SUPPLEMENTARY MATERIAL

Chemical composition, UV/Vis absorptivity, and antioxidant activity of essential oils from bark and leaf of *Phoebe* zhennan S. K. Lee & F. N. Wei

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The chemical composition of essential oil (EO) from bark and leaf of *P. zhennan* was identified by GC-MS. The compounds of α-calacorene, □-cadinol, □-eudesmol and d-cadinene were found in the essential oils from both bark and leaf. The UV-Vis spectra results indicated the EO could completely absorbed the UV light at the wavelength range of 200-370nm, revealing that EO had great potential as additives for manufacturing UV light blocking products. The radical DPPH scavenging activity assay showed that both the bark and leaf EO possessed strong DPPH radical scavenging activity of 90.25% and 82.10% respectively, which provides an important theoretical guiding in exploiting the value of *P. zhennan* bark and leaf.

Keywords: *Phoebe* zhennan; Essential oils; UV/Vis absorptivity; Antioxidant activity

3. Experimental

Materials

The bark and leaves samples used in this study were collected from *P. zhennan* trees living on campus of Sichuan Agricultural University, Ya'an. The species of the samples were carefully identified with reference to the voucher specimen (No.15-1120-02) in the herbarium of Sichuan Agricultural University. The size was 40-60 mesh. The samples were air dried prior to the analysis. All chemicals used in this investigation were of reagent grade and obtained from commercial sources.

Essential oil (EO) extraction

In experiments of steam distillation, a quantity of 150g of *P. zhennan* bark and leaves were weighed and added into the 2000mL extraction flask with 1000g distilled water, respectively. Oily water separating installation and steam distiller (steam distillation apparatus) were used with heating mantle, and the distillation was carried out for 10 h at the temperature of 100^{o}C.The essential oil was collected and stored under refrigeration.

Chemical composition identification

The chemical compositions of the EO were identified by GC-MS (Agilent 7890A). The GC conditions were: the gas chromatograph equipped with a fused capillary column (HP-5MSHP-5MS, 5 % Phenyl Methyl Silox, L = 30 m, i.d. 0.25 mm, film thickness 0.25 lm) with 5 % phenyl and 95 % dimethylpolysiloxane as the stationary phase; injection mode was split at split rate 35; the column was held at 50℃for 3 min and then heated to 150℃at the rate of 5℃ min^{-1} for 10 min, and thereafter heated to 250℃at 10℃min^{-1}, for 5 min. Essential oil constituents were identified by using total ion chromatograms as well as fragmentation patterns. The carrier gas was helium at a flow rate of 1.5 mL min-1. The MS conditions were: EI (electron impact ionization) of 70 eV.

The spectrum was compared with a standard spectrum library (NIST) to obtain possible molecular structures and to determine the percentage contribution of

individual elements. Relative percentages were obtained by integration and summation of peak areas.

Ultraviolet/ Visible (UV/Vis) Spectroscopy Analysis

The transmittance of the diluted EO using ethanol was analysed using a double beam ultraviolet visible spectrophotometer (UNICO, UV-4802). The samples were scanned from 190 to 900 nm.

Antioxidant activity

The antioxidant activity of the bark and leaf EO from *P. zhennan* wood was determined using the DPPH-free radical scavenging method in accordance with a modified method by Xu *et al*.. Briefly, 2.5 mL of 60μmol/L DPPH dissolved in methanol was added to 0.5 mL EO that had been diluted with ethanol into a series of concentration gradients. The mixture was mixed evenly and incubated in the dark at ambient temperature for 30 min. Thereafter, the absorbance of the sample ($A_{sample)}$ was measured using UV-vis spectrometer at 517 nm. The control sample ($A_{control}$) was performed by adding the DPPH solution to 0.5 mL of ethanol solution. The percentage of DPPH free radical scavenging activity was determined according to the formula:

$$\text{DPPH scavenging rate (\%)} = [(A_{control} - A_{sample})/ A_{control}] \times 100 \quad (1)$$

Each sample was carried out in triplicate. The IC_{50} value is the effective concentration that 50% of the DPPH radical is scavenged by the sample. For the measurement of the IC_{50}, analysis of regression was performed to obtain the relationship between the concentration and the DPPH scavenging rate. The regression equation was calculated and used to calculate the IC_{50} value.

