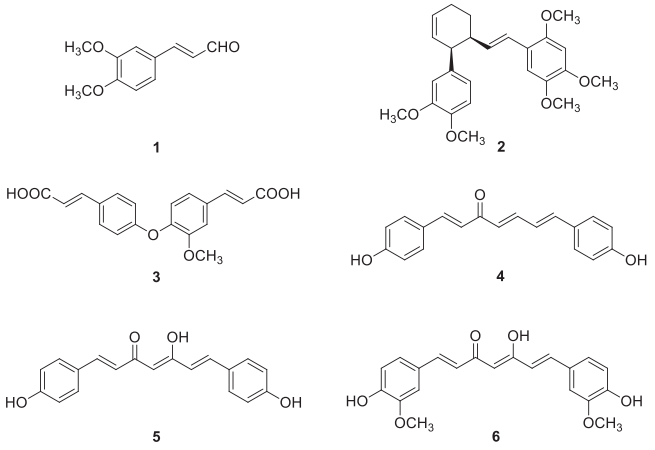

Fig. 1. Structures of compounds from the rhizome of *Zingiber cassummunar* Roxb.

compounds were exhibited in Fig. 1. **3** and **4** were firstly isolated from *Z. cassumunar* Roxb.

(*E*)-3-(3,4-Dimethoxyphenyl)-2-propenal (**1**), yellow amorphous solid, was identified by comparing the experimental spectroscopic date with the data previously reported (Ito et al., 2001). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.68 (1H, *d*, *J* = 7.8 Hz, H-3′), 7.42 (1H, *d*, *J* = 15.8 Hz, H-1′), 7.17 (1H, *dd*, *J* = 8.3, 2.0 Hz, H-6), 7.08 (1H, *d*, *J* = 2.0 Hz, H-2), 6.90 (1H, *d*, *J* = 8.3 Hz, H-5), 6.62 (1H, *dd*, *J* = 15.8, 7.8 Hz, H-2′), 3.93 (6H, *s*, 3, 4−OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 194.1 (C-3′), 153.4 (C-1′), 152.5 (C-4), 149.9 (C-3), 127.5 (C-1), 127.2 (C-6), 124.0 (C-2′), 111.6 (C-2), 110.3 (C-5), 56.5 (3, 4−OCH_3).

cis-3-(3,4-Dimethoxyphenyl)-4-[(*E*)-2,4,5-trimehoxystyryl]cyclohex-1-ene (**2**), white solid, was identified by comparing the experimental spectroscopic date with the data previously reported (Amatayakul et al., 1979; Kuroyanagi et al., 1980). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.80 (1H, *d*, *J* = 8.2 Hz, H-5′), 6.75 (1H, *dd*, *J* = 8.2, 2.0 Hz, H-6′), 6.71 (1H, *s*, H-6‴), 6.71 (1H, *d*, *J* = 2.0 Hz, H-2′), 6.60 (1H, *dd*, *J* = 16.0, 0.9 Hz, H-2″), 6.47 (1H, *s*, H-3‴), 5.94–6.02 (1H, *m*, H-1), 5.79 (1H, *ddt*, *J* = 10.0, 4.5, 2.1 Hz, H-2), 5.53 (1H, *dd*, *J* = 16.0, 9.2 Hz, H-1″), 3.87 (3H, *s*, OCH_3), 3.85 (3H, *s*, OCH_3), 3.78 (3H, *s*, OCH_3), 3.77 (6H, *s*, OCH_3), 3.52 (1H, *s*, H-3), 2.68–2.81 (1H, *m*, H-4), 2.13–2.29 (2H, *m*, H-5), 1.59–1.74 (2H, *m*, H-6); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 150.8 (C-2‴), 148.9 (C-3′), 148.1 (C-4‴), 147.6 (C-4′), 143.4 (C-5‴), 134.1 (C-1′), 133.0 (C-1″), 129.3 (C-2), 128.2 (C-1), 122.8 (C-2″), 122.1 (C-6′), 118.9 (C-1‴), 113.7 (C-2′), 110.3 (C-5′), 109.6 (C-6‴), 97.9 (C-3‴), 56.8 (OCH_3), 56.5 (OCH_3), 56.2 (OCH_3), 56.0 (OCH_3), 55.9 (OCH_3), 45.9 (C-3), 43.0 (C-4), 25.0 (C-6), 24.4 (C-5).

