

## ORIGINAL RESEARCH

# Chinese Propolis: Ultrasound-assisted enhanced ethanolic extraction, volatile components analysis, antioxidant and antibacterial activity comparison

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## Abstract

This study was aimed to enhance the extraction yield of propolis samples using ultrasound technology, analyze the volatile compounds, and compare the antioxidant and antimicrobial effect of propolis extracts of different areas. Four propolis samples were collected from different regions of China, namely: Linqing, Shandong Province (LSP); Yingchun, Heilongjiang Province (YHP); Changge, Henan Province (CHP); and Raohe, Heilongjiang Province (RHP). The ultrasound extracts of CHP and RHP showed a higher total phenolic content (TPC) of  $201.78 \pm 4.60$  mgGAE/g and  $166.071 \pm 1.53$  mgGAE/g, total flavonoid content (TFC) of  $519.77 \pm 29.90$  and  $341.227 \pm 10.82$  mg quercetin/g respectively, as well as high antioxidant and antibacterial activity. Conventional extraction showed 15%–20% lower yield for TPC ranging from  $72.02 \pm 1.99$  to  $155.95 \pm 3.69$  mg GAE/g, TFC ranges from  $129.675 \pm 6.82$  to  $412.83 \pm 12.14$  mg quercetin/g, with lower antibacterial activity. The antioxidant activity of propolis extracts was determined by assays of reducing power, DPPH\*, FRAP\*, TEAC\*, hydroxyl radical scavenging activity and superoxide anion scavenging activity. Collectively, the antioxidant activities of extracts from CHP and RHP were higher than those of the other two extracts (YHP and LSP). All the extracts showed high antimicrobial activity on *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus subtilis*, but no effect on *Escherichia coli*. A total of 150 compounds in propolis were detected by GC/MS. Terpenes (RHP 34%, YHP 5%, LSP 18%, and CHP 12%) and alcohols (RHP 12%, YHP 13%, LSP 12%, and CHP 10%) showed the highest relative content among all other extracts.

## KEYWORDS

antibacterial activity, antioxidant activity, HS-SPME-GC-MS, propolis, ultrasound-assisted extraction

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## 1 | INTRODUCTION

Propolis is a material collected by bees from the sap of trees and mixed with their saliva to seal and sterilize the hive (Reis et al., 2019). Generally, propolis is composed of 50% resins, 30% vegetable balsams, 10% wax, 5% aromatic and essential oils, and 5% pollens and other substances (Rufatto et al., 2017). The chemical composition varies depending on the source of the plant. Propolis exhibits a broad spectrum of biological activities, including anticancer, antibacterial (Kasiotis et al., 2017) and antioxidant properties (Yuan et al., 2020). Many studies have indicated a particular interest in the antioxidant activities of propolis using various assays. These assays are mainly spectrophotometric, which include free radical scavenging (Pobiega et al., 2019), ferric reducing antioxidant power (Ahmed et al., 2017; Gülçin et al., 2010), and electrochemical methods (Rebiai et al., 2011). A lot of results have described the antimicrobial and antibacterial activity of propolis extracts, noting that phenolic compounds, terpenoids and nonaromatic acids are primarily responsible for the inhibiting microorganism growth (Bayram et al., 2017; Martinotti & Ranzato, 2015; Yuan et al., 2020).

The essential oils extracted from propolis exhibited antimicrobial activity mostly against gram-positive bacteria and fungi (Franchin et al., 2016). The primary polyphenol (flavonoid) content in propolis fluctuates qualitatively and quantitatively depending on the plant's environment and ecology (Dantas Silva et al., 2017). Headspace solid-phase microextraction (HS-SPME) has been proposed as a dependable tool for the analysis of volatile organic compounds (Mohtar et al., 2018), because it removes many drawbacks: (a) organic extraction, (b) higher cost and (c) prolonged extraction time.

In this study, we focus on enhanced extraction of propolis compound using a novel technology to attain a higher yield at the cost of relatively less energy and time. Ultrasound wave-mediated energized medium (solvent) endorsed easy recovery of the active compounds from the samples. Ultrasound-assisted extraction was done from propolis to analyze the volatile compounds using HS-SPME and GC-MS to evaluate their antioxidant and antibacterial activities, *in vitro*. Results of this study prove that the proposed technology may be economically and efficiently superior for the industrial use in the manufacturing of functional foods.

## 2 | MATERIAL AND METHODS

### 2.1 | Materials

Gallic acid, vanillin, quercetin, ethanol, methanol, HPLC-grade acetonitrile (ACN), acetic acid, Folin-Ciocalteu reagent, aluminum chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). The purity of standard compounds was checked by HPLC-DAD analysis and was higher than 98%. All the other reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). *Bacillus subtilis* (No. ATCC10160), *Staphylococcus*

*aureus* (No. ATCC25923), *Listeria monocytogenes* (No. ATCC19120) were purchased from the Microbial Strain Preservation Center, China. *Escherichia coli* (DH5 $\alpha$ ) was prepared and stored at  $-20^{\circ}\text{C}$ .

### 2.2 | Sample collection, preparation, and ultrasound-assisted extraction of propolis

#### 2.2.1 | Shandong province propolis (LSP)

Samples were collected with the help of beekeepers from the area of Songlin Town, Linqing City, Shandong Province (N36 $^{\circ}$ 53'52.12" E115 $^{\circ}$ 51'8.89") from the plant species *Ginkgo biloba* L., *Populus alba* L., *opulus canadensis* Moench, and *Salix babylonica*. The bee species used was *Apis cerana* during May.

#### 2.2.2 | Raohe, heilongjiang province propolis (RHP)

Crude propolis samples were collected from Raohe city, Heilongjiang Province (N47 $^{\circ}$ 34'26", E134 $^{\circ}$ 20'16"). Main floras of the area include *Fraxinus mandshurica* Rupr., *Populus* L., *Quercus mongolica* Fisch, *Tilia tuan* Szyszyl., and *Betula*. Sampling was done in June and the main bee species present at that time was *Apis mellifera*.

#### 2.2.3 | Changge, henan province propolis (CHP)

Samples were collected by beekeepers from the location of Nanxi Town, Changge City, Henan Province (N34 $^{\circ}$ 12'59.04", E114 $^{\circ}$ 05'56.40") during April and common plants there were *Paulownia fortunei*, *Populus tomentosa*, *opulus canadensis* and Moench, *Populus alba* L. Bee species in this area were *Apis mellifera* and *Ligustica Spinola*.

#### 2.2.4 | Yingchun, heilongjiang province propolis (YHP)

Yingchun Town, Hulin City, Heilongjiang Province (N46 $^{\circ}$ 03'2.13", E132 $^{\circ}$ 56'50.10") was the last location for propolis sample collection during August and the bee species found there was *Apis mellifera*. Typical floras of that area were *Fraxinus mandshurica* Rupr., *Phellodendron amurense* Rupr, *Juglans mandshurica*, *Quercus mongolica* Fisch. Ledeb, *Ulmus pumila* L., and *Tilia tuan* Szyszyl.

#### 2.2.5 | Initial processing

Propolis samples after collection were crushed using a chilled mortar at freezing temperature, then sieved using a sieve size of 40 mesh and kept at  $-20^{\circ}\text{C}$  once powdered. A pulverized propolis sample (5 g) was added to 100 ml of 95% ethanol and placed

in an ultrasonic device (Jiangsu Jiangda Wukesong Biological Technology Co., Ltd., Jiangsu, China) at 220 W and 40 kHz for 30 min. After three extractions, the extract was subjected to suction filtration and filtered to a volume of 300 ml to obtain the extract for further analysis. After ultrasound extraction, the mixtures were centrifuged for 10 min at 1,644 g (TGL-16M High-speed Desktop Refrigerated Centrifuge, Changsha Xiangyi Centrifuge Instrument Co., Ltd.). The supernatant was filtered using vacuum filtration and ethanol was added up to 250 ml. Supernatants of different extracts were divided into two parts: one part was prepared for detection and the other part was concentrated using a rotary evaporator at 40°C (R-210 BUCHI Labortechnik AG, Flawil, Switzerland). Subsequently, samples were shifted to a vacuum-drying oven to remove any residual solvent (DZF-6050 Shanghai Yiheng Scientific Instrument Co., Ltd.). All measurements were carried out in triplicate.