Table S1. Chemical composition of EO from leaf of *P. zhennan*

No.	Compound name	RT（min）	Area（%）
1	1S-□-Pinene	6.119	1.774
2	Camphene	6.52	4.921
3	Fenchol	11.51	3.509
4	Isoborneol	12.797	1.128
5	Borneol	13.117	6.204
6	□-Terpineol	13.878	4.164
7	L-Borneol acetate	16.556	4.074
8	□-Cubebene	19.103	4.496
9	Caryophyllene	20.293	1.031
10	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.□,4a.□,8a.□)-	21.815	4.809
11	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	21.906	2.764
12	b-Eudesmene	22.095	3.017
13	(-)-a-Selinene	22.313	4.505
14	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.□,4a.□,8a.□)-	22.439	5.535
15	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	22.799	2.434
16	d-Cadinene	23.057	16.167
17	a-Murulene	23.388	1.576
18	□-Calacorene	23.537	3.647
19	Espatulenol	24.453	2.91
20	g-Gurjunene	24.601	1.775
21	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	25.643	1.163
22	Selineno	25.757	3.594
23	□-Cadinol	25.998	5.082
24	□-Eudesmol	26.244	3.651
25	a-Eudesmol	26.312	6.072

Table S2. Chemical composition of EO from bark of *P. zhennan*

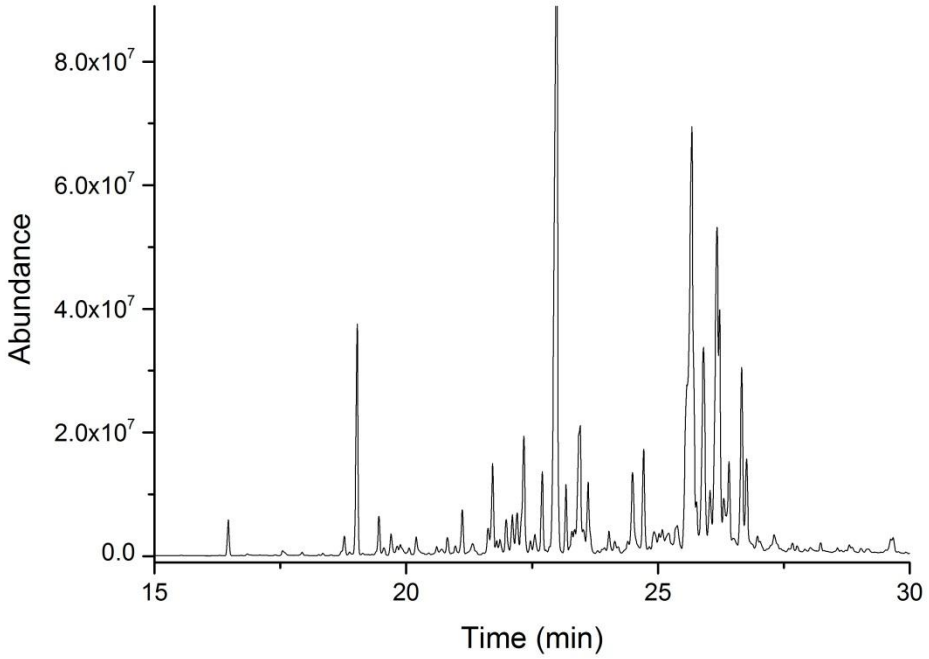
No.	Compound name	RT(min)	Area(%)
1	Copaene	19.028	4.710
2	□-Caryophyllene	21.117	0.888
3	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	21.717	1.992
4	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.□,4a□□,8a.□)-	22.341	3.048
5	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	22.702	1.605
6	d-Cadinene	22.988	21.228
7	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	23.177	1.254
8	□-Calacorene	23.451	4.250
9	a-Elemol	23.617	1.802
10	2,2-Dimethylpropionic acid, 4-methoxybenzyl ester	24.498	2.445
11	Germacrene B	24.716	2.319
12	Calarene	25.671	21.341
13	□-Cadinol	25.906	6.156
14	Copaene	26.038	1.320
15	□-Eudesmol	26.175	9.624
16	□-Cadinol	26.226	4.073
17	Benzene, 1-(2-butenyl)-2,3-dimethyl-	26.415	1.769
18	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	26.667	3.972
19	15-Copaenol	26.764	2.116

