Supporting Information

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Effect of Drying on the Quantity and Composition of *Artemisia monosperma* Essential Oil and Exploring the Bronchodilator Effect Using Guinea Pig Tracheal Muscles

Faten F. Bin Dayel [1*], Najeeb Ur Rehman [1], Mohammad Ayman A. Salkini [2], Abdulaziz F. Al Dosari [3], Abdulaziz S. Saeedan [1] and Maged S. Abdel-Kader [2,4*]

[1]Department of Pharmacology and Toxicology, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia

[2]Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia

[3]College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia

[4]Department of Pharmacognosy Faculty of Pharmacy, Alexandria University, Alexandria 21215, Egypt

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S1:Materials and Methods

S1.1. Chemicals and Reagents

The following chemicals of analytical grade were obtained from Sigma Company, St. Louis, MO, USA; Chloroform, ether and sodium sulphate, carbamylcholine (CCh), loperamide, acetylcholine perchlorate (ACh), isoprenaline, verapamil and papaverine. Details of reagents (salts) to prepare physiological buffer solution (Tyrod) are as follows; potassium chloride (Sigma Co), sodium chloride, calcium chloride, glucose, sodium bicarbonate magnesium sulphate, potassium and dihydrogen phosphate (Merck, Germany).

S.1.2. Preparation of A. Monosperma Oil

A sample of 100 g of the fresh plant and 100 g of shade dried aerial parts (acquired from 250 gm fresh plants) were used for *A. monosperma* essential oil (AMEO) preparation. The reduced size plant materials were subjected to hydro-distillation using Clevenger apparatus for 6 hours. The oil layers were collected with yield of 0.77% and 0.5% w/w of fresh and dry plants, respectively. The oil samples were kept at 4 0C for analysis and biology.

S.1.3. GC-MS Analysis

Chloroform was used as a solvent to dilute oil in order to obtain 1 ppm concentration. Injection of 1 µL of the solutions to Agilent GC/MS instrument Model 7890 MSD were performed with the help of autosampler adjusted to splitless mode. Helium (99.999% purity) was the carrier gas adjusted to flow rate = 1.2 mL/min. HP5MS 30 m length column with 0.25 mm i.d. and 0.25 µm thickness was used in the analysis. The Injector temperature was set at constant temperature of 280 °C. The instrument was programmed with a start temperature of 70 °C for 5 min, then gradual raising of the temperature at rate = 2 °C/min till 120 °C where it was hold for 2 mins followed by increasing rate of 15 °C/min to 290 °C that was kept for 2 mins. The ionization in the mass spectrometer was performed at 70 eV, mass range was set between 30-600 daltons. The ion-source temperature was adjusted to 280 °C.

S.1. 4. GC/FID Analysis

Chromatograms obtained under conditions similar to the GC/MS analysis were generated using FID detector. Peak quantification was recorded by automatically measuring the area of each peak.

S.1.5. Animals

Guinea pigs weighing around 0.6 kg of either male or female were obtained from the animal care unit of KSU and housed at the Animal Care Unit at the College of Pharmacy, PSAU, KSA. The accommodation of the guinea pigs was regulated to a constant temperature range of 23-25 °C. Free *access to drinking water and commercial grade animal nourishment were provided to the animals*. All the ex vivo experiments performed adhere to the guidelines established by the Institute of Laboratory Animal Resources, Commission on Life Sciences, NRC. The PSAU Bio-Ethical Research Committee (BERC) approved the study protocol under the reference number BERC-001-12-19.