### 2.3 | Determination of Total Phenolic Content (TPC)

TPC was determined by the Folin–Ciocalteu assay (Hernandez Zarate et al., 2018). Samples were mixed with diluted Folin–Ciocalteu solution and 500 µl of 20% Na<sub>2</sub>CO<sub>3</sub> solution, mixture was kept in the dark for 1 hr at room temperature and centrifuged at 872.1 g for 10 min, and absorption was measured at 750 nm using UV spectrophotometer (Cary 100, Agilent Technologies, Santa Clara CA, USA). Gallic acid at various concentrations was used as a standard to construct a standard curve. TPC was denoted as mg of Gallic acid equivalent (GAE)/grams of propolis extract using a calibration curve of 50–250 mg of gallic acid/mL.

### 2.4 | Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined using colorimetric method based on aluminum chloride complex formation (Hernandez Zarate et al., 2018). Briefly, 0.5 ml of 5% AlCl<sub>3</sub> mixed with 2 ml of extracts in 25 ml volumetric flask distilled water was used to adjust the volume. Mixture was left in the dark at room temperature for 30 min, and absorption was measured at 425 nm using UV spectrophotometer. Quercetin was used as reference to plot a standard curve. Results were expressed as mg of quercetin of propolis extract.

## 2.5 | Antioxidant assays

### 2.5.1 | DPPH scavenging assay

0.2 ml of DPPH solution (0.1 mM) was mixed with Propolis extracts at various concentrations (0–5 mg/ml) using vortex. The reference compound (Trolox) was used to draw a standard curve. Samples were then incubated in the dark for 30 min at room temperature, and

absorbance was measured at 517 nm using UV- spectrophotometer (Wen et al., 2020). The equation used for DPPH scavenging activity was as follows:

$$\text{DPPHscavenging activity (\%)} = [1 - (Ab_{s0} - Ab_{s1}) / Ab_{s0}] \times 100$$

Where:

Abs<sub>0</sub> = absorbance of the control sample;

Abs<sub>1</sub> = absorbance in the presence of samples tested.

### 2.5.2 | Reducing power assay

Propolis extract was diluted in different concentrations (0–5 mg/ml) mixed with 0.1 ml of phosphate-buffered saline (0.20 M, 6.6 pH) and 0.1 ml of 1% potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>]. Samples were vortexed and left for incubation at 50°C for 20 min. After incubation 0.1 ml of 10% trichloroacetic acid was added to the samples to decrease the pH of the reaction medium to 4.0. Finally, 0.04 ml of 0.6 M FeCl<sub>3</sub> was added and absorbance was measured at 700 nm using a UV spectrophotometer. Each sample was replicated thrice (Woźniak et al., 2019).

### 2.5.3 | Ferric reducing antioxidant power (FRAP)

All reagents were prepared according to Ding, Jiang, et al. (2019) with some modifications. FRAP reagent was prepared by adding 25 ml of 300 mM acetate buffer (pH 3.6) with 2.5 ml of 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) solution (in 40 mM HCl) and 2.5 ml of 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O solution. The newly prepared reagent was heated at 37°C. The FRAP was done using the method of (Benzie & Strain, 1996). Crude extracts of propolis were dissolved in ethanol (95%) with a concentration of 50 mg/ml and diluted to 100, 80, 60, 40, 20, and 0 µg/ml. Aliquots (100 µg/ml) of newly prepared samples were mixed with 0.5 ml of FRAP reagent. Ferrous sulfate ion concentration (10–100 µM) was used as the standard, and the absorbance was measured at 593 nm. Results were expressed as µM ferrous sulfate per gram of propolis extract.

### 2.5.4 | Total Equivalent Antioxidant Capacity (TEAC)

The method used to determine the total antioxidant capacity was ABTS by consulting the method of Osés et al. (2020). Trolox at different concentrations was used as the standard. ABTS cations preparation includes oxidization of ABTS solution in water with the treatment of potassium persulphate (molar ratio 1:0.35) and kept in the dark for 12–16 hr. Subsequently, the solution was diluted by adding 0.1 M potassium phosphate buffer (pH 7.4) to provide the absorbance of 0.70 ± 0.02 at 734 nm. The sample (200 µl) was added to 1.8 ml of the reagent, mixed and incubated at room temperature. After every 10 min, absorbance was read using a spectrophotometer.

Distilled water was used as a control. Results were expressed in terms of TEAC, as  $\mu\text{M TEAC/g}$  of propolis extract.

### 2.5.5 | Hydroxyl radical scavenging activity

The hydroxyl scavenging activity of samples was determined by following the method of Dai et al. (2017). A 1 ml sample (0.5–2.5 mg/ml), 0.5 ml 9 mmol/L  $\text{FeSO}_4$ , 1 ml 9 mmol/L salicylic acid ethanol solution, 1 ml 4.4 mmol/L  $\text{H}_2\text{O}_2$  and 2 ml  $\text{H}_2\text{O}$  were mixed at 37°C for 60 min, followed by absorbance measurement at 510 nm ( $\text{Abs}_1$ ). Distilled water instead of the sample was used to measure the absorbance value  $\text{Abs}_0$ , whereas distilled water without  $\text{H}_2\text{O}_2$  was used to measure the absorbance value  $\text{Abs}_2$ . The equation used for hydroxyl radical scavenging rate was as follows:

$$\text{Hydroxyl scavenging activity (\%)} = [1 - (\text{Abs}_1 - \text{Abs}_2) / \text{Abs}_0] \times 100$$

### 2.5.6 | Superoxide anion scavenging activity

The hydroxyl scavenging activity of samples was determined by adopting the method of Wen et al with slight modifications. A sample solution of 0.5–2.5 mg/ml + 2.8 ml 0.1 mol/L of Tris-HCl buffer solution at pH 8.2 was used, with double distilled water as blank. Add 0.1 ml of 3 mmol/L pyrogallol solution in water bath and start timer, preheat it at 25°C, mix it quickly to measure absorbance using spectrophotometer at 325 nm every 30 s and finish measuring after 5 min. The antioxidant activity tests were compared to standard antioxidants such as butylatedhydroxytoluene (BHT). As the regression equation of absorbance with time, the slope is the autoxidation rate of pyrogallol. The inhibition rate of the samples to the superoxide anion was calculated as follows:

$$\text{Superoxide anionscavenging activity (\%)} = [(V_0 - V_1) / V_0] \times 100$$

Where:

$V_0$  = The self-oxidation rate ( $\Delta A/\text{min}$ ) of pyrogallol in the control group.

$V_1$  = The self-oxidation rate ( $\Delta A/\text{min}$ ) of pyrogallol in sample group.

## 2.6 | Determination of the antibacterial activity of flavonoid extracted from propolis samples

### 2.6.1 | Bacterial suspension preparation

*Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* were activated in LB solid media. An inoculating ring was used to collect bacterial strains, which were then cultured for 18 hr in liquid media with continuous shaking. The concentration of the various bacterial suspensions was adjusted to 10<sup>6</sup>–10<sup>7</sup> CFU/ml for further analysis (Wen et al., 2020).

### 2.6.2 | Bacterial Growth Inhibition determination assay

A sterilized spatula was used to drop and coat 120  $\mu\text{l}$  of bacterial suspension uniformly. Filter paper soaked with antimicrobial propolis extract was pasted on the plate containing bacteria on agar medium. Each plate was pasted three times. Two plates were cultured in a constant temperature incubator at 37°C for 24 hr to determine the size of the bacteriostatic zone (Kalogeropoulos et al., 2009). The size of the inhibited area was measured (mm).

## 2.7 | Determination of volatile compounds by HS-SPME GC-MS

Analysis of the volatile compounds in propolis was done using headspace solid-phase microextraction (HS-SPME) combined with GC-MS. HS-SPME was done using a manual holder and fiber (050/30  $\mu\text{m}$  DVB/CAR/PDMS, 24Ga). In a 15 ml flat bottom head vial, 0.5 g of sample was placed and sealed with a magnetic crimp cap and PTFE/silicone septa (Supelco). A thermostatic bath was used to heat the sample at 60°C for 10 min. The SPME device was introduced into the sealed vial by manually diffusing the septum and then displayed to the headspace extraction for 30 min. The SPME fiber was quickly inserted into the GC injector.

