Supporting Information

Rec. Nat. Prod. **X:X (2018) XX-XX**

Comparison of Chemical Composition, Antifungal and Antibacterial Activities of Two Populations of *Salvia macilenta* Boiss. Essential Oil

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S.1. Experimental Details

S.1.1. Extraction of essential oil

The essential oil of each specimen was extracted from 150 g of crushed tissue by hydrodistillation, using a Clevenger-type system. The sample was placed in a 2 L flask comprising 1 L of water that was heated to 100°C for 2.5 hours with a heating jacket to vaporize the essential oil that was eventually condensed and gathered. Essential oil samples were dried over anhydrous sodium sulfate and stored at 4°C until analysed of components.

S.1.2. Gas chromatography-mass spectrometry

GC analysis of the essential oil was done using a Thermoquest-Finnigan instrument armed with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was consumed as the carrier gas at a constant current of 1.1 mL/min. The oven temperature was kept at 60ºC for 1 min, then programmed to 250ºC with speed of 4ºC/min, and then held for 10 min. The injector and detector (FID) temperatures were held at 250ºC and 280ºC, respectively.

GC-MS analysis was accomplished on a Thermoquest-Finnigan Trace GC-MS system equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was elevated from 60ºC to 250ºC at a velocity of 5ºC /min, and then held at 250ºC for 10 min, transfer line temperature was 250ºC. The quadrupole mass spectrometer was scanned over 45-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA. Recognition of the specific ingredients was based on comparison of their GC-MS retention indices (RIs) on nonpolar and polar columns, specified relative to the retention times of a series of n-alkanes (C_6-C_{24}), with those of valid compounds or retention time data presented in the literatures. All components identities were authenticated by contrasting the RIs of the components with those of reference standards or with printed data [1].

S.1.3. Antimicrobial activity assay

Antibacterial activity: In vitro antibacterial properties of essential oils was determined against *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 as Gram positive bacteria and *Pseudomonas aeruginosa* PTCC1430 and *Escherichia coli* ATCC 25922 as Gram negative bacteria. Designation of MIC value was accomplished by broth micro-dilution procedure as recommended by CLSI (Clinical Laboratory and Standard Institute) with some modifications [2]. Briefly, a serial dilution of each essential oil was made in a concentration range of 64 to 0.125 μg/mL in sterile 96 wells trays containing Mueller-Hinton broth medium supplemented by 0.5% Tween 80 as co-solvent. Normal saline was utilized for production of inoculants having turbidity equal to 0.5 McFarland standards. The inoculants of the microbial strains were generated from freshly cultured bacteria that were regulated to 0.5 McFarland standard turbidity and were further diluted (1:100) using MHB medium just before adding to the serial diluted samples. Plates incubated for 24 h at 37°C. MIC values were recorded as the lowest concentrations which could inhibit visible growth of microorganisms [2]. Minimum bactericidal concentrations (MBCs) were specified by culturing of 100 μl of each no-growth well onto nutrient agar trays and incubation at proper temperature. MBC values were recorded as the lowest concentration which resulted in killing of 99.9 % of microorganism. Each test was done in triplicate and Cefixime was utilized as standard antibacterial agent.

S.1.4. Antifungal activity: Two fungi: *Aspergillus flavus* (Lab Isolate) also *Candida albicans* ATCC 10231 and *Candida Krusei* ATCC 5295 were tested in this portion of research. Calculation of MIC was accomplished by broth micro-dilution manner as introduced by CLSI with some modifications

[3]. In brief, a serial dilution of each essential oil was made in a concentration range of 64 to 0.125 µg/mL in sterile 96 wells trays containing RPMI pH 7 supplemented by 2% (w/v) Dextrose, MOPS (0.165 M) and 0.5% Tween 80 as co-solvent for oil. The inoculants of the yeast strain were manufactured from freshly cultured yeast that were adjusted to 0.5 McFarland standard turbidity and were further diluted (1:1000) using mentioned medium just before increasing to the serial diluted specimens. For *A. flavus* examined strain, Potato dextrose agar plates were applied as medium for conidial growth. Plates were incubated at 35°C for 7 days. After that, a 1:50 diluted stock of conidia was applied for inoculation of plates containing diluted oils. Trays incubated for 48 h at 30°C. MIC values were recorded as the lowest concentrations which could inhibit visible growth of microorganisms [2]. Minimum fungicidal concentrations (MFCs) were determined by culturing of 100 µl of each no-growth well onto Sabouraud dextrose agar trays and incubation at appropriate temperature. MFC values were recorded as the lowest concentration which resulted in killing of 99.9 % of tested microorganism. Each test was accomplished in triplicate and Amphotericin B is used as standard antifungal agent.

Table S1. Antimicrobial activities of two populations of *Salvia macilenta Boiss.*(mg/mL)

		S. aureus	***E. faecalis***	***E.coli***	***P.aaeruginosa***	***C. albicans***	***A. flavus***	***C. Krusei***
Baluchistan sample	MIC	2	32	8	>64	>64	>64	>64
	MBC	8	64	16	>64	>64	>64	>64
Kerman sample	MIC	2	16	4	64	>64	>64	>64
	MBC	64	64	16	>64	>64	>64	>64
Cefixim	MIC	1	4	4	64	-	-	-
	MBC	8	32	16	>64	-	-	-
Amphotericin B	MIC	-	-	-	-	0.0005	0.004	-
	MBC	-	-	-	-	0.001	0.008	-
Nystatin	-	-	-	-	-	-	-	0.0078
	-	-	-	-	-	-	-	0.0078

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