S.1.6. Guinea Pig Tracheal muscles

Cervical dislocation was performed to scarify the trachea of guinea pigs following a forceful jerk at the trachea of the animals, which were sacrificed with their heads suspended in an erect position between the middle and index figure. After being dissected, the tracheal tube was maintained in Kreb's solution, an appropriate physiological buffer. Seven to eight distinct tissues were isolated from the tracheal tube, with each tissue ring being exposed through a longitudinal incision in the cartilage that was perpendicular and facing the smooth muscle. The tracheal tissue was then mounted in a 10 mL tissue buffer supplemented with Kreb's solution, kept at 37 °C aeriated with carbogen (95% O_2 and 5% CO_2). The buffer components were (mM): KCl 4.7, KH_2PO_4 1.3, NaCl 118.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0 and glucose 11.7 with pH 7.4. Each tracheal strip was subjected to a constant tension of 1 g for the duration of the experiment. A 60-minute equilibration period was observed prior to application of the tested materials. During this time the buffer was replaced every 15 minutes. For tissue stabilization, CCh (1 µM) was employed until constant superimposable contractions were accomplished. The relaxation caused by AMEO of the dried aerial parts and standards was determined by adding increasing concentrations to get concentration-dependent responses.

S.1.7. Determination of the mechanism of the Bronchodilator's effect

In order to investigate the existence of calcium channel blocking (CCB) activity of AMEO of the dried aerial parts, the standard Kreb's solution in the tissue-organ baths was gradually substituted a Kreb's solution that is rich in K+ and lacks Ca+2 after the tissues were mounted and stabilized [1]. Following that, concentration response curves (CRCs) for Ca+2 were constructed in the presence and absence of different AMEO concentrations.

Verapamil served as the positive control. Phosphodiesterase inhibitory-like activity of AMEO was investigated by constructing inhibitory CRCs of isoprenaline against CCh (1 µM)-induced contractions [2]. To examine the potentiating effect of AMEO on the inhibitory activity of isoprenaline, the experiments were replicated while oil was present. The resulting responses were then compared to those obtained using papaverine, the standard drug [3]. An isometric transducer was used to achieve the responses on an organ bath (emkaBATH, France) with iox software (2.10.8.6) installed.

S.1.8. Statistical Analyses

Results are presented as mean ± standard error of the mean (SEM, n represents the number of experiments). EC50 with 95% confidence intervals (CI) represents the median effective potency. A p-value of less than 0.05 was accounted as significant result. Relaxation concentration response curves (CRCs) were inspected using non-linear regression utlizing the GraphPad program (GraphPAD, San Diego, CA, USA).