The GC-MS analysis was performed according to the method of (Kasiotis et al., 2017) with some modifications. An Agilent 6,890 GC connected to 5973N MSD mass spectrometer framework supplied with an HP5-MS slender section (60 m  $\times$  0.25 mm i.d., 0.32  $\mu\text{m}$  film thickness) was used. Analysis of the sample was done with the column held initially at 40°C for 2 min, inclined at a rate of 10–60° and then contained for 5 min. After 2 min the temperature was straightly stepped up to 280°C with a 3°C/min heating ramp and holding at 280°C for 10 min. The carrier gas was Helium with a 1.0 ml/min flow rate. The infusion was performed in pulse splitless mode, and at injector temperature 250°C. Full scans EI (Electron Impact) spectra were recorded from 30 to 450 m/z (mass/charge) with two scans per second. The ionization voltage was 70 eV with 230°C temperature and the ionization source for EI-MS in positive mode.

The component identity in the extracts was referred to by the correlation of their maintenance records and mass spectra with those stored on the PC library and with those distributed in the literature. NIST05 library sources were utilized to match the identified compounds.

## 2.8 | Statistical analysis

The data were expressed as the mean  $\pm$  standard deviation (SD), analyzed by one-way analysis of variance (ANOVA) and Tukey's test was used for comparison of the means using Minitab 18 (Minitab Inc., State College, PA, USA). Origin Pro software (2018) was used for graphical presentation. All experiments were performed in triplicate.

### 3 | RESULTS AND DISCUSSION

#### 3.1 | Total phenolic and flavonoid contents of various extracts of propolis samples

According to the results in Table 1, TPC and TFC varied from 89.343 to 201.786 mg GAE/g of and 166.227 to 519.769 mg quercetin/g of ultrasound-assisted extracts respectively for propolis in different areas of China with significant difference ( $p < .05$ ). In comparison with conventional extraction, TPC varied from  $72.02 \pm 1.99$  to  $155.95 \pm 3.69$  mg GAE/g, TFC varied from  $129.675 \pm 6.82$  to  $412.83 \pm 12.14$  mg GAE/g, that is, 15%–20% lower than the ultrasound-assisted extraction. Molnár et al. (2017) and Haile and Lin (2011) reported that TPC of propolis of different regions of Europe (Hungary and Bulgaria) and China (Zhejiang, Hubei, Hebei) varies approximately from 200 to 300 mg GAE/g, while TFC content reported is lower than that in these locations of China and TPC is higher or comparable with these values. (Kumazawa et al., 2004) reported that the TPC in propolis extract from Shandong, China was  $433.8 \pm 1.7$  mg GAE/g, which is higher than the present study results. The reason could be due to climate change, seasonal change, and time of collection that may vary with the phenolic compounds of propolis samples.

Total flavonoid content and TPC of YHP are lower as compared to those of other areas. Propolis has a wide range of phenolic compounds, mostly flavonoids (Kurek-Górecka et al., 2013). The variations of flavonoid compounds of propolis are primarily due to the different chosen regional floras collected by honey bees. It has been found that flavonoid and other phenolic contents inhibit the growth of cancer and the development of heart disease. (Ahangari et al., 2018).

Biological activities of propolis are chiefly due to the presence of flavonoid contents in the sample (Ahangari et al., 2018). Our results have clearly shown that the difference in biological activities of samples is strictly due to the variation in flavonoid contents.

#### 3.2 | DPPH free radical scavenging activity of various Propolis sample extracts

2,2-diphenyl-1-picrylhydrazyl has been broadly used to determine the free radical scavenging activity of multiple samples (Benhanifia et al., 2013). In Figure 1(a), all extracts of propolis samples showed significant DPPH scavenging activity. Figure 1(a) shows that the order of action of DPPH free radical scavenging activity is  $\text{CHP} > \text{RHP} > \text{LSP} > \text{YHP}$ . The samples with more DPPH activity have higher phenolic contents. Phenols in the ethanolic extracts of propolis are for the antioxidant activity of propolis because of their biological properties, which is responsible in scavenging reactive oxygen species (Osés et al., 2020). YHP and LSP have higher phenolic content as compared to other samples. Phaniendra, Jestadi, and Periyasamy (2015) reported that propolis samples from China and Brazil have vigorous DPPH free radical scavenging activity ranging from  $98.80 \pm 1.0\%$  to  $70.50 \pm 1.2\%$ . Similar results were also published by (Hatano et al., 2012), which demonstrate that Chinese propolis has more vigorous DPPH free radical activity than Brazilian propolis.

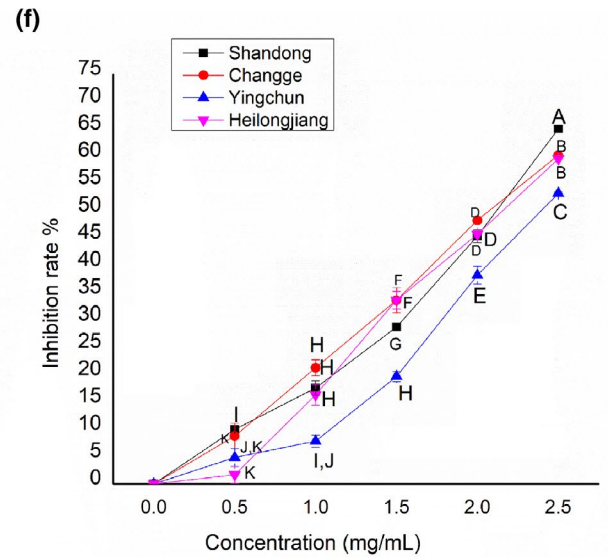
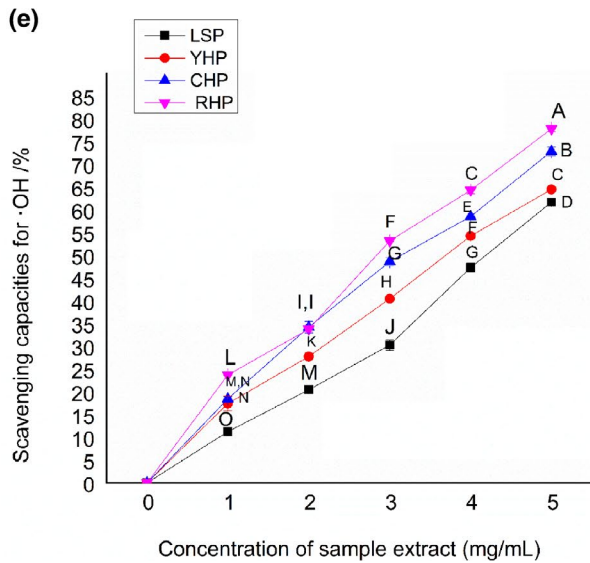
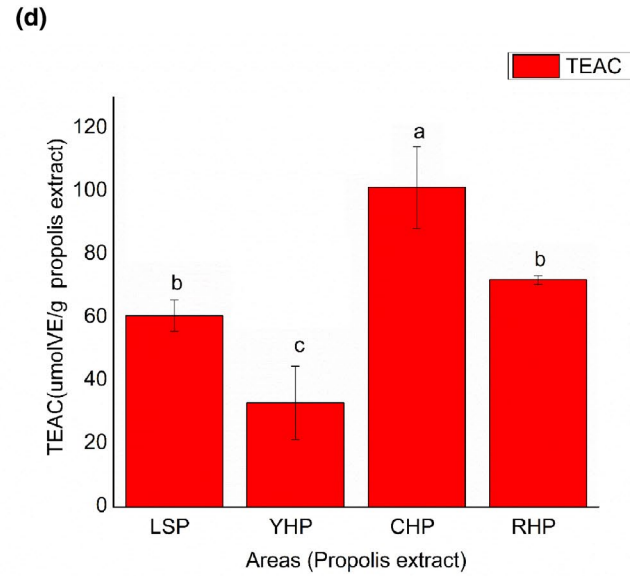
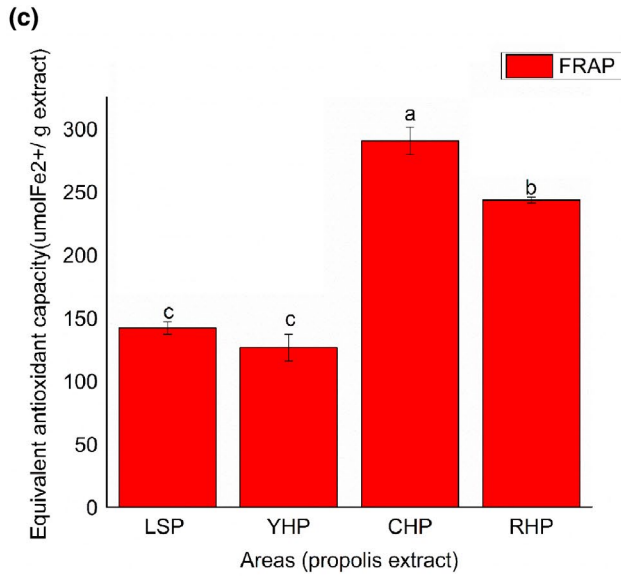
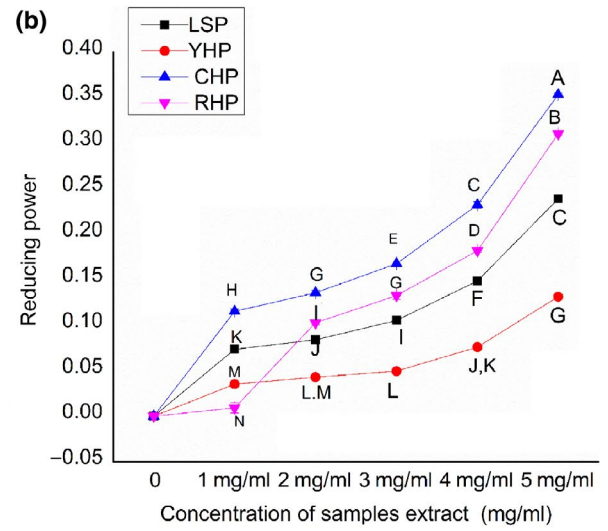
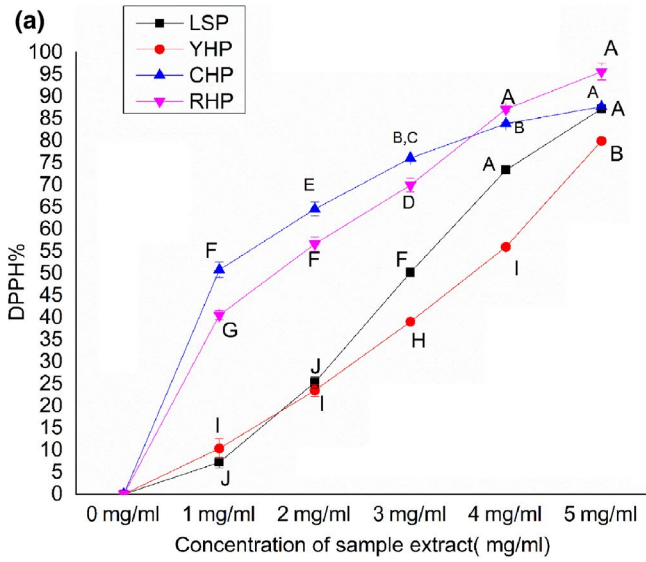
#### 3.3 | Reducing power activity of different propolis extracts of samples

