Stem barks of bauhinia acuminata | analysis

Materials and Methods

Plant collection

The stem barks of *Bauhinia acuminata* L. were collected from Rajshahi university campus, Bangladesh, in the month of September, 2013. The plant was authenticated by a taxonomist of Department of Botany, University of Rajshahi. A voucher specimen (Voucher No. MN-13) was deposited to the herbarium in the Department of Botany, University of Rajshahi. The stem barks were then washed separately with fresh water to remove dirty materials and were shade dried for several days with occasional sun drying. The dried barks were then ground into coarse powder by grinding machine and the materials were stored in dark at room temperature for future use.

Extract preparation

The extraction was performed according to method described by Alam el al. (Alam et al., 2002). About 400 gm of dried powdered stem bark were taken in an amber colored reagent bottle (2. 5-liter capacity) and the materials were soaked in 2. 0 liter of 100% methanol. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No. 1 filters paper and were concentrated with a rotary evaporator (Bibby Sterlin Ltd, UK) under reduced pressure at 45°C temperature to afford crude extract of the bark. The crude extract was mixed with 90% methanol in water to obtain a slurry of satisfactory volume of 100ml. The slurry was taken in a separating funnel and added equal amount of different partitioning solvent sequentially as like n-hexane, chloroform, ethyl acetate and finally water. The funnel was shaken vigorously and

allowed to stand for a few minutes for separation of the compounds according to their partition co-efficient and thus extracts were prepared for the experiment purpose. The process was repeated three times. At last, the different fractionated parts were evaporated using rotary evaporator at 40 ^{0} C to obtain n-hexane fraction (NHF, 1. 12 gm), chloroform fraction (CHF, 1. 33 gm), ethyl acetate fraction (EAF, 2. 27 gm) and aqueous fraction (AQF, 8. 86 gm) respectively.

Chemicals

1, 1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide, catechin (CA), ferrous ammonium sulphate, butylated hydroxytoluene (BHT), gallic acid (GA), ascorbic acid (AA), $AlCl_3$, trichloro acetic acid (TCA), sodium phosphate, sodium nitrate, ammonium molybdate, 2-deoxy ribose, sodium hydroxide, EDTA and $FeCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA); potassium acetate, phosphate buffer, thiobarbituric acid(TBA), HCl, H_2SO_4 , H_2O_2 were purchased from Sigma-Aldrich, vinblastine sulphate (VBS) from Cipla India, folin-ciocalteus's phenol reagent and sodium carbonate were obtained from Merck (Dam-stadt, Germany).

Determination of total phenolics

Total phenolic contents in the extracts were determined by the Folin-Ciocalteu method described by Singleton et al. (Slinkard & Singleton, 1977). 40 µl of the extract/fractions (250µg/ml) were taken in test tubes and to each 3. 16 ml of water was added to make up the volume 3. 2ml. 200 µl of folin-Ciocalteu (Undiluted) reagent solution was added into the test tubes and kept for 5-8min. 600 µl of sodium carbonate (20%) solution was added into the test tubes and shake to mix. The test tubes were incubated for 2 hours

at 20ËšC to complete the reaction. Then the absorbance of the solution was measured at 765 nm using a spectrophotometer (Shimadzu, USA) against blank solution. A typical blank solution contains all reagents except plant extract or standard solution. Standard Gallic acid solutions (50-250Î¼g /ml) concentrations were also treated as above. The total content of phenolic compounds in plant methanol extract and in different fractions was expressed as Gallic acid equivalent (GAE)/gm of dry extract in respect to standard gallic acid curve equation (y = 0. 0008x – 0. 005, R^2 = 0. 975).

Determination of total flavonoids

Total flavonoids were estimated using aluminum chloride colorimetric assay described by Zhishen et al. (Zhishen, Mengcheng, & Jianming, 1999). To 0. 5 ml of samples/standard, 150 µl of 5% sodium nitrate and 2. 5 ml of distilled water were added. After 5 min, 0. 3 ml of 10% $AlCl_3$ was added. At 6 min, 1 ml of 0. 001M NaOH and 0. 55 ml distilled water was added to the mixture and left at RT for 15 min. Absorbance of the mixtures was measured at 510 nm. Total flavonoid contents were expressed in terms of catechin equivalent, CAE /gm of dry extract in respect to standard curve equation (y = 0. 0178x+0. 0524, R^2 = 0. 9862).

Determination of free radical scavenging activity

DPPH radical scavenging activity

Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay (DRSA) as described by Braca et al. (Braca et al., 2001). Aliquots of 2. 5mL of methanolic solution containing sample at different concentration was mixed with 2. 5 ml of 0. 008% DPPH solution in methanol. The reaction mixture was vortexed thoroughly and left in the dark at room

temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference. Percentage DPPH radical scavenging activity (% DRSA) was calculated by the following equation,

$\% DRSA = (A_0 - A_1 / A_0) \times 100$

Where,

A_0 = Absorbance of control and

A_1 = Absorbance of sample.

IC_{50} values denote the concentration of sample required to scavenge 50% DPPH free radicals.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of extract was assessed by the method described by Zhang et al. (Zhang & He). Aliquot of 1. 0 ml of 0. 1mmol/L H_2O_2 and 1. 0 ml of various concentrations of extracts were mixed. Followed by 2 drops of 3% ammonium molybdate, 10 ml of 2M H_2SO_4 and 7. 0 ml of 1. 8 mol/L KI. The mixed solution was titrated with 5. 09mmol/L $Na_2S_2O_3$ until yellow color disappeared. The extent of scavenging of hydrogen peroxide was calculated as:

$\% scavenging\ of\ hydrogen\ peroxide = [(V_0 - V_1) / V_0] Ã- 100$

Where,

V_0 = Volume of $Na_2S_2O_3$ solution used to titrate the control sample in the presence of hydrogen peroxide (without sample),

V_1 = Volume of $Na_2S_2O_3$ solution used in the presence of samples.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity (HRSA) of the extracts was determined by the method of Halliwell et al. (Halliwell, Gutteridge, & Aruoma, 1987) with a slight modification. 100 µl of various concentrations of extracts were mixed with 100 µl of hydrogen peroxide (10mmol/L). To this 200 µl premixed FeCl3 (100mmol/L) and EDTA (100mmol/L) solution (1: 1; v/v) was added. Followed by addition of 500 µl of 2. 8mmol/L 2-deoxyribose in phosphate buffer (PH7. 4) and finally the reaction was triggered by adding 100 µl ascorbate (300mmol/L). Then the reaction mixture was incubated at 37. 5ËšC for 1 hour. To the above reaction mixture 2 ml of TCA (2. 8% w/v aqueous solution) and 2 ml of TBA (1% w/v aqueous solution) was added. The final reaction mixture was heated for 15min in boiling water bath, cooled and absorbance was taken at 532nm using a spectrophotometer.

The hydroxyl radical scavenging capacity was evaluated with the inhibition percentage of 2-deoxyribose oxidation on hydroxyl radicals. The percentage of hydroxyl radical scavenging (%HRSA) activity was calculated according to the following formula:

$\% HRSA = [A_0 – (A_1 - A_2]$ Ã-100/A_0

where A_0 is the absorbance of the control without a sample. A_1 is the absorbance after adding the sample and 2-deoxyribose. A_2 is the absorbance of the sample without 2-deoxyribose.

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