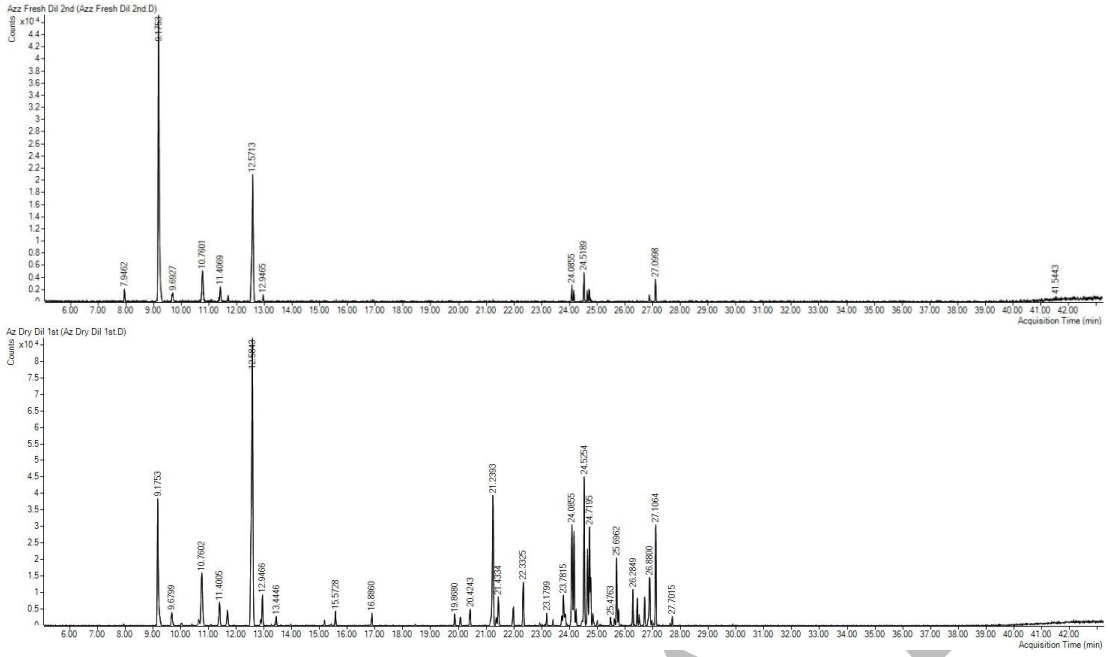

Figure S1 : Composition of AMEO from fresh and dry plant materials.

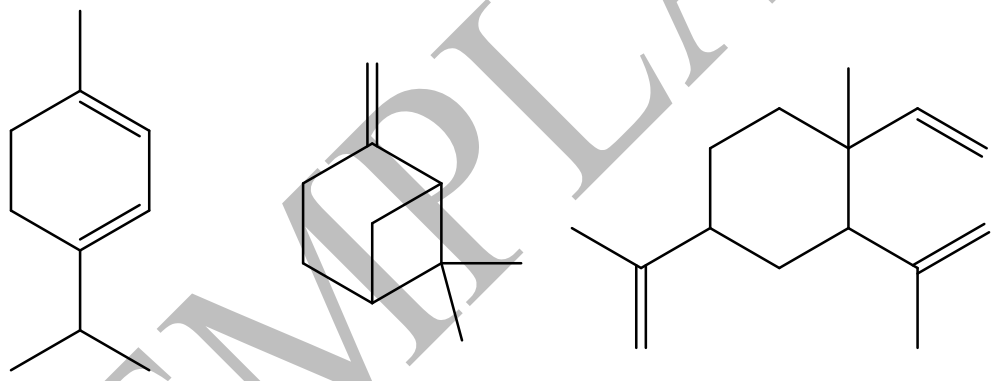

Figure S2 : Chemical structure of some AMEO components.

Table S1: Composition of AMEO of fresh and dry aerial parts.

	Common name	RT	RI measured	RI reported
1.	Sabinene	7.9	965	968 [4]
2.	β-Pinene	9.2	975	979 [5]
3.	β-Myrcene	9.7	983	991 [5]
4.	α-phellandrene	9.9	1005	1003 [5]
5.	α-Terpinene	10.3	1017	1017 [5]
6.	L-Limonene	10.8	1027	1029 [5]
7.	β-trans-Ocimene	11.4	1047	1050 [5]
8.	cis-Ocimenol	15.6	1150	1156 [6]
9.	Pulegone	19.9	1237	1235 [7]
10.	Citronellol acetate	20.4	1359	1356 [8]
11.	β-Elemene	21.2	1388	1391 [5]
12.	α-Isocomene	21.4	1392	1394 [9]
13.	β-Caryophyllene	21.7	1422	1419 [5]
14.	*cis*-Arbusculone	21.9	1457	1464 [4]
15.	(Z,Z)-α.-Farnesene	22.3	1463	1462 [10]
16.	α-Zingiberene	23.8	1490	1487 [8]
17.	(+)-Bicyclogermacrene	24.1	1497	1500 [5]
18.	α-Muurolene	24.2	1499	1500 [5]
19.	Shyobunone	24.5	1519	1522 [9]
20.	α-Cadinene	24.6	1538	1543 [9]
21.	α-Calacorene	24.7	1560	1558 [4]
22.	β-Caryophyllene oxide	25.8	1574	1574 [7]
23.	τ-Cadinol	26.9	1644	1649 [7]
24.	α-Cadinol	27.1	1660	1654 [5]

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