Various studies have shown that Chinese propolis has high reducing activity of phenolic compounds. Greek, Polish, and Slovenian propolis have high reducing power (Yang et al., 2011). In this study, propolis samples collected from different areas of China showed varying reducing power in varying dilutions. The sample with the highest reducing power activity was CHP; the sample with the lowest reducing power was YHP. The order of activity of all the samples, as shown in Figure 1(b), is  $\text{CHP} > \text{RHP} > \text{LSP} > \text{YHP}$ .

**TABLE 1** Total polyphenols and flavonoid content of different Chinese propolis extracts

Ultrasound-assisted extracted sample	Total polyphenolic contents of propolis extracts (TPC) mg/g	Total Flavonoid content of propolis extracts (TFC) mg/g
Raohe Heilongjiang propolis (RHP)	$166.07 \pm 1.54$	$341.00 \pm 10.82$
Yingchun Heilongjiang propolis (YHP)	$89.34 \pm 1.76$	$166.22 \pm 8.59$
Linqing Shandong propolis (LSP)	$154.09 \pm 12.03$	$235.60 \pm 39.70$
Change Henan propolis (CHP)	$201.78 \pm 4.60$	$519.77 \pm 29.90$
Conventional extraction:		
Raohe Heilongjiang propolis (CRHP)	$128.56 \pm 2.18$	$254.75 \pm 6.82$
Yingchun Heilongjiang propolis (CYHP)	$72.02 \pm 1.99$	$129.675 \pm 5.52$
Linqing Shandong propolis (CLSP)	$120.43 \pm 8.14$	$179.70 \pm 10.25$
Change Henan propolis (CHP)	$155.95 \pm 3.69$	$412.83 \pm 12.14$





**FIGURE 1** (a). DPPH activity of ultrasound-assisted extract of propolis of different areas. Means that do not share a letter are significantly different. Figure 1(b). Reducing power rate of ultrasound-assisted extract of propolis of different areas. Means that do not share a letter are significantly different. Figure 1(c) FRAP assay of ultrasound-assisted extract of propolis of different areas. Means that do not share a letter are significantly different. Figure 1(d) TEAC assay of ultrasound-assisted extract of propolis of different. Means that do not share a letter are significantly different. Figure 1(e) Hydroxyl radical scavenging activity of ultrasound-assisted extract of propolis of different. Means that do not share a letter are significantly different. Figure 1(f) Superoxide anion scavenging activity of ultrasound-assisted extract of propolis of different areas. Means that do not share a letter are significantly different

### 3.4 | Ferric Reducing Antioxidant Power (FRAP) of propolis extracts

The antioxidant power of propolis samples from different regions of China was determined by using ferric, reducing antioxidant power (FRAP). Figure 1(c) shows significant differences ( $p < .05$ ) between LSP and all other areas except for YHP samples. The propolis extracts of CHP and RHP also showed significant differences ( $p < .05$ ) in the FRAP activity from all other areas. The FRAP values of ultrasound-assisted extract samples ranged from  $126 \pm 10.60$  to  $290.34 \pm 10.80$   $\mu\text{mol/g}$  of extract. Figure 1(c) shows the FRAP activity of different samples among them CHP extracts showed the strongest FRAP activity ( $290.348 \pm 10.80$   $\mu\text{mol/g}$  of extracts). The order of activity of propolis samples from different areas of China is  $\text{CHP } 290.35 \pm 10.80 > \text{RHP } 243.35 \pm 2.40 > \text{LSP } 141.93 \pm 4.90 > \text{YHP } 126.47 \pm 10.60$ . (Socha et al., 2015) previously reported the FRAP activity of extracts from Shandong, China ( $89.20 \pm 3.80$   $\mu\text{g/ml}$ ), Henan, China ( $34.5 \pm 1.1$ )  $\mu\text{g/mL}$ , and different areas of Brazil ranges from  $45.40 \pm 2.40$   $\mu\text{g/m}$  to  $32.10 \pm 0.50$   $\mu\text{g/mL}$ , which is slightly lower than our results.

### 3.5 | TEAC of Ultrasound-assisted extracts of Chinese propolis from different areas

The Propolis antioxidant effect was determined by Trolox equivalent antioxidant capacity (TEAC) per gram of propolis sample. According to the results shown in Figure 1(d), all Propolis samples showed significant difference ( $p < .05$ ) whereas, in comparison with the propolis samples of RHP and LSP was slightly insignificant ( $p < .05$ ,  $p < 10\%$ ). The extracts of propolis extracts from CHP, RHP and YHP areas are significantly different from their mean in comparison with all the other areas. ( $p < .05$ ). The order of activity of different samples collected is  $\text{CHP} > \text{RHP} > \text{LSP} > \text{YHP}$ , clearly shown in Figure 1(d). TEAC of propolis extracts ranged from  $36.76 \pm 11.6$  to  $106.73 \pm 12.9$   $\mu\text{mol Trolox/g}$  of extracts. Hatano et al. (2012) reported that the TEAC values of Brazilian propolis ranged from 8,491.5 to 8,773.6  $\mu\text{mol TEAC g}^{-1}$  propolis extracts. Augusto-Obara et al. (2019) indicated that the TEAC values of Tunsanian propolis ranged from 109.76 to 252.9  $\mu\text{mol TEAC/g}$ . Gargouri et al. (2019) evaluated the TEAC activity of extracted propolis samples from northeast Spain which ranged from 560–1,430 L mol Trolox/g.

