Supplementary Materials: Secondary metabolites profiling, biological activities and computational studies of *Abutilon figarianum* Webb (Malvaceae)

Hammad Saleem, Muhammad Sarfraz, Hafiz Muhammad Ahsan, Umair Khurshid, Syed Asif Jahanzeb Kazmi, Gokhan Zengin, Marcello Locatelli, Irshad Ahmad, Hassan H. Abdallah, Mohamad Fawzi Mahomoodally, Kannan RR Rengasamy, Nafees Ahemad

1. Material and Methods

1.1. Total Phenolic and Flavonoid Content

Total phenolic and flavonoid contents were evaluated in crude extract and its fractions as previously elaborated (Grochowski et al., 2017) by means of a well-known procedure as follow. 1 mL of diluted (1:9, v/v) Folin–Ciocalteu reagent (1 mL) was added to 0.25 mL of the sample solution (1 mg/mL). After 3 min, 0.75 mL of a Na_2CO_3 solution (1%) was added and the mixture was left at room temperature for 2 h in an incubator. Absorbance was measured at 760 nm. Total flavonoid contents were estimated by adding 1 mL of aluminium chloride (2%) in methanol to 1 mL of sample solution (1 mg/mL). The mixture was incubated for 10 min at room temperature and the absorbance reading was taken at 415 nm. The outcomes of total phenolic constituents were reported as equivalents of gallic acid (mg GAE/g extract) while the results of total flavonoid constituents were recorded as equivalents of quercetin (mg QE/g extract).

1.2. Antioxidant Activities

The estimation of free radical scavenging activity by two methods (DPPH, ABTS), reducing power assay (FRAP, CUPRAC) followed by phosphomolybdenum (total antioxidant capacity) and metal chelating activity (ferrous ion chelation) was done by utilizing techniques narrated by [1].

1.2.1. DPPH Free Radical Scavenging Activity

For this antioxidant activity, the DPPH solution (0.267 mM 4 mL, 0.004% methanol solution) was mixed with 1 mL sample solution followed by 30 min of incubation and ultimately absorbance was measured at 517 nm. Unit of measurement was in mg of trolox equivalents/g of dry extract (TEs/g extract).

1.2.2. ABTS Radical Cation Scavenging Activity

The reaction of 7 mM ABTS solution with 2.45 mM potassium persulfate led to the formation of $ABTS^+$ radical cation. 1 mL of test solution was mixed with 2 mL of ABTS solution followed by measurement of absorbance at 734 nm after 30 min. The articulation of results was done in mg of trolox equivalents/g of dry extract (TEs/g extract).

1.2.3. Phosphomolybdenum Method

In this assay, 3 mL of reagent solution which comprised of (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was mixed in 0.3 mL of sample solution and the resultant absorbance was measured at 695 nm after 90 min of incubation. Measuring units were in mmol of trolox equivalents/g of dry extract (TEs/g extract).

1.2.4. Cupric Ion Reducing (CUPRAC) Method

This method was executed by taking 0.5 mL of the sample solution and mixing it with [CuCl2 (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM), NH_4Ac buffer (1 mL, 1 M, pH 7.0)] and after 30 min of

incubation, the absorbance was estimated at 450 nm. The measurement of results was done in mg of trolox equivalents/g of dry extract (TEs/g extract).

1.2.5. Ferric Reducing Antioxidant Power (FRAP) Method

This method involved the addition of 0.1 mL of sample solution to 2 mL reagent in acetate buffer (0.3 M, pH 3.6), 2, 4, 6-tris (2-pyridyl)-s-triazine (TPTZ) (10 mM) in 40 mM HCl and ferric chloride (20 mM) in (v/v/v) ratio of 10:1:1 leading to the measurement of absorbance at 593 nm after an incubation period of 30 min. The articulation of results was done in mg of trolox equivalents/g of dry extract (TEs/g extract).

