SHORT REPORT

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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

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Abstract: *Artemisia monosperma* is a plant with many traditional uses including some affecting smooth muscles. The *A. momosperma* essential oil (AMEO) prepared by hydrodistillation from fresh and dry aerial parts was compared qualitatively and quantitatively using GC/MS. The drying process affected the yield and composition of AMEO. The bronchodilator potential was explored using isolated guinea-pig trachea in *ex-vivo* organ bath setup. In the tracheal contractions induced by different spasmogens, AMEO was able to completely relax contractions induced by carbachol (CCh; 1 µM) and high K+ (80 mM) at closely related doses ($p > 0.05$) indicating a dual inhibition of phosphodiesterase enzyme (PDE) and voltage-mediated L-type Ca^{++} channels blocker (CCB) as papaverine. The current study provides scientific support to the medicinal use *A. monosperma* in respiratory disorders.

Keywords: *Artemisia monosperma*; essential oil; GC/MS; *ex-vivo*; Guinea pig trachea; mechanism.

1. Plant Source

Artemisia monosperma was collected in April, 2023 from Al-Jubail region (26°56'26.2"N 49°30'22.8"E) eastern part of Saudi Arabia. The plants were authenticated by Dr. Mona Alwahibi, Botany and Microbiology Department, College of Science at KSU. A voucher specimen #MSA 11723 was preserved at the Department of Pharmacognosy, College of Pharmacy, PSAU.

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2. Previous Studies

In Saudi Arabia deserts *A. monosperma* grows up to 1 meter in height [1]. *A. monosperma* is reputed to have antispasmodic effect. In Jordan, the leaves are applied to induce abortion [2]. The plant is also used traditionally to treat diabetes, rheumatic pain and fever [2, 3].

3. Present Study

A sample of 100 g of the fresh aerial parts and 100 g of dried aerial parts obtained from 250 g of fresh sample after drying for two weeks under controlled lab conditions were used for *A. monosperma* essential oil (AMEO) preparation by hydro-distillation using Clevenger apparatus for 6 hours. The yield based on the sample weight used for oil preparation was 0.77% and 0.5% w/w of fresh and dry aerial parts, respectively. The components of the AMEO were determined using GC/MS analysis as well as comparison of the retention indices with the values of the National Institute of Standards and Technology database (Table 1, Table S1, Figures S1 and S2). The AMEO of the fresh aerial parts was rich in monoterpenes. β-pinene (48.7%), α-terpinene (25.3 %) and L-limonene (6.2 %). Previous analysis of AMEO from fresh aerial parts, leaves stems all contains β-pinene as the major component. Many components were also in common especially shyobunone [4- 7]. However, differences exist due to environmental factors. The drying process took place gradually where enzymatic activity can keep going till the moisture contents reached a low critical level to stop it. The enzyme activity optimally requires 45 % moisture contents or more. Both enzymatic activity and the more volatility of the lighter monoterpenes are accounted for the changes in the oil composition of the oil derived from dry aerial parts [8]. The most dramatic loss was in the β-pinene % that decreased to 9.7 while the percentage of heaviour components such as the sesquiterpenes (+)-Bicyclogermacrene, α-Muurolene, τ-Cadinol and α-Cadinol increased. The percentage of β-Elemene increase may be attributed to both less volatility and enzymatic activity during the drying process.

Table 1. Composition of AMEO of fresh and dry aerial parts

No	Common name	% Fresh	% Dry
1.	Sabinene	1.3	-
2.	β-Pinene	48.7	9.7
3.	β-Myrcene	1.2	0.8
4.	α-phellandrene	0.9	0.9
5.	α-Terpinene	25.3	22.8
6.	L-Limonene	6.2	4.5
7.	β-trans-Ocimene	2.4	1.9
8.	cis-Ocimenol	0.1	0.7
9.	Pulegone	0.1	0.6
10.	Citronellol acetate	0.1	0.9
11.	β-Elemene	1.7	8.9
12.	α-Isocomene	0.1	1.7
13.	β-Caryophyllene	0.6	2.5
14.	*cis*-Arbusculone	0.2	0.9
15.	(Z,Z)-α.-Farnesene	0.5	2.7
16.	α-Zingiberene	0.5	2.9
17.	(+)-Bicyclogermacrene	1.3	5.2
18.	α-Muurolene	1.0	4.5
19.	Shyobunone	1.4	6.7
20.	α-Cadinene	1.5	3.5
21.	α-Calacorene	0.9	6.5
22.	β-Caryophyllene oxide	-	0.6
23.	τ-Cadinol	0.6	2.9
24.	α-Cadinol	1.9	4.2
Monoterpenes hydrocarbons		**86.0**	**40.6**
Oxygenated monoterpenes		**0.3**	**2.2**
Sesquiterpenes hydrocarbons		**9.7**	**46.0**
Oxygenated sesquiterpenes		**2.5**	**7.7**
Total		**98.5**	**96.5**