### 3.6 | Hydroxyl radical scavenging activity of samples extract

The extracted samples hydroxyl scavenging activity was reviewed using the Fenton reaction, and the trend is shown in Figure 1(e). The extracted samples showed significantly different scavenging activity except CHP and YHP area extracted samples with a concentration of 1 mg/ml ( $p < .01$ ). All the samples showed the highest activity with a concentration of 5 mg/ml. Propolis samples extract from RHP showed the highest activity of 77.81%. The order of activity was  $\text{RHP} > \text{CHP} > \text{YHP} > \text{LSP}$ . Previously, Bonvehí and Gutiérrez (2011) concluded that the hydroxyl scavenging activity of Chinese and Brazilian propolis is  $59.61 \pm 19.92$   $\mu\text{g}\cdot\text{mL}^{-1}$  for Chinese propolis and  $54.42 \pm 10.32$   $\mu\text{g}\cdot\text{mL}^{-1}$  for Brazilian propolis and Chinese propolis showed a lower hydroxyl scavenging activity than Brazilian propolis.

### 3.7 | Superoxide anion scavenging activity of extract of propolis of different areas

The developing of pathophysiological conditions and redox cell signalling is significantly completed by production of superoxide radical species. In most of the biological reaction medium, these species are comparatively unreactive.  $\text{O}_2^{\bullet-}$  is also a precursor of many strong oxidants. Figure 1(f) presents the inhibition rate of ultrasound-assisted extracts of different areas with variable concentrations. The scavenging activity was  $\text{CHP} > \text{RHP} > \text{LSP} > \text{YHP}$ . Though our data is higher, some of the previous results by de Francisco et al. (2018) studies superoxide scavenging activity of two kinds of propolis samples from Brazil showed  $48.08 \pm 4.28$ , 138.29 mg/ml and  $34.0 \pm 2.0$  mg/ml.

### 3.8 | Antibacterial activity of different Chinese propolis extract

The ultrasound-assisted extract of propolis was used to determine the antibacterial activity of propolis. Our previous research revealed that the antibacterial activity of Chinese propolis increased by using ultrasound to extract propolis. Moreover, the extraction of flavonoids and polyphenols yield also increased (Ding, Wu Chen, et al., 2019). The results of the antibacterial assay

**TABLE 2** Antibacterial activity of ultrasound-assisted and conventional extracts of different Chinese Propolis

Propolis extracts (Ultrasound -assisted extracts)	Inhibition zone diameter (mm)			
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Listeria</i>	<i>E.coli</i>
Raohe Heilongjiang propolis (RHP)	16.05 ± 0.54	17.12 ± 0.91	21.58 ± 3.60	0
Yingchun Heilongjiang propolis (YHP)	15.07 ± 0.11	18.27 ± 0.25	18.95 ± 0.16	0
Linqing Shandong propolis( LSP)	16.33 ± 1.03	15.40 ± 0.54	16.77 ± 1.02	0
Changege Henan propolis (CHP)	16.67 ± 0.65	15.27 ± 0.16	17.70 ± 0.75	0
Conventional extraction				
Raohe Heilongjiang propolis (CRHP)	15.62 ± 0.23	15.77 ± 0.35	18.45 ± 0.24	0
Yingchun Heilongjiang propolis (CYHP)	14.28 ± 0.29	17.59 ± 0.21	17.12 ± 0.37	0
Linqing Shandong propolis(CLSP)	14.76 ± 0.20	15.02 ± 0.37	14.89 ± 0.86	0
Changege Henan propolis (CCHP)	15.25 ± 0.29	14.53 ± 0.31	15.74 ± 0.51	0

of selected bacterial strains (Table 2) show the highest and the lowest inhibition in the growth of these bacteria. The extracts of RHP and CHP showed the highest inhibition of *Listeria monocytogenes* than any other samples because of higher phenolic and flavonoids concentration (Table 1). LSP and YHP also showed significant effects on the inhibition of bacterial growth. Pazin et al. (2017) reported the antimicrobial activity of Turkish propolis on microorganisms like *Bacillus subtilis* (20 ± 2 mm), *Staphylococcus aureus* (18 ± 3 mm), and *Escherichia coli* (10 ± 2 mm) with the minimum inhibition concentration of 20–25 µg/ml. Moreover, Bayram et al. (2017) worked on different samples from Chile and Spain on the same bacterial strains. It is evident from the present study that gram-positive bacteria were more sensitive to Chinese propolis extract, as shown in Table 2 (RHP : *Listeria monocytogenes* 21.58 ± 3.6 mm > *Staphylococcus aureus* 17.12 ± 0.91 mm > *Bacillus subtilis* 16.05 ± 0.54 mm > *E.coli* 0; YHP : *Listeria monocytogenes* 18.95 ± 0.16 mm > *Staphylococcus aureus* 18.27 ± 0.25 mm > *Bacillus subtilis* 15.07 ± 0.11 mm > *E.coli* 0; LSP *Listeria monocytogenes* 16.77 ± 1.02 mm > *Staphylococcus aureus* 15.40 ± 0.54 mm > *Bacillus subtilis* 16.33 ± 1.03 mm > *E.coli* 0; CHP *Listeria monocytogenes* 17.70 ± 0.75 mm > *Staphylococcus aureus* 15.27 ± 0.16 mm > *Bacillus subtilis* 16.67 ± 0.65 mm > *E. coli* 0), so Chinese propolis has very little or no effect on gram-negative bacteria (*E. coli*). In contrast extract from conventional procedure showed antimicrobial activity but the effect was lower than ultrasound-assisted extracts; that is, the highest activity was from YHP: *Staphylococcus aureus* 17.59 ± 0.21 mm and *Listeria monocytogenes* 17.12 ± 0.37 mm to 0 mm for *E.coli* as both ultrasound-assisted and conventional extraction did not show any effect on *E.coli* (Table 2). Gram-negative bacteria have a complex chemical structure, and their flexible cell wall contains polysaccharide; these characteristics account for its toxicity in microorganisms. Another main reason for the resistance against propolis extract is the presence of the multidrug obstruction pumps, which expels the entrance of external poisons over the external layer (Tukmechi et al., 2010). Likewise, this bacterial group has a higher lipid ration than that detected in gram-positive bacteria (Revilla et al. (2017). Mohdaly et al. (2015) also reported

no effect on *E. coli* and other gram-negative bacteria in his research using Brazilian propolis extract. However, other authors have reported propolis extract from Argentina that affects *E. coli* (Dantas Silva et al. (2017). The antibacterial mechanism of propolis is linked to some of its constituents such as higher concentration of flavonoids components like Galangin, pinocembrin and pinobanksin which possess higher antimicrobial activity (Popova et al., 2017). The reason behind the different antibacterial activity of propolis is the collection region and species of bees. Flavonoids affect the membrane of the bacteria, causing permeability alteration within the inner microorganism membrane (Przybyłek & Karpiński, 2019).

### 3.9 | Analysis of volatile compounds in raw propolis by HS-SPME GC-MS

The chemical composition of volatile compounds depends mainly on the flora and collection site (Wojtyczka et al., 2013). Previously, Salatino, Fernandes-Silva, Righi, and Salatino (2011) determined 99 compounds from propolis samples collected from Italy by using HS-SPME GC-MS. In our study, a total of 150 compounds were identified in the propolis samples collected from different regions of China.

According to the results (Table 3), LSP contains 58 kinds of substances, accounting for 38% of the total peak area. CHP comprises 51 substances, accounting for 34.0% of the total peak area. RHP contains 74 elements, accounting for 49.3% of the total peak area. YHP contains 41 kinds of substances, accounting for 27.3% of the total peak area.

For acid substances, LSP contains the highest content of acid materials, accounting for 11.51% of the total peak area. CHP, RHP, and YHP have 7.96%, 3.25%, and 6.35% acid relative contents, respectively. The variability in acid materials may be related to regional and climatic differences and the plants growing in each region.

