SUPPLEMENTARY MATERIAL

Phenolic Profiling of Different *Jasminum* Species Cultivated in Egypt and their Antioxidant Activity

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Abstract

This study focused on the profiling of phenolic constituents in 80% methanolic extracts of the leaves of some *Jasminum* species cultivated in Egypt and their antioxidant activities. Phenolic profiling was performed by total phenolic contents, total flavonoid contents and HPLC-DAD for selected species in comparison to authentic standards. DPPH assay was used to estimate the antioxidant activities of *Jasminum azoricum* L., *Jasminum humile* L., *Jasminum multiflorum* (Burm.f.) Andrew, *Jasminum officinale* L., *Jasminum sambac* (Ait) L. "Arabian Nights cultivar" and *Jasminum sambac* (Ait) L. "Grand Duke of Tuscany cultivar". *Jasminum multiflorum* showed the highest antioxidant activity among selected species with IC_{50} of 34.8 µg/ml. *J. multiflorum* showed high concentrations of hydroxytyrosol, protocatechuic acid, hydroxybenzoic acid, kaempferol-*3*-*O*- neohesperidoside, and quercetin-*3*-*O*-glucoside with concentrations of 977.1 µg/g, 2224.7 µg/g, 714.8 µg/g, 1738.8 µg/g, and 4356.1 µg/g, respectively.

Keywords: DPPH, *Jasminum*, HPLC, Total phenolics, Total flavonoids

Experimental

Plant Material

Dry leaves (50 g) of each of *Jasminum azoricum* L., *Jasminum humile* L., *Jasminum multiflorum* (Burm.f.), *Jasminum officinale* L., *Jasminum sambac* Ait. (Arabian nights) and *Jasminum sambac* Ait. (Grand Duke of Tuskany) selected from Keram farms El-Tall El-Kebeer, Egypt, (GPS coordinations- $30^{o}33`57.7``N\ 30^{O}30`20.7``E$) were collected March, 15th, 2017, extracted separately with 80 % methanol by percolation (4 x 5 L) and concentrated using rotary evaporator at 45ºC to yield 10.0 g, 9.5 g, 12.6 g, 11.8 g, 13.1g, 11.4 g residues, respectively. The identity of the plant was kindly confirmed by Prof. Dr. Abdel Megeed Ali Abdel Megeed, Prof. of Plant Taxonomy, Flora and Phytotaxonomy Research Department, Horticultural Research Institute, Agricultural Research Center, Dokki, Cairo, Egypt. Voucher specimens (3.10.16.5), (3.10.16.1), (3.10.16.4), (3.10.16.3), (3.10.16.2) and (3.10.16.6) for *Jasminum azoricum (J. azoricum), Jasminum humile (J. humile*), *Jasminum multiflorum* (*J. multiflorum*), *Jasminum officinale* (*J. officinale*), *Jasminum sambac* "Arabian nights" cultivar (*J. sambac* (A)) and *Jasminum sambac* "Grand Duke of Tuscany" cultivar, (*J. sambac* (G)) respectively are kept at the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Total Phenolics and Total Flavonoids

Total Phenolic

The total phenolic content was estimated by Folin – ciocalteu method; for standard calibration curve, weigh accurately 10 mg standard gallic acid and dissolve in 10 mL distilled water (stock solution), to (2, 4, 6, 8, 10) mL of the stock solution add 1.5 mL Folin ciocalteu reagent and 10

mL distilled water, after 5 minutes add 4 mL 20 % sodium carbonate solution make up to 25 mL with distilled water and incubate for 30 minutes and record absorbance at 765 nm, 1 mL of each extract was mixed with the same reagent.

The absorbance at 765 nm against standard blank was recorded using UV/VIS spectrophotometer after 30 minutes at room temperature in triplicate for each reading, total phenolic content was expressed as mg gallic acid equivalent (GAE) (Laghari et al., 2012)

Total Flavonoid

Total flavonoid content was estimated via colorimetry using aluminum chloride reagent, quercetin was used to make calibration curve. 10 mg quercetin was dissolved in methanol and diluted to (5 – 100) µg, 10 % aluminum chloride and 1 M potassium acetate solutions were prepared, to 1 mL of each extract stock add 1.5 mL methanol, 0.1 mL aluminum chloride, 0.1 mL potassium acetate and 2.8 mL distilled water and mix well, blank was prepared in the same way using distilled water instead of sample. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm against standard blank using UV/VIS spectrophotometer. Total flavonoid content in the supplied sample expressed as mg quercetin equivalent (QE) (Dahija et al., 2014).