1-Feruloyloxy cinnamic acid (**3**), yellow amorphous solid, was elucidated by spectroscopic methods and comparing the experimental spectroscopic date with the data previously reported (Huang et al., 2000). 1H NMR (500 MHz, MeOD) δ: 7.55 (2H, *dd*, *J* = 15.8, 2.5 Hz, H-7, 7′), 7.47 (2H, *d*, *J* = 8.5 Hz, H-3′, 5′), 7.19 (1H, *d*, *J* = 2.0 Hz, H-3), 7.09 (1H, *d*, *J* = 8.3, 2.0 Hz, H-5), 6.81 (1H, *dd*, *J* = 8.5 Hz, H-6), 6.80 (2H, *dd*, *J* = 8.5, 2.5 Hz, H-2′, 6′), 6.61 (2H, *d*, *J* = 8.5 Hz, H-6), 3.89 (3H, *s*, OCH_3); ^{13}C NMR (125 MHz, MeOD) δ: 184.9 (C-9′), 184.7 (C-9), 161.1 (C-1′), 150.4 (C-2), 149.4 (C-1), 142.1 (C-7), 141.9 (C-7′), 131.2 (C-5′), 128.6 (C-4), 128.0 (C-4′), 124.1 (C-5′), 122.2 (C-8), 122.0 (C-6′), 116.9 (C-6′), 116.6 (C-6′), 111.7 (C-3), 56.4 (2−OCH_3).

(*1E,4E,6E*)-1,7-Bis(4-Hydroxyphenyl)-1,4,6-heptatrien-3-one (**4**), yellow amorphous solid, was identified by spectroscopic methods and comparing the experimental date with the data previously reported (Jang et al., 2004). ^{1}H NMR (500 MHz, CD_3COCD_3) δ: 7.57 (1H, *d*, *J* = 15.8 Hz, H-1), 7.56 (2H, *d*, *J* = 8.6 Hz, H-2′, 6′), 7.49 (1H, *dd*, *J* = 15.3, 10.6 Hz, H-5), 7.42 (2H, *d*, *J* = 8.6 Hz, H-2″, 6″), 7.02 (1H, *d*, *J* = 15.8 Hz, H-2), 6.98 (1H, *d*, *J* = 15.3 Hz, H-7), 6.93 (1H, *dd*, *J* = 15.3, 10.6 Hz, H-6), 6.86 (2H, *d*, *J* = 8.6 Hz, H-3′, 5′), 6.82 (2H, *d*, *J* = 8.6 Hz, H-3″, 5″), 6.59 (1H, *d*, *J* = 15.3 Hz, H-4); ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 188.7 (C-3), 160.7 (C-4′), 159.6 (C-4″), 144.0 (C-5), 142.9 (C-1), 142.0 (C-7), 131.2 (C-2′, 6′), 129.9 (C-2″, 6″), 129.2 (C-4), 129.1 (C-1′), 127.7 (C-1″), 125.4 (C-6), 123.6 (C-2), 116.8 (C-3′, 5′), 116.7 (C-3″, 5″).

Bisdemethoxycurcumin (**5**), yellow amorphous solid, was identified by spectroscopic methods and comparing the experimental date with the data previously reported (Kim et al., 2003; Syu et al., 1998). ^{1}H NMR (500 MHz, CD_3COCD_3) δ: 7.61 (2H, *d*, *J* = 15.8 Hz, H-1, 7), 7.57 (4H, *d*, *J* = 8.6 Hz, H-2′, 6′, 2″, 6″), 6.91 (4H, *d*, *J* = 8.6 Hz, H-3′, 5′, 3″, 5″), 6.67 (2H, *d*, *J* = 15.8 Hz, H-2, 6), 5.98 (1H, *s*, H-4); ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 184.3 (C-3, 5), 160.3 (C-4′, 4″), 140.8 (C-1, 7), 130.8 (C-2′, 6′, 2″, 6″), 127.5 (C-1′, 1″), 121.8 (C-2, 6), 116.6 (C-3′, 5′, 3″, 5″), 101.6 (C-4).

Curcumin (**6**), yellow amorphous solid, was identified by spectroscopic methods and comparing the experimental date with the data previously reported (Kim et al., 2003). ^{1}H NMR (500 MHz, $CDC1_3$) δ: 7.60 (2H, *d*, *J* = 15.8 Hz, H-l, 7), 7.12 (2H, *dd*, *J* = 8.0, 2.0 Hz, H-6′, 6″),7.05 (2H, *d*, *J* = 2.0 Hz, H-2′, 2″), 6.93 (2H, *d*, *J* = 8.0 Hz, H-5′, 5″), 6.48 (2H, d, *J* = 15.8 Hz, H-2, 6), 5.80 (1H, *s*, H-4), 3.95 (6H, *s*, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 183.4 (C-3), 148.0 (C-4′, 4″), 146.9 (C-3′, 3″), 140.7 (C-l, 7), 127.8 (C-l', 1″), 123.0 (C-6′, 6″), 121.9 (C-4, 6), 115.0 (C-5′, 5″), 109.7 (C-2′, 2″), 101.4 (C-4), 56.1 (3′, 3″−OCH_3).