Linqing, Shandong Province contains the highest content of esters, accounting for 3.25% of the total peak area, followed by YHP, where the content of esters accounted for 1.81% of the total peak area. CHP and RHP have 1.1% and 1.04% of relative ester contents



TABLE 3 Volatile compounds in propolis samples determined by GC-MS

Time/min	Flavor substances	Chemical formula	Raohe Heilongjiang (RHP) Relative content %	Yingchun Heilongjiang (YHP)	Linqing Shandong (LSP)	Change Henan (CHP)
<b>Acids</b>						
23.27	Acetic acid	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	2.06	1.26	-	0.73
27.79	Butyric acid	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	-	-	0.14	-
32.78	Citric acid	C <sub>5</sub> H <sub>8</sub> O <sub>2</sub>	-	-	3.04	1.41
44.38	benzoic acid	C <sub>7</sub> H <sub>6</sub> O <sub>2</sub>	-	6.35	8.33	5.82
32.93	2-Buten-1-ol-3-methyl Acid(relative content%)	C <sub>5</sub> H <sub>8</sub> O	1.19 3.25	7.61	- 11.51	- 7.96
<b>Number of acid substances/one</b>						
			2.00	2.00	3.00	3.00
<b>Esters</b>						
7.2	Ethyl Acetate	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	-	0.02	-	-
15.51	4- Pentene-1-acetate	C <sub>7</sub> H <sub>12</sub> O <sub>2</sub>	-	-	-	0.14
17.52	Prenyl acetate	C <sub>7</sub> H <sub>12</sub> O <sub>2</sub>	-	-	0.10	-
17.32	2-methyl-2-buten-1-ol ester	C <sub>7</sub> H <sub>12</sub> O <sub>2</sub>	-	-	0.14	-
22.93	Ethyl octanoate	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	-	-	-	0.29
23.19	Methyl formate	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	-	-	1.11	-
27.3	3-methyl-2-buten-1-ol benzyl ester	C <sub>10</sub> H <sub>16</sub> O <sub>2</sub>	-	-	0.93	-
30.42	Acetate	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	-	1.26	-	-
32.48	Phenylacetate	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	0.68	-	-	0.67
37.32	Phenylpropanol acetate	C <sub>9</sub> H <sub>12</sub> O	0.36	0.43	-	-
47.1	Dioctyl adipate	C <sub>22</sub> H <sub>42</sub> O <sub>4</sub>	-	-	1.07	-
<b>Ester (relative content/%)</b>			1.04	1.71	3.35	1.10
<b>Number of esters</b>			2.00	4.00	4.00	3.00
<b>Alcohols</b>						
7.28	Ethanol	C <sub>2</sub> H <sub>6</sub> O	-	-	-	1.63
13.15	Ethyl octinol	C <sub>10</sub> H <sub>18</sub> O	-	0.23	-	-
15.97	Prenol	C <sub>5</sub> H <sub>8</sub> O	0.42	0.32	-	-
17.44	3-methyl-2-buten-1-ol	C <sub>5</sub> H <sub>10</sub> O	-	0.43	0.65	0.53
17.68	Acid 2-isoamyl alcohol	C <sub>7</sub> H <sub>12</sub> O <sub>2</sub>	0.15	-	-	-
19.62	2-methyl-2-buten-1-ol	C <sub>5</sub> H <sub>10</sub> O	0.42	0.32	0.96	0.30
19.82	2-Buten-1-ol. 3-methyl-	C <sub>5</sub> H <sub>10</sub> O	0.53	-	-	-
21.38	2-Isopropyl-5-methyl-1-heptanol	C <sub>11</sub> H <sub>24</sub> O	-	0.42	-	-
25.78	Linalool	C <sub>10</sub> H <sub>18</sub> O	-	-	0.89	-

(Continues)

TABLE 3 (Continued)

Time/min	Flavor substances	Chemical formula	Raohe Heilongjiang (RHP) Relative content %	Yingchun Heilongjiang (YHP)	Linqing Shandong (LSP)	Change Henan (CHP)
29.78	Terpineol	C <sub>10</sub> H <sub>18</sub> O	0.13	-	-	1.76
33.65	Benzyl Alcohol	C <sub>7</sub> H <sub>8</sub> O	5.27	12.3	6.70	-
34.46	Phenylethanol	C <sub>8</sub> H <sub>10</sub> O	7.85	6.89	7.05	6.65
38.04	Guaiol	C <sub>15</sub> H <sub>26</sub> O	1.54	1.22	4.12	1.29
38.94	Cedarol	C <sub>15</sub> H <sub>26</sub> O	0.59	1.07	0.49	0.35
39.12	tetrahydro- $\alpha$ , $\beta$ -5-Trimethyl-5-(4-methyl-3-cyclohexene)-2	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	-	-	0.65	0.87
39.24	Bismuth oleyl alcohol	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	-	-	1.23	-
40.03	Agarospinol	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	0.56	-	1.90	-
40.3	$\Delta$ -red bisabolol	C <sub>15</sub> H <sub>26</sub> O	1.18	1.14	3.57	-
40.56	Guai-1(10)-en-11-ol	C <sub>15</sub> H <sub>26</sub> O	0.57	-	-	-
40.8	$\alpha$ -Cadinol	C <sub>15</sub> H <sub>26</sub> O	-	0.29	-	-
40.89	Beta-camphorol	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>	-	1.78	2.97	1.41
41.6	Cinnamyl alcohol	C <sub>9</sub> H <sub>10</sub> O	1.900.	1.02	1.18	1.89
	Alcohols (relative content/%)		19.21	26.18	31.18	13.16
	Number of alcohols		12.00	13.00	13.00	10.00
Terpenes						
10.25	Terpene	C <sub>10</sub> H <sub>16</sub>	-	-	0.12	0.06
10.41	$\alpha$ -Pinene	C <sub>10</sub> H <sub>16</sub>	0.58	-	-	-
13.07	Bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methylene-, (1S) -	C <sub>10</sub> H <sub>16</sub>	0.16	-	-	-
11.52	Terpene	C <sub>10</sub> H <sub>16</sub>	-	-	0.02	-
14.53	Myrcene	C <sub>10</sub> H <sub>16</sub>	-	-	0.02	-
15.37	Carene -4	C <sub>10</sub> H <sub>16</sub>	0.03	-	-	-
15.83	Citrus limene	C <sub>10</sub> H <sub>16</sub>	0.37	-	0.10	0.10
17.48	Bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1-methylethyl) -, (1 $\alpha$ , 2 $\alpha$ , 5 $\alpha$ ) -	C <sub>10</sub> H <sub>18</sub> O	0.17	-	-	-
18.68	Terpinolene	C <sub>10</sub> H <sub>16</sub>	0.21	-	-	-
24.17	Long-leafene	C <sub>15</sub> H <sub>24</sub>	-	-	-	1.11
24.47	Zelenene	C <sub>15</sub> H <sub>24</sub>	-	-	1.91	-
24.57	(+) - cyclodecene	C <sub>15</sub> H <sub>24</sub>	-	-	-	0.31
24.63	Cedrene	C <sub>15</sub> H <sub>24</sub>	0.63	-	-	-

(Continues)

TABLE 3 (Continued)

