Supporting Information

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Chemical Composition, Antibacterial and Antioxidant Activities of Essential oil from *Leonurus pseudomacranthus* Kitag

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S1: *Isolation and identification of the essential oil*

The fresh plant material (500g) was hydrodistilled for four hours using a Clevenger apparatus to give the essential oil (0.53g, 0.106% w/w). The oil was stored at 4°C until analyses.

Gas chromatography analysis:

The essential oil sample was analyzed using a Gas chromatograph (Hewlett Packard-6890 system) coupled with a flame ionization detector (GC-FID) and equipped with a HP-5MS capillary column (30 m×0.25 mm, 0.25 µm film thickness). GC oven temperature was programmed as follows: 60°C for 1 min, programmed to 200°C at 8°C/min, 200°C for 5 min and then increased to 280°C at 5°C/min and hold at 280°C for 2 min. The injector and detector (FID) temperature were maintained at 250°C and 270°C, respectively. The carrier gas used was helium at a constant flow rate of 1.2 mL/min; the injection volume was 0.2 µL.

Gas chromatography- mass spectrometry

Analyses of the essential oil were carried out on a Hewlett Packard 6890 gas chromatograph (Agilent) fitted with a HP-5MS fused silica column, coupled with a Hewlett Packard 5975C mass selective detector. GC parameters were the same as those mentioned for GC-FID. The injection volume was 0.2 µL of 1% solution prepared in n-hexane with split ratio 1:50. Mass spectra were acquired in EI mode at 70 eV. The mass range was from m/z 50 to 550.

Identification of components

The components of the essential oil were identified by their retention indices (relative to C_{10}–C_{30} n-alkanes, under the same experimental conditions), computer matching with NIST MS Search 2.2 Mass Spectral Database for GC-MS and comparisons of their mass spectra with those of authentic samples or with data already available in the literature [1, 2].

S2: ***Antibacterial activity test:***

The antibacterial activity of the investigated essential oil was tested against four bacteria strains, including the Gram positive bacteria, *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538) and the Gram negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853).

Antibacterial activity was assessed using the disc agar diffusion method recommended by CLSI [3]. Filter paper discs (6 mm in diameter) were impregnated with 10 µL of the essential oil or chloramphenicol as a positive control and incubated at 37°C for 24 h. Antibacterial effect was assessed by measuring the diameter of the inhibition zone, which visibly presents the

absence of bacterial growth, including the 6 mm disk. All the tests were repeated in triplicate.

The MIC values were performed in the 96 well-microplates using the microdilution assay according to the literature previously described by Ellof (1998) with slight modifications [4]. The essential oil was diluted and transferred into each well (100 µL per well). Chloramphenicol was used as the reference antibiotic control. The inoculum was added to all wells (100 µL per well). The 12th well was considered as growth control (it contained only the culture medium and strain). The plates were incubated at 36 °C for 18 h. 20 µL of 1% TTC (2,3,5-triphenyl tetrazolium chloride) aqueous solution was used as an indicator of microbial growth [5]. Experiments were carried out in triplicates to minimise the experimental error.

S3: *Antioxidant activity test:*

The essential oil was subjected to screening for the possible antioxidant activity by three methods namely DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay, ABTS (2,20-azinobis-3-ethylbenzothiazoline-6-sulphonate) radical cation scavenging assay and ferric reducing antioxidant power (FRAP) assay.

Free Radical Scavenging Activity by DPPH Method

The DPPH radical scavenging activity was measured according to our previous publication [6] with some modification. A methanol solution of DPPH was prepared immediately before the assay. A volume of 100 µL of different concentrations of the essential oil was added to 100 µL of 0.1 mM DPPH solution in a 1:1 ratio. An equal volume of methanol was added in the control test. The reaction mixture were shaken and allowed to stand for 30 min at shade. The absorbance of the samples was measured by a 96-well microplate reader at 517 nm. BHT (butylated hydroxytoluene) and Trolox were used as the standard reference compounds. The experiment was repeated for three times. The percentage of the DPPH radical scavenging activity was calculated according the following equation:

$$\text{Scavenging effect (\%)} = (A_0 - A_1)/A_0 \times 100\% \quad (1)$$

Where A_0 is the absorbance of the control solution, and A_1 is the absorbance of the test solution. The antioxidant activity was expressed as IC_{50} value.

ABTS Radical Cation Scavenging Assay

The ABTS radical cation scavenging assay was performed according to the previously reported procedure with some modifications [7]. Briefly, a stock ABTS aqua solution (7mM) was reacted with potassium persulfate aqua solution (2.45mM) and kept overnight in dark at room temperature for 16 h. Trolox and BHT were used as positive control and the essential oil were diluted in DMSO at appropriate concentrations. Prior to use in the assay, the ABTS radical cation solution was diluted with phosphate buffer saline (PBS) to an initial absorbance of about 0.700 (±0.02) at 734nm. Afterwards, in a 96-well plate 160μL of ABTS radical cation solution and 40 μL of positive control or samples were added, incubated in the dark for 10 min at room

temperature, and the absorbance was measured at 734 nm. A negative control containing 40 μL DMSO and 160 μL ABTS was performed each time. The ABTS radical cation scavenging effect was calculated by the following formula:

$$\text{Scavenging effect (\%)} = (A_0 - A_1)/A_0 \times 100\% \quad (2)$$

where A_0 is the absorbance of control without sample and A_1 is the test sample with ABTS radical cation solution.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power (FRAP) of the essential oil was quantified using the method proposed by Benzie and Strain (1996) [8], with slight modifications. The FRAP reagent was prepared by mixing sodium acetate buffer (300 mmol/L, pH 3.6), a solution of TPTZ (10 mmol/L) in 40 mmol/L HCl, and 20 mmol/L $FeCl_3 \cdot 6H_2O$ using the proportion 10:1:1 (v/v/v). A 40 µL of appropriately diluted sample extract and 160 µL of FRAP reagent were mixed in a 96-well plate and incubated at 37 °C for 40 min in the dark. In the case of the blank, 40 µL methanol was added to 160 µL FRAP reagent. The absorbance of the resulting solution was measured at 593 nm using a plate reader. An analytical curve with different concentrations of Trolox (linearity: 0.1–40 µg/mL; $R^2 = 0.999$) was plotted to quantify the ferric reducing antioxidant power of the essential oil. The potential antioxidant activity was expressed as Trolox equivalent antioxidant capacity in µmol Trolox × g^{-1}.

S4: References

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