Table S3. DPPH radical scavenging activity of bark and leaf EO of *P. zhennan*

DPPH	Regression equation	IC_{50} (mg/mL)

Bark	y=0.2301ln(x)-0.3563 R^2=0.9877	41.32
Leaf	y=0.2276ln(x)-0.3842 R^2=0.9731	48.66

A



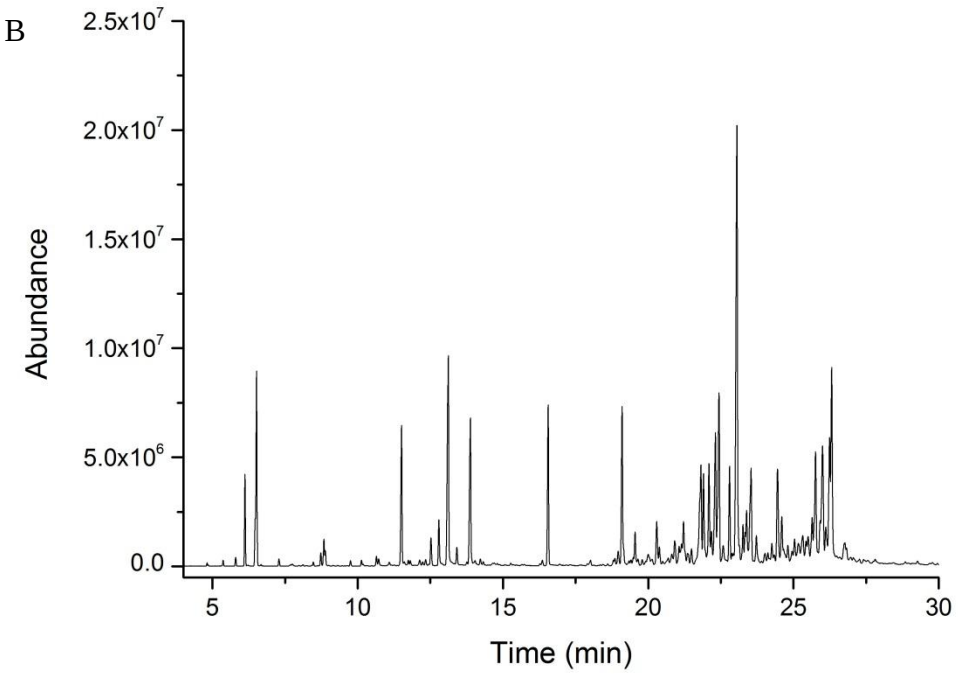


Figure S1. GC-MS chromatogram of EO of *P. zhennan* (A) Bark and (B) Leaf

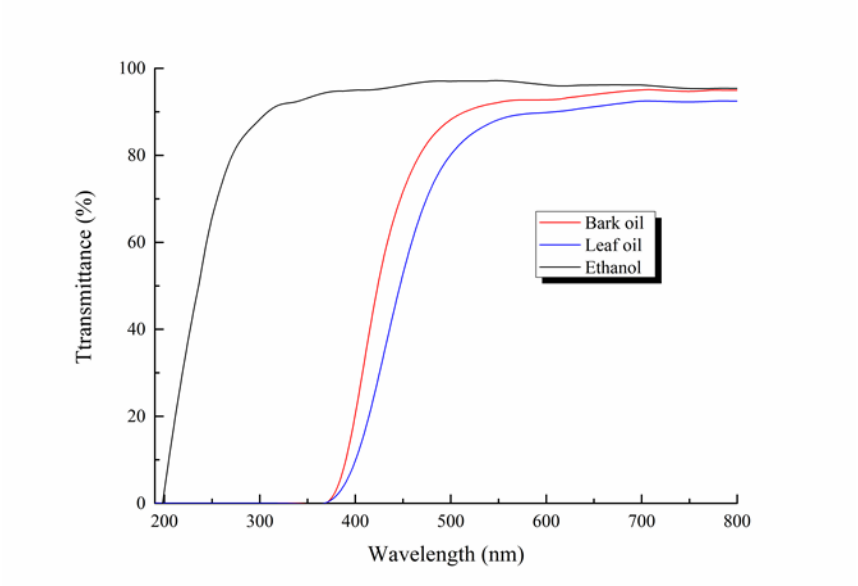