Time/min	Flavor substances	Chemical formula	Raoh Heilongjiang (RHP) Relative content %	Yingchun Heilongjiang (YHP)	Linqing Shandong (LSP)	Change Henan (CHP)
24.72	Tricyclo [5.4.0.0 (2.8)] undec-9-ene. 2.6.6.9-tetramethyl-	C <sub>15</sub> H <sub>24</sub>	1.23	-	0.83	-
25.84	[1S-- octahydro-4-methyl-8-vinyl-7- (1-methylethyl) -1.4-methanol-1H-indole	C <sub>15</sub> H <sub>24</sub>	-	-	-	0.60
25.88	Hexamethyl dewabenzene	C <sub>12</sub> H <sub>18</sub>	-	-	0.63	-
25.91	1.4-Methano-1H-indene. octahydro-4-methyl-8-methylene-7- (1-methylethyl) -, [1S- (1à. 3aa. 4à. 7à. 7aa)] -	C <sub>15</sub> H <sub>24</sub>	0.58	-	-	-
26.71	α-Bergamotene	C <sub>15</sub> H <sub>24</sub>	0.25	--	-	-
26.87	Alpha-cedarene	C <sub>15</sub> H <sub>24</sub>	-	-	0.29	-
27.13	Kuromatsuene	C <sub>15</sub> H <sub>24</sub>	13.18	-	-	-
27.37	α-Guaiene	C <sub>15</sub> H <sub>24</sub>	0.61	-	-	-
27.2	1.2.3.4.5.6.7.8-octahydro-1.4-dimethyl-7- (1-methylvinyl) indole	C <sub>15</sub> H <sub>24</sub>	-	-	0.34	0.63
27.53	Caryophyllene	C <sub>7</sub> H <sub>6</sub> O	3.88	-	1.35	3.35
27.61	Aristolochia	C <sub>10</sub> H <sub>18</sub> O	-	-	0.89	-
28	Tetracyclo [5.3.0.0 < 2.6 > 0.0 < 3.10 >] deca-4.8-diene	C <sub>10</sub> H <sub>10</sub>	0.65	0.77	-	--
28.67	(E)-β-farnesene	C <sub>15</sub> H <sub>24</sub>	0.64	-	0.54	-
29.31	Pacitaxel	C <sub>15</sub> H <sub>24</sub>	-	-	0.25	0.85
29.46	Cedarene	C <sub>15</sub> H <sub>24</sub>	0.63	-	1.55	0.34
29.47	α-Caryophyllene	C <sub>15</sub> H <sub>24</sub>	0.98	-	-	-
29.63	1H-Benzocycloheptene. 2.4a. 5.6.7.8-hexahydro-3.5.5.9-tetramethyl-, (R) -	C <sub>15</sub> H <sub>24</sub>	3.16	-	-	-
29.77	γ-Muuroleone	C <sub>15</sub> H <sub>24</sub>	0.43	--	-	-
30.26	γ-Muuroleone	C <sub>15</sub> H <sub>24</sub>	0.13	-	-	-
30.3	1.2.3.4.5.6.7.8-octahydro-1.4-dimethyl-7- (1-methylvinyl) indole	C <sub>15</sub> H <sub>24</sub>	-	-	-	0.67
30.4	Guai-1(10),11-diene	C <sub>15</sub> H <sub>24</sub>	0.56	-	-	-
30.45	(R)-β-Bisabolene	C <sub>15</sub> H <sub>24</sub>	0.6	-	-	-
30.64	Terpene	C <sub>15</sub> H <sub>24</sub>	-	-	0.64	0.94
30.66	Eudesma-4 (14). 11-diene	C <sub>15</sub> H <sub>24</sub>	0.36	-	-	-

(Continues)

TABLE 3 (Continued)

Time/min	Flavor substances	Chemical formula	Raoh Heilongjiang (RHP) Relative content %	Yingchun Heilongjiang (YHP)	Linqing Shandong (LSP)	Change Henan (CHP)
30.74	(+)- $\alpha$ -Cedarwood terpenes	C <sub>15</sub> H <sub>24</sub>	1.26	-	-	-
31.28	$\beta$ -cadinene	C <sub>15</sub> H <sub>24</sub>	0.5	-	-	-
31.34	$\alpha$ -curcumene	C <sub>15</sub> H <sub>22</sub>	4.41	-	2.27	4.66
33.04	cis-Calamenene	C <sub>15</sub> H <sub>22</sub>	0.27	-	-	-
34.69	$\alpha$ -dihydro-pyrene	C <sub>15</sub> H <sub>20</sub>	0.31	0.47	0.90	-
34.87	$\alpha$ -Calacorene	C <sub>15</sub> H <sub>20</sub>	0.31	-	-	-
36.93	3,4-dimethoxy styrene	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	0.56	-	-	-
37.23	Tricyclo [4,4,0,0 (2,7)] dec-8-ene-3-methanol, $\Delta$ , $\Delta$ , 6,8-tetramethyl-, stereoisomer	C <sub>15</sub> H <sub>24</sub> O	0.62	-	-	-
39.63	$\gamma$ -Eudesmole	C <sub>15</sub> H <sub>26</sub> O	-	1.07	-	-
39.86	2-Naphthalenemethanol, 1.2.3.4.4a, 5.6.7-octahydro- $\Delta$ , $\Delta$ , 4a, 8-tetramethyl-, (2R-cis) -	C <sub>15</sub> H <sub>26</sub> O	0.99	0.59	-	-
40.1	Guaiacene	C <sub>15</sub> H <sub>24</sub>	--	-	-	0.33
40.39	1.2.3.3a, 4.5.6.7-octahydro- $\Delta$ , $\Delta$ , 3,8-tetramethyl-5-methanol	C <sub>15</sub> H <sub>26</sub> O	-	0.45	1.59	-
40.87	$\alpha$ -Eudesmol	C <sub>15</sub> H <sub>26</sub> O	1.61	-	-	-
41.06	2-Naphthalenemethanol, decahydro- $\Delta$ , $\Delta$ , 4a-trimethyl-8-methylene-, [2R-(2 $\Delta$ , 4a $\Delta$ , 8a $\Delta$ )] -	C <sub>15</sub> H <sub>26</sub> O	1.79	-	-	-
Terpenes (relative content/%)			42.34	3.35	14.12	13.89
Number of terpenes			48	5.00	18	12
Olefins						
5.11	1,4-pentadiene	C <sub>5</sub> H <sub>8</sub>	-	-	0.01	-
15.96	3-methyl-2-butene	C <sub>5</sub> H <sub>8</sub> O	-	-	0.30	0.38
16.35	Bicyclo [3.1.0] hex-2-ene, 4-methyl-1-(1-methylethyl) -	C <sub>10</sub> H <sub>16</sub>	0.17	-	-	-
25.79	2-methyl-5-(1,5-dimethyl-4-hexenyl)-1,3-cyclohexadiene	C <sub>15</sub> H <sub>24</sub>	-	-	-	-
26.96	2,6-Dimethyl-6-(4-methyl-3-pentenyl)-2-enebicyclo [3.1.1] hept-2-ene	C <sub>15</sub> H <sub>24</sub>	-	-	1.57	-
29.53	(R)-2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-1H-benzocyclobutene	C <sub>15</sub> H <sub>24</sub>	-	-	-	2.87

(Continues)

TABLE 3 (Continued)

Time/min	Flavor substances	Chemical formula	Raohel Heilongjiang (RHP) Relative content %	Yingchun Heilongjiang (YHP)	Linqing Shandong (LSP)	Change Henan (CHP)
30.35	--1-methyl-4- (1-methylene-5-methyl-4-hexenyl) cyclohexene	C <sub>15</sub> H <sub>24</sub>	-	-	-	0.52
	Olefins (relative content/%)		0.17	0	1.87	3.77
	Olefins (relative content/%) Number of olefins/ one		1.00	3.00	3.00	3.00
Aromatic Hydrocarbons						
17.67	Styrene	C <sub>8</sub> H <sub>8</sub>	0.6	0.23	0.23	0.26
18.27	P-cymene	C <sub>10</sub> H <sub>14</sub>	0.27			
18.47	Isopropyltoluene	C <sub>10</sub> H <sub>14</sub>			-	0.01
21.27	1.3-Cyclohexadiene. 1.5.5.6-tetramethyl-	C <sub>10</sub> H <sub>16</sub>	0.05			
24.33	6.7-Dimethyl-1.2.3.5.8.8a-hexahydronaphthalene	C <sub>12</sub> H <sub>18</sub>		0.23	0.23	-
24.7	Cyclosativene	C <sub>15</sub> H <sub>24</sub>	0.34			
25.27	Longicyclone	C <sub>15</sub> H <sub>24</sub>	1.09			
26.9	Spiro [4.5] dec-7-ene. 1.8-dimethyl-4-(1-methylethenyl) -. [1S- (1à. 4à. 5à)] -	C <sub>15</sub> H <sub>24</sub>	0.22			
29.22	4-Methoxystyrene	C <sub>10</sub> H <sub>18</sub> O	0.85	0.45		
29.61	(1à. 4aa. 8aa)-1.2.4a. 5.8.8a-hexahydro-4.7-dimethyl-1- (1-methylethyl) naphthalene	C <sub>15</sub> H <sub>24</sub>		0.62	3.30	0.25
30.24	(1à. 4aa. 8aa) - 1.2.3.4.4a. 5.6.8a-octahydro-7-methyl-4-vinyl-1- (1-methylethyl) naphthalene	C <sub>15</sub> H <sub>24</sub>			1.42	-
31.12	[1S- (1à. 4aa. 8aa)] - 1.2.4a. 5.8.8a-hexahydro-4.7-dimethyl-1- (1-methylethyl) naphthalene	C <sub>15</sub> H <sub>24</sub>		0.83	0.67	0.42
37.07	Cyclodextrin	C <sub>15</sub> H <sub>24</sub> O			0.34	-
39.4	Phenol. 4-ethyl-	C <sub>8</sub> H <sub>10</sub> O		2.25		
39.63	(2R-cis)-1.2.3.4.4a. 5.6.7-octahydro-à. à. 4a. 8-tetramethyl-2-methanol	C <sub>15</sub> H <sub>26</sub> O			1.57	0.52
40.55	Hydroxy-5-methylacetophenone	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>		0.83		
40.69	[2R- (2à. 4aa. 8aa)] - 1.2.3.4.4a. 5.6.8a-octahydro-à. à. 4a. 8-tetramethyl-2-methanol	C <sub>15</sub> H <sub>26</sub> O		1.51	2.62	1.27