3.3. Anti-inflammatory activity

Macrophages, as a kind of phagocytes, can widely participate in the inflammatory response in the body. When suffering the outsider incitement, abundant NO can be produced by macrophages. Excessive NO can react with superoxide anion (O^{2-}) to produce nitrite peroxide, which will lead to local tissue damage and promote the occurrence of inflammatory diseases. This study evaluated the inhibitory effect of NO production in accordance with the Griess method using the mouse mononuclear macrophage cell (RAW 264.7). The anti-inflammatory bioassay results of compounds **1**-**6** were showed in Table 2. Among these compounds, **1** (IC_{50} = 22.67 ± 2.36 μM), **3** (IC_{50} = 7.00 ± 0.22 μM), **4** (IC_{50} = 15.40 ± 0.63 μM), **5** (IC_{50} = 5.47 ± 0.09 μM), and **6** (IC_{50} = 7.16 ± 0.05 μM) indicated significant anti-inflammatory activities which were superior to the positive control L-NMMA (IC_{50} = 46.56 ± 2.39 μM) at a low toxicity

Table 2
Inhibitory effects of compound **1**-**6** on NO generation induced by LPS in Mouse Peritoneal Macrophages (RAW264.7).

Compounds	Concentration (μM)	Inhibitory rate (%)	IC_{50} (μM)
L-NMMA	50	51.94 ± 1.48	46.56 ± 2.39
1	50	79.44 ± 1.14	22.67 ± 2.36
2	25	15.09 ± 2.11	–
3	12.5	84.93 ± 1.26	7.00 ± 0.22
4	50	93.14 ± 1.03	15.40 ± 0.63
5	12.5	89.53 ± 0.96	5.47 ± 0.09
6	12.5	92.98 ± 0.64	7.16 ± 0.05

level (toxicity data are not listed in the article.). **2** (inhibitory rate 15.09% in 25 μM concentration) exhibit weak activity in this bioassay. For the first time, compound **3** and **5** with their most prominent anti-inflammatory activity were found in the present study. From previous research, the IC_{50} of **4** and **6** were 8 and 11 μM respectively in same anti-inflammatory model (Jang et al., 2004; Nakamura et al., 2009), which were consistent with the results of this study. The results indicate that *Z. cassumunar Roxb.* has potential value in the prevention and treatment of inflammatory diseases.

3.4. Antioxidant activity

When the free radicals exceed the human body's antioxidant defense ability, damaged molecules and cells will increase sharply then oxidative damage will occur, which may lead to inflammation, dry skin, dark yellow, relaxation, wrinkles and so on. Therefore, the screening of natural products with free radical scavenging activity has certain significance for the development of anti-aging cosmetics. The antioxidant activity of compounds **1**-**5** were measured by using DPPH scavenging assay (Table 3). Among them, both compound **1** and **2** didn't show antioxidant activity, but **3** exhibited strong antioxidant activity with the antioxidant rate of 94.16% at the concentration of 100 μg/ml and 50% free radicals can be scavenged at the concentration of 22.95 μg/ml. Compound **4** and **5** also showed a certain activity for DPPH radical scavenging (56.27% and 35.20%) at the concentration of 100 μg/ml, which belong to the analogues of curcumin. The antioxidant activity of curcumin **6** and its analogues have been extensively investigated(Anand et al., 2008; Niu et al., 2012), but DPPH scavenging activity of **3** was firstly reported in this study.

3.5. Promoting HDFa collagen secretion activity

Collagen is the most abundant protein in animals, accounting for 70% of human skin protein. Once there is a lack of collagen in the skin, the collagen fibers will be cross-linked and solidified, as well as the intercellular mucopolysaccharides will be reduced, also the skin will lose elasticity and become thin and aging. Type I collagen is the main type of collagen in the skin, and the secretion level of type I collagen can be reflected by the secretion level of type I procollagen. Therefore, in this assay, the increase rate of type I collagen secretion was calculated by detecting type I procollagen. As can be seen from the results in Table 4, all these compounds didn't show cytotoxic effects for the HDFa by the MTS colorimetric assay except for compound **6** which showed weak cytotoxicity. Compounds **1**, **3**, **5**, and **6** didn't show promoting effects for the collagen secretion. Compound **2** and **4** showed potent promoting collagen secretion activity with EC_{50} of 11.43 ± 1.08 and 8.50 ± 1.31 μg/mL, respectively. The collagen-promoting activity of *Z. cassumunar* Roxb has not been mentioned in the literature. The collagen-promoting activity of compound **2** and **4** were found in this study and reported for the first time.