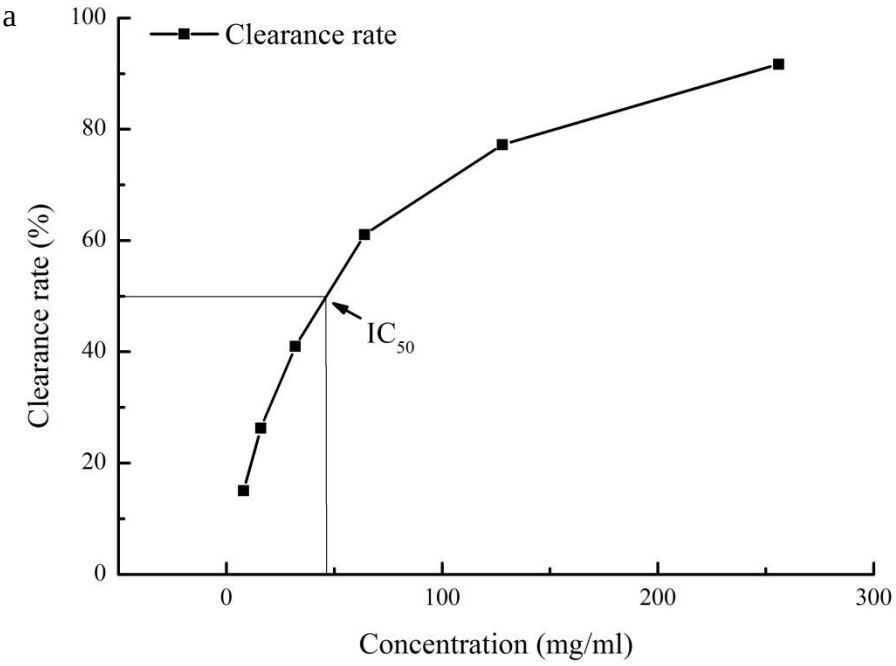


Figure S2. UV-Vis spectra of ethanol and 5% (v/v) EO from bark and leaf of *P. zhennan*

a


Clearance rate
Clearance rate (%)
Concentration (mg/ml)
IC50
0
20
40
60
80
100
0
100
200
300

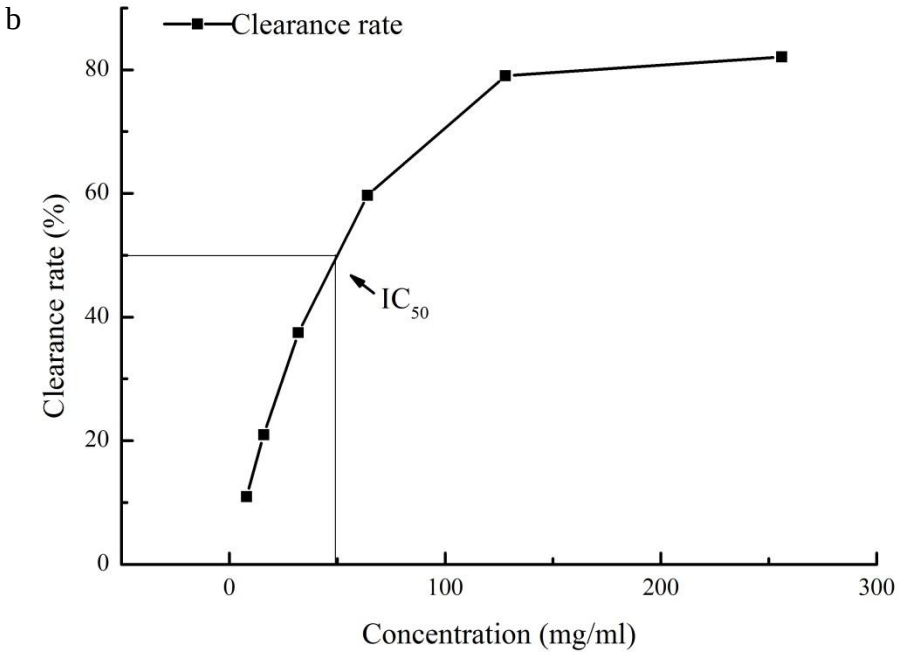


Figure S3. Radical DPPH scavenging activity of bark (a) and leaf (b) EO