1.2.6. Metal Chelating Activity on Ferrous Ions

This method was effectuated by adding 0.05 mL of a solution of $FeCl_2$ (2 mM) to 2.0 mL of sample solution. Later 0.2 mL of ferrozine (5 mM) was utilized in order to instigate the reaction followed by measurement of absorbance at 562 nm after 10 min of incubation and the expression of results was done in milligrams of ethylenediaminetetraacetic acid equivalents per gram of dry extract (EDTAEs/g extract).

1.3. Enzyme Inhibition Assays

The potential inhibitory capacity of all the concentrates against the enzymes including acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, α-amylase and α-glucosidase were explored making use of standard bio-assays [1].

1.3.1. Cholinesterase

In either of the enzyme substrates i.e. [acetylthiocholine iodide (15 mM ATCI) or butyrylthiocholine chloride (1.5 mM BTCl, 25 µL)], 50 µL sample solution was added along with DTNB (3 mM 125 µL) and 25 µL enzyme solution (0.265 u/mL AChE or 0.026 u/mL BChE) in Tris-HCl buffer (pH 8.0). Reaction was allowed to be completed in 15 min of incubation and absorbance was measured at 405 nm. The results were articulated as mg of galantamine equivalent/g of dry extract (GALAEs/g extract).

1.3.2. α-Amylase

25 µL of sample solution was mixed with 50 µL of the α-amylase solution (10 u/mL) already dissolved in phosphate buffer (pH 6.9 with 6 mM sodium chloride). The above reaction mixture was added to 50 µL of the starch solution (0.05%) and the reaction was made to stop by adding 25 µL of HCl (1 M). Finally, 100 µL of the iodine-potassium iodide solution was incorporated into the above mixture. The absorbance reading was logged after an incubation period of 10 min. Millimoles of acarbose equivalents per gram of dry extract (ACAEs/g extract) were the units in which measurements were made.

1.3.3. α-Glucosidase

This assay comprehended with the addition of 50 µL of sample solution in equal concentrations 50 µL of glutathione (0.5 mg/mL) and α-glucosidase solution (0.2 u/mL) in phosphate buffer (pH 6.8) and PNPG (10 mM). The reaction was stopped after 15 min with the addition of 50 µL of sodium carbonate solution (0.2 M) and ultimately absorbance was measured at 400 nm. The expression of results was done in millimoles of acarbose equivalents per gram of dry extract (ACAEs/g extract).

1.3.4. Tyrosinase

40 µL of tyrosinase solution (200 u/mL) was mixed with 25 µL of the sample solution. To the above mixture, phosphate buffer (40 mM, 100 µL, pH 6.8) was added in a 96-well microplate. Temperature was retained at 25 °C during the incubation period of 15 min. Addition of l-DOPA (10

mM, 40 μL) led to the actuation of reaction. The incubation period was set for 10 min at room temperature and the absorbance was estimated at 492 nm. Milligrams of kojic acid equivalents per gram of dry extract (KAE/g extract) were the units in which results were expressed.

1.4. Phytochemical Composition (UHPLC-MS Analysis)

The profiling of secondary metabolites in the methanolic extract was estimated by employing the standard RP-UHPLC-MS analysis method as described previously [2]. Agilent 1290 Infinity UHPLC system paired to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer interfaced with a dual ESI source was incorporated with the column Agilent Zorbax Eclipse XDB-C18 (2.1 x 150 mm, 3.5 μm). 4 °C temperature was set and maintained for auto-sampler and 25 °C for column respectively. 0.1% formic acid solution in water was selected as mobile phase (A), while acetonitrile and 0.1% formic acid solution as mobile phase (B) with a flow rate of 0.5 mL/min. 1.0 μL of plant extract dissolved in methanol (HPLC grade) was inserted for 25 min followed by 5 min of post-run time. For nebulization, nitrogen gas was availed with a flow rate of 25 L/h and for drying purpose its flow rate was adjusted to 600 L/h with temperature being set at 350 °C. The capillary voltage and fragmentation voltage were maintained at 3500 V and 125 V respectively [2]. Similarly, polyphenolic composition in all the extracts were also determined using HPLC analyses as previously reported [3].

References:

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