Table 3
DPPH radical scavenging activity of compounds isolated from Z. cassumunar Roxb.

Compounds	Concentration (μg/mL)	Scavenging rate (%)	IC_{50} (μg/mL)
Trolox	25	95.49 ± 0.08	4.52 ± 0.36
1	100	−1.76 ± 1.00	–
2	100	8.05 ± 0.03	–
3	100	94.16 ± 0.01	22.95 ± 0.87
4	100	56.27 ± 0.03	–
5	100	35.20 ± 0.03	–

3.6. Tyrosinase inhibitory activity

Tyrosinase is the main rate-limiting enzyme in melanin production, and its activity is positively correlated with the amount of melanin synthesis. Dopa is converted to dopaquinone catalyzed by tyrosinase. There are two metabolic pathways for dopaquinone. The first one continues to convert to melanin, and the second one interacts with cysteine and turns to brown melanin. If the sample can inhibit the activity of tyrosinase, it can indirectly inhibit the formation of melanin. Accordingly, the effect of compounds **1**-**6** on mushroom tyrosinase were analyzed by this assay and the results were summarized in Table 5. α-Arbutin has been widely used as a cosmetic raw material, and it was selected as positive control in this experiment. Compounds **1** (17.34%), **3** (15.88%), and **5** (18.47%) showed similar tyrosinase inhibitory activity as α-arbutin(22.16%), but compound **2** and **6** showed no activity. It is worth mentioning that the tyrosinase inhibitory activity of compound **4** was 1.9 times that of α-arbutin, which was first discovered in the present study, and the tyrosinase inhibition of *Z. cassumunar* Roxb has not been reported in the literature as well.

4. Conclusion

Z. cassumunar Roxb, as a traditional Dai medicine, is widely used by local people in the treatment of superficial gastritis, spasmodic pain, indigestion and so on. Many previous studies have been focused on the chemical constituents and their medicinal activity. While in the present study, new biological activities, such as DPPH radical scavenging, HDFa collagen secretion promotion, tyrosinase inhibition, and NO generation inhibition, were found from PE and EtOAc extracts of the plant. Specific active compounds which mainly responsible for the above mentioned bioactivity were isolated by bioassay-guided fractionation and their chemical structures were elucidated by spectroscopic methods. HDFa collagen secretion promoting activity of *cis*-3-(3,4-dimethoxyphenyl)-4-[(*E*)-2,4,5-trimehoxystyryl]cyclohex-1-ene (**2**) and (*1E*,*4E*,*6E*)-1,7-bis (4-hydroxyphenyl)-1,4,6-heptatrien-3-one (**4**), tyrosinase inhibiting activity of **4**, and NO generation inhibiting activity of 1-feruloyloxy cinnamic acid (**3**) and bisdemethoxycurcumin (**5**), were discovered for the first time. These results suggest that the rhizome of *Z. cassumunar* Roxb. has remarkable cosmetic potentials in functional cosmetics research and development for its anti-aging, skin-whitening, and anti-inflammation activities. It also provides a new way of thinking for the basic and applied research of traditional ethnic medicine resources.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Table 4
Promoting collagen secretion activity of compounds from *Z. cassumunar* Roxb.

Compounds	Concentration (μg/mL)	HDFa viability (%)	Increasing rate of collagen secretion (%)	EC_{50} (μg/mL)
TGF-β	0.01	110.22 ± 0.11	60.13 ± 0.53	0.004 ± 1.04
1	10	135.76 ± 1.17	−31.32 ± 1.00	–
2	10	83.60 ± 0.09	46.77 ± 1.03	11.43 ± 1.08
3	10	125.08 ± 0.86	−19.96 ± 1.54	–
4	10	104.80 ± 2.14	52.94 ± 0.38	8.50 ± 1.31
5	10	138.78 ± 3.77	−51.57 ± 2.76	–
6	10	46.05 ± 1.43	−80.57 ± 0.77	–

Table 5
Tyrosinase inhibitory activity of compounds from *Z. cassumunar* Roxb.

Compounds	Concentration (μg/mL)	Inhibitory rate (%)
α-Arbutin	100	22.16 ± 0.04
1	100	17.34 ± 0.03
2	100	−17.02 ± 0.01
3	100	15.88 ± 0.07
4	100	42.56 ± 0.46
5	100	18.47 ± 0.13
6	100	−29.62 ± 0.06

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