Supporting Information

Essential Oil from *Hedyotis chrysotricha*: Chemical Composition, Cytotoxic, Antibacterial Properties and Synergistic Effects with Streptomycin

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S1: *Isolation and identification of the essential oil*

The extraction of essential oil from aerial parts of *H. chrysotricha* was performed by the hydrodistillation method (Clevenger-type apparatus), the plant material (600 g) was previously crushed and subjected to hydrodistillation for 4 h. The experiments were performed in triplicate. The oil was stored at 4 °C for further analysis.

The compositions of the essential oil were identified by GC-FID and GC-MS, following the protocol as previously described [1]. The chromatographic analysis of essential oil was performed using an Agilent 6890 gas chromatograph equipped with a flame ionization detector (FID). The used column is capillary column HP-5MS (30 m × 0.25 mm i.d.; 0.25 μm film thickness). The GC oven temperature program was as follows: the initial temperature was 60 °C for 1 min, increased at 6 °C/min to 200 °C for 5 min, and 5 °C/min up to 280 °C, being maintained for 2 min. Helium (1.2 mL/min) was used as the carrier gas. The injector and detector temperatures were set at 250 °C and 280 °C, respectively. The injection volume was 0.2 μL. Gas chromatography-mass spectrometry (GC-MS) analysis was carried out using an Agilent 6890A/5975C GC-MS system. 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 50:1. Mass spectra were acquired in EI mode at 70 eV. The mass scan range was 50-550 *m/z*.

The identification of the essential oil chemical composition was performed based on the comparison of retention indices obtained (relative to C_7-C_{30} *n*-alkanes, under the same experimental conditions) and the compounds mass spectra with those from the NIST 14 library, as well as by comparisons of their mass spectra with data already available in the literature [2-4].

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S2: *Antibacterial Activity Test*

Four bacteria strains were used to assess the antibacterial activity of the essential oil: *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) of the essential oil were determined according to the micro-dilution method as previously described [5].

S3: *Evaluation of Synergistic Effects*

The synergistic activity was quantified by checkerboard assay in 96-well microtiter plates [6]. The interaction results were performed by the fractional inhibitory concentration index (FICI), defined as the sum of the MIC of the combined substances divided by the MIC of the isolated substances and categorized as: Synergism (FICI $\leqslant$ 0.5), additive (0.5 < FICI $\leqslant$ 1), indifferent (1 < FICI < 2), or antagonism (FICI $\geqslant$ 2).

S4: *Cytotoxic Activity Test*

The *in vitro* screening of cytotoxic effects of the essential oil against tested cell lines (HepG2, MCF-7, A-549, HCT-116, LO2) were estimated in terms of growth inhibition percentage and expressed as IC_{50}. The MTT assay was performed to determine the cell viability as described in a previous report [1].

S5: *Statistical Analysis*

All biological experiments were performed in triplicate. The statistical analysis was conducted using IBM SPSS software (version 21.0). The statistical significance of differences between controls and experimental groups was evaluated using Student's t-test. $P < 0.05$ was considered statistically significant.

S6: References

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[4]   P. J. Linstrom and W.G. Mallard (2014). NIST Chemistry WebBook, NIST Standard Reference Database Number 69. (http://webbook.nist.gov).

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