The antispasmodic effect of *A. monosperma* encourage us to study the bronchodilator effect of its oil as we were interested in studying such activity in many traditional plants [2]. Smooth muscle relaxant effect of many essential oils correlated to their terpene contents such as β-pinene and τ-Cadinol [9- 12]. The AMEO was evaluated against CCh and high K+ evoked bronchospasm using the well-established guinea-pig tracheal muscles model [13]. CCh stimulates the muscarinic (M3) receptors leading to induced bronchoconstriction [14]. Solutions with K^+ concentration more than 25 mM could open the voltage-gated L-Type Ca^{++} channels causing depolarization leads to tracheal contractions [15]. AMEO was able to suppress both CCh and high K^+ initiated tracheal muscles contractions in a concentration-dependent manner with EC_{50} = 0.24 mg/mL (0.21 – 0.28, n=4) and 0.28 mg/mL (0.24 – 0.32, n=4), respectively (Figure 1). Papaverine is an inhibitor of both Ca^{++} channels and PDE expressed similar behaviour with EC_{50} = of 11 µM (0.86 – 13.42, n=5) and 12.20 µM (10.42 – 14.86, n=5), respectively (Figure 1)[16]. The standard Ca^{++} channel blocker verapamil [17], was highly selective in blocking K^+ contractions resulted from the opening of the voltage-gated L-Type Ca^{++} channels with EC_{50} = 0.86 µM (0.74 – 0.98, n=5) and 16.14 µM (14.24 – 18.56, n=5), respectively (Figure 1) [18]. These finding indicated that AMEO expressed the airways relaxant activity via CCB and PDE inhibitory mechanisms in a fashion resembles that of papaverine.

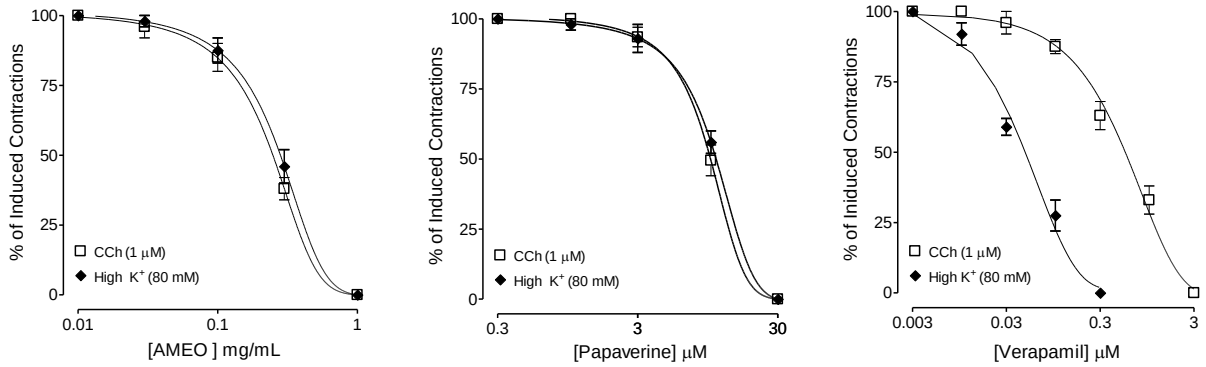

Figure 1. Concentration-response curves of AMEO, papaverine and verapamil, suppression of carbachol (CCh; 1 µM) and high K^+ (80 mM) initiated contractions guinea-pig tracheal muscles preparations. Values shown are mean ± SEM, n=4-5.

AMEO along with the two standards verapamil and papaverine were challenged against Ca^{++} induced bronchospasm (Figure 2). The three tested entities were able to markedly attenuate the contraction as well as reduce maximum response indicating their effect on the Ca^{++} channels. The three entities were again tried for their effect on isoprenaline relaxant effect against CCh bronchoconstriction.

Both AMEO and papaverine expressed potentiation of the iosprenaline relaxation proving the co-existence of PDE inhibitory like activity (Figure 3). Verapamil did not show any potentiation to isoprenaline relaxation (Figure 3). Isoprenaline is a nonselective β-adrenoceptor agonist resulted in airways relaxation by raising the intra-cellular cAMP concentration. Respiratory tract increase in cAMP concentration can result from two possible mechanisms: β2–agonistic activity and PDE inhibition [19]. The demonstrated enhancement of isoprenaline inhibitor action by AMEO indicate the presence of PDE inhibitor mechanism on the airways relaxant mechanism. It is reported that PDE inhibitors potentiate the isoprenaline relaxant effect [20]. Based on these findings the presence of β2–agonistic activity cannot be excluded. The beneficial role of PDE inhibitors in the management of asthma is well established [21].