(Continues)



TABLE 3 (Continued)

Time/min	Flavor substances	Chemical formula	Raoh Heilongjiang (RHP) Relative content %	Yingchun Heilongjiang (YHP)	Linqing Shandong (LSP)	Change Henan (CHP)
	Aromatic hydrocarbons (relative content/%) Amount of aromatic hydrocarbons/ one		3.42	6.72	10.38	2.73
	Aromatic hydrocarbons (relative content/%) Amount of aromatic hydrocarbons/ one		7.00	7.00	8.00	6.00
<b>Aldehydes</b>						
12.07	Hexanal	C <sub>6</sub> H <sub>12</sub> O			0.09	0.03
12.53	Trans-2-methyl-2-butenal	C <sub>5</sub> H <sub>8</sub> O	0.09	0.18	0.19	0.14
21.83	Furfural	C <sub>9</sub> H <sub>18</sub> O			0.57	0.21
23.78	Furfural	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>			-	0.04
21.99	Nonanal	C <sub>9</sub> H <sub>18</sub> O	0.46			
25.54	Benzaldehyde	C <sub>7</sub> H <sub>6</sub> O	1.65	2.56	1.35	1.34
28.13	BETA-cyclic citral	C <sub>10</sub> H <sub>16</sub> O			0.76	-
29.57	2-hydroxybenzaldehyde	C <sub>15</sub> H <sub>24</sub>			-	-
37	Benzaldehyde, 4-methoxy-	C <sub>8</sub> H <sub>8</sub> O <sub>2</sub>		0.43		
37.33	Cinnamaldehyde	C <sub>9</sub> H <sub>8</sub> O	0.69	0.64		0.68
48.09	Vanillin	C <sub>8</sub> H <sub>8</sub> O <sub>3</sub>	-	1.21		
50.19	Benzyl Benzoate	C <sub>14</sub> H <sub>12</sub> O <sub>2</sub>	-	1.14		
	Aldehydes (relative content/%)		2.89	6.16	2.96	2.44
	Number of aldehydes/ piece		4.00	6.00	5.00	6.00
<b>Others</b>						
4.99	Hexane	C <sub>6</sub> H <sub>14</sub>		0.01	0.03	-
5.02	Acetone	C <sub>3</sub> H <sub>6</sub> O			-	0.08
8.47	2,2,4,6,6-pentamethylheptane	C <sub>12</sub> H <sub>26</sub>		0.02	0.03	0.10
9.49	Decane	C <sub>10</sub> H <sub>22</sub>		0.02		
12.42	3,3-dimethylhexane	C <sub>8</sub> H <sub>18</sub>			0.01	-
16.38	Octane	C <sub>10</sub> H <sub>18</sub> O	0.45	0.23	0.86	0.63
18.94	Tridecane	C <sub>13</sub> H <sub>28</sub>	0.15			
20.2	Methylheptenone	C <sub>8</sub> H <sub>14</sub> O	0.08		0.08	0.06
21.77	N-tetradecane	C <sub>14</sub> H <sub>30</sub>	0.11		-	-
23.52	à-Methyl-à-[4-methyl-3-pentenyl] oxiranemethanol	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	0.15			

(Continues)

TABLE 3 (Continued)

Time/min	Flavor substances	Chemical formula	Raohe Heilongjiang (RHP) Relative content %	Yingchun Heilongjiang (YHP)	Linqing Shandong (LSP)	Change Henan (CHP)
27.96	10.10-dimethyl-2,6-divinyl-bicyclo [7.2.0] undecane	C <sub>15</sub> H <sub>24</sub>		-	-	0.14
28.73	[1R-(1R*,4Z,9S*)]-4,11,11-Third 8-vinyl-bicyclo [7.2.0] undecane	C <sub>15</sub> H <sub>24</sub>		-	-	0.63
36.3	phenol	C <sub>6</sub> H <sub>6</sub> O	0.13			
36.5	Benzene, 1,2-dimethoxy-4-(1-propenyl)	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	0.1			
36.78	Phenol, 4-ethyl-2-methoxy-	C <sub>9</sub> H <sub>12</sub> O <sub>2</sub>		5.29		
39.73	4-octadecylmorpholine	C <sub>22</sub> H <sub>45</sub> NO			-	-
40.72	Hydroxy-2-methylacetophenone	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	0.55			
41.14	isoanlyl epoxide	C <sub>15</sub> H <sub>24</sub> O			-	1.43
43.34	2,4,6-tri-tert-butylphenol	C <sub>18</sub> H <sub>30</sub> O			-	-
43.82	1-(4-hydroxy-3,5-dibutylphenyl)-2-methyl-3-morpholinone	C <sub>22</sub> H <sub>35</sub> NO <sub>3</sub>			-	-
44.48	N,N'-benzoic acid-hexenediamide	C <sub>21</sub> H <sub>22</sub> N <sub>2</sub> O <sub>6</sub>	1.90		-	-
	Other categories (relative content/%)		3.62	5.57	1.01	3.07

of peak area, respectively. Although the content of the esters is relatively low, they are essential for the flavor, thus they were analyzed under the same detection conditions to obtain variable results.

Alcohol is the main contributors to propolis flavors. The content of alcohol in propolis in different regions is relatively high. Contents determined were 32.36%, 16.68%, 19.21%, and 27.43%, respectively, with no significant geographic variations. The terpenes ratio in RHP was the highest, accounting for 42.34%. CHP and LSP contained 3.35%, 13.95% and 14.24% terpenes relative content, respectively.

A very low content of olefins was found in the samples. The relative content of olefins in CHP was 3.77% of the total peak area. The relative content of olefins in LSP was 1.88%.

The aromatic substances in propolis contain bactericidal and bacteriostatic effects. The relative proportion of aromatic substances in LSP, CHP, RHP, and YHP accounted for 10.38%, 2.73%, 6.72%, and 3.42% of the total peak area, respectively. Aldehydes are also one of the active compounds in propolis, but the proportion and type are relatively small. The aldehydes in LSP, CHP, RHP, and YHP accounted for 2.96%, 2.44%, 2.89%, and 6.16%, respectively.

## 4 | CONCLUSION

It can be concluded from the explained results that the polyphenolic compounds in propolis chiefly vary according to their botanical origin and environmental ecology of the plant. Ultrasound-assisted extraction can increase the yield of TFC and TPC and thus may strongly enhance the antimicrobial activity of these extracts. Chinese propolis has high antioxidant and antimicrobial activity; variability in antioxidant activity of Chinese propolis is strictly related to variability in different amounts and kinds of phenolic compounds. Chinese propolis also contains much higher flavonoids than the other chemical substances such as alcohols, terpenes, aromatic acids, ketones, hydrocarbons, aliphatic acids, and their esters, thus they are very active as antioxidant agents. Chinese propolis may be used in natural antibacterial agents when extracted by ultrasound-assisted extraction, as it is time-saving and increases the extraction yield. Further studies about its uses and functional efficacy are warranted.

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## CONFLICT OF INTEREST

There are no conflicts of interest.

## ETHICAL APPROVAL

This study does not involve any human or animal testing.

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