Antioxidant Activity

Freshly prepared (0.004%w/v) methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10 ^{o}C in the dark. A methanol solution of the test compound

was prepared. A 40 uL aliquot of the methanol solution was added to 3 ml of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized for16 min. The absorbance of the DPPH radical without antioxidant control and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

PI = [{(AC- AT)/ AC} x 100]

Where AC = Absorbance of the control at t = 0 min and AT = absorbance of the sample, DPPH at t = 16 min. (Lee et al., 1998).

The 50% inhibitory concentration (IC_{50}), the concentration required to inhibit DPPH radical by 50%, was estimated from graphic plots of the dose response curve.

HPLC-DAD

Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was a Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.7 ml/min for a total run time of 60 min and the gradient program was as follows: 100% B to 80 % B in 20 min, 80 % B to 50 % B in 20 min, 50 % B to 0% B in five min. and 100% A for five min. The injection volume was 50 µL and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. Flavonoids were determined at 360

nm. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards (Dey and Kuhad, 2014).

Reference:

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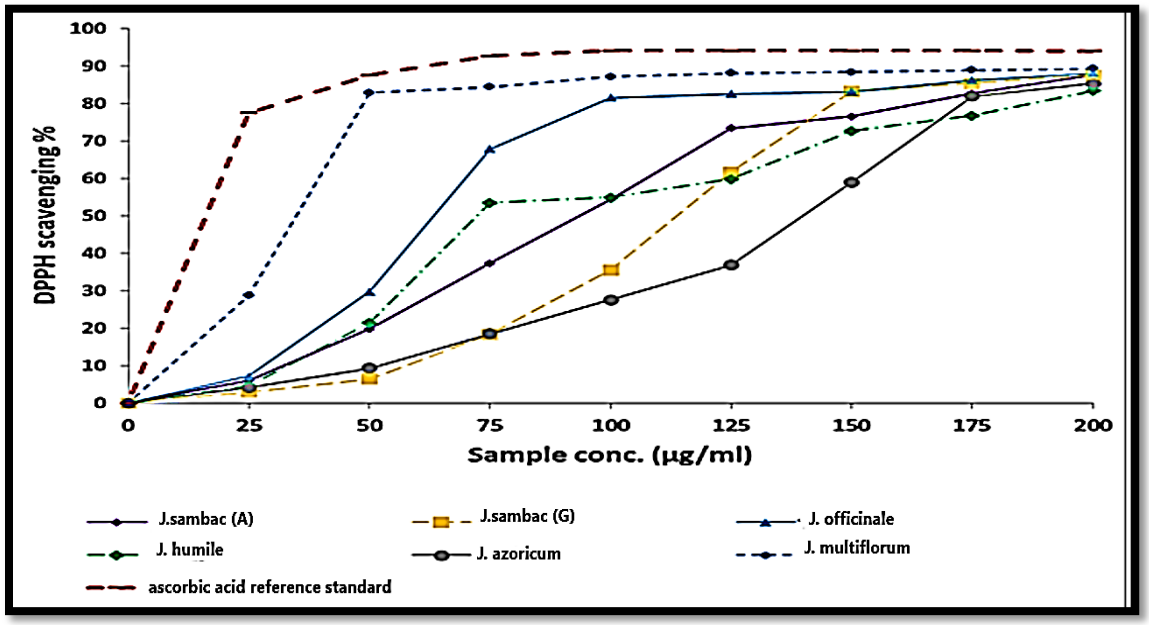

Figure S1: DPPH scavenging % of different concentrations in (µg/ml) of leaves methanolic extracts of *J. azoricum*, *J. humile*, *J. multiflorum*, *J. officinale*, *J. sambac* (A), and *J. sambac* (G), using ascorbic acid reference standard.