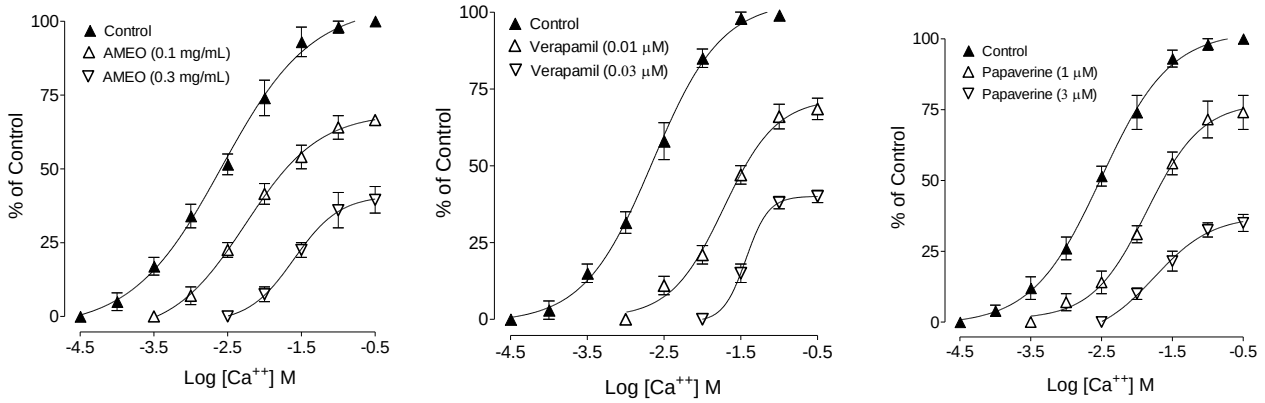

Figure 2. Concentration-response curves of Ca^{++} with or without increasing concentrations of the AMEO, verapamil and papaverine using guinea-pig tracheal muscle preparations. Values shown are mean ± SEM, n=4-5.

Their major drawback is the cardiac stimulation effect [22]. Interestingly, Ca^{++} antagonists expressed beneficial action in the treatment of bronchoconstriction [23] and in contrary to PDE inhibitors they exhibit suppressant action on the cardiac muscle [24]. The combination of Ca^{++} channel blocker as well as PDE inhibitor(s) components in AMEO is perhaps implied by the Nature to oppose the tachycardia accompanying with the use of PDE inhibitors alone. This finding is very supportive for the concept that natural remedies known to possess synergistic and/or side effect neutralizing potential. This is added to the cost effectiveness offering merit in evidence-based studies [25]. The presence of the binary inhibition effect on both PDE and Ca^{++} channels is most probably responsible for the medicinal application of AMEO as spasmolytic agent.

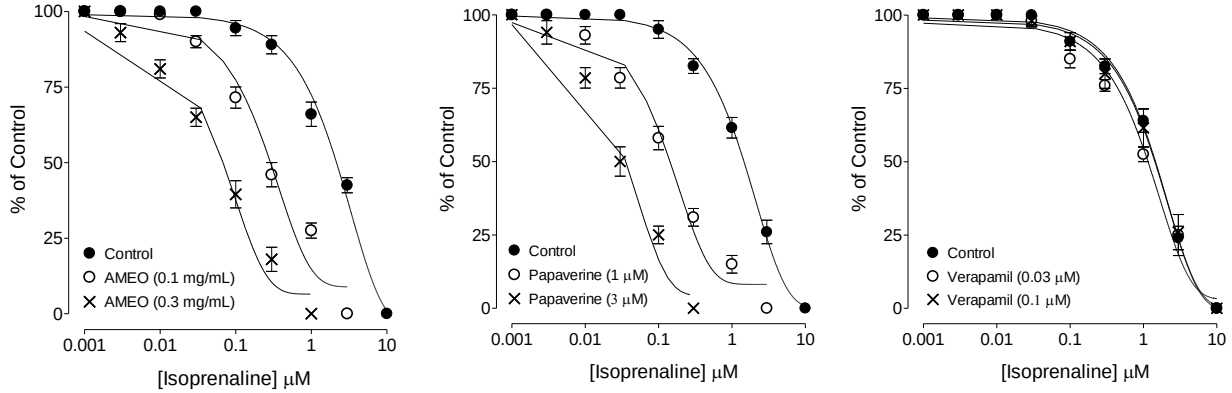

Figure 3. Concentration-response curves of isoprenaline relaxant effect against carbachol (CCh)-mediated contractions with or without different concentrations of AMEO, papaverine and verapamil using guinea-pig tracheal muscles preparations. Values shown are mean ± SEM, n=4-5.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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