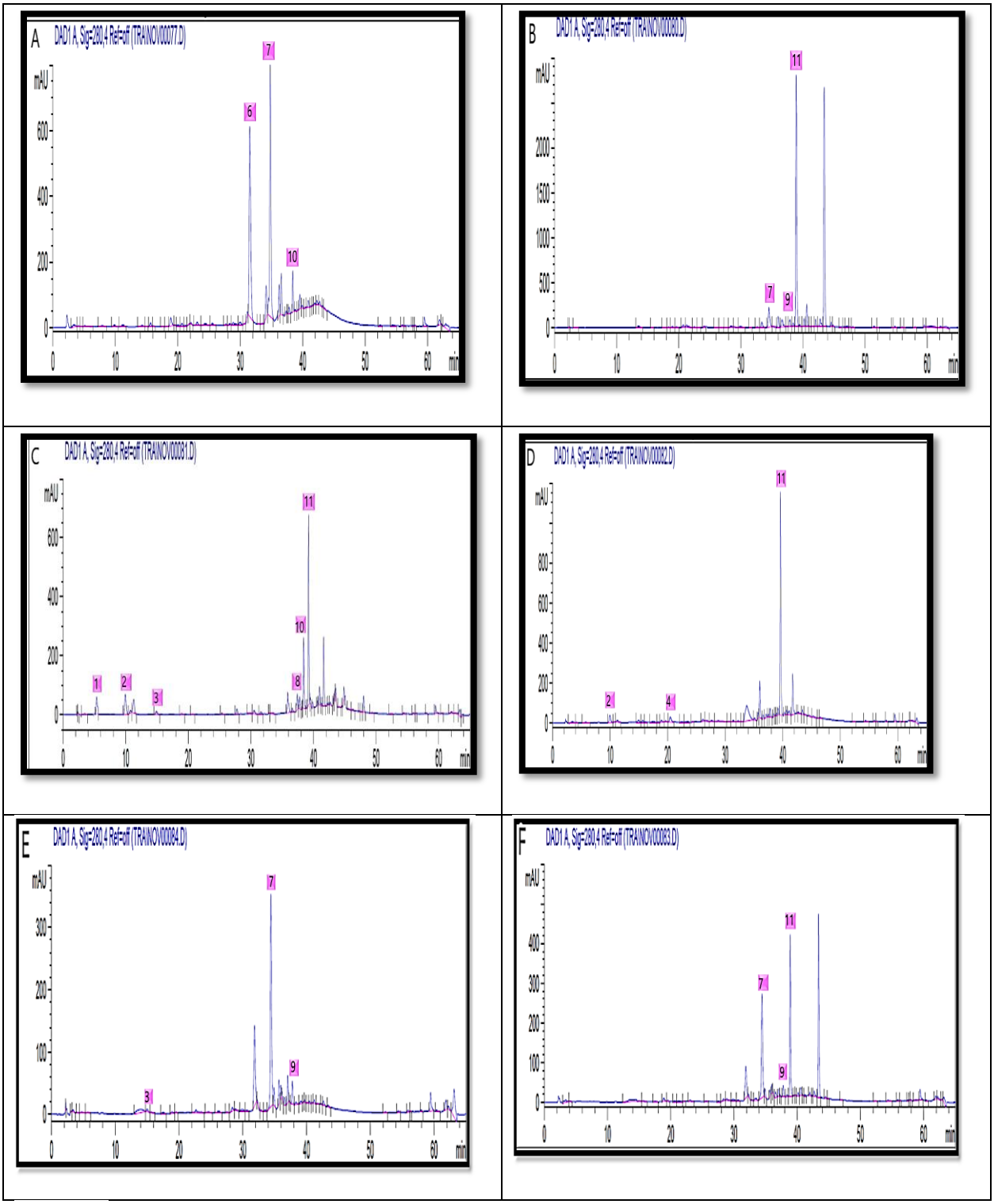

Figure S2: Chromatogram of HPLC-DAD analysis of phenolic compounds in leaves extracts of; (A) *J. azoricum*, (B) *J. humile,* (C), *J. multiflorum, (*D) *J. officinale,* (E) *J. sambac* (A), (F) *J. sambac* (G) at 280 nm