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Full Length Research Paper

In vitro antioxidant, total phenolic content and preliminary toxicity studies of *Gmelina philippensis* chem.

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Investigation with the crude methanol extract of leaves and flowers of *Gmelina philippensis* chem. as well as petroleum ether, carbon tetrachloride and dichloromethane soluble partitionates were carried out to evaluate antioxidant activity. It was evaluated by analyzing the bleaching rate of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and phosphomolybdenum total antioxidant assay using, butylated hydroxytoluene (BHT) and ascorbic acid (ASA) as standard antioxidants. The total phenolic content was also determined and expressed in gallic acid equivalent (mg of GAE/g of sample). A great variance was observed for polyphenol content as well as antioxidant activity (1.354 to 10.179 mg GAE/g and DPPH IC_{50} 9.75 to 35.25 µg/ml) depending on the nature of the solvent used to fractionate the crude extracts. The result demonstrated that carbon tetrachloride soluble fraction of leaves extract revealed the highest amount of phenolic compounds (10.179 mg GAE/g) and also had significant antioxidant activity (IC_{50} 9.75 µg/ml). A positive correlation was seen between total phenolic content and total antioxidant activity of leaves and flowers of *G. philippensis* chem. having correlation coefficient (R^2) values of 0.9878 and 0.9385, respectively. The general toxicity of the extractive was studied by brine shrimp lethality bioassay and from the results (LC_{50} 0.902 to 0.487 µg/ml).

Key words: Antioxidant, brine shrimp lethality bioassay, *Gmelina philippensis* chem., phenolic content, verbenaceae.

INTRODUCTION

Gmelina philippensis chem. (Synonyms- Gmelina hystrix, Bengali name- Badhara, Korobi, Family- Verbenaceae) is a small tree having 3 to 8 m tall with pendant branches. Rhomboid-elliptic leaves are 5 to 7.5 cm long, 3 to 4 cm broad, entire or slightly lobed, smooth. Exotic flowers comprised of yellow blossoms which emerge from a pendant structure of overlapping bracts. The flower resembles parrot's beak (Common name- Parrot's Beak, Hedgehog). The fruit is fleshy, smooth, yellow, pear-shaped, and about 2 cm long (flowersofindia). It is a native of Philippine islands, India and S.E. Asia. Also it is distributed in United States, Australia, Vietnam, Thailand, Malaysia, Indonesia, Myanmar, and Bangladesh (Helfrich and Rimpler, 2000). In the Philippines, juice of the fruit is applied to eczema of the feet. It is further mentioned as a leech repellent. In Peninsular Malaysia, the fruit pounded with lime is applied as a poultice to the throat as a remedy for coughs. In Indo-China, the juice of the roots is used as a purgative and in treating fatigue. The extract of the roots is used internally as a stimulant, resolvent, and in treating diseases of the joints and nerves. Likewise, an extract of the leaves is employed externally (Chung, R.C.K., 2001). In the present study, the organic soluble materials of methanolic extract of leaves and flowers were used to investigate the antioxidant activity in terms of total phenolic content, free radical scavenging activity, and preliminary toxicity studies of *G. philippensis chem.* for the first time. Attempt has been taken to establish a correlation between the total antioxidant activity and total phenolics in the extractives.

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MATERIALS AND METHODS

Collection and extraction of plant material

The leaves and flowers of *G. philippensis* chem. were collected in March 2010 from the Dhaka. A voucher specimen for this collection has been deposited in Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession Number: 35547). The collected plant materials were chopped, dried and powdered. 900 gm leaves and 300 gm flowers powder was extracted separately with methanol at room temperature for 7 days. The extracts were filtered using Whatman filter paper (number: 1) and concentrated in a rotary evaporator at reduced temperature and pressure. The concentrated methano extract were partitioned by modified Kupchan method (Wagenen et al., 1993) using pet-ether, carbon tetrachloride, dichloromethane and subsequent evaporation of solvent yielded from leaves : pet-ether (PEL) 3.0 gm, carbon tetrachloride (CTCL) 3.5 gm, dichloromethane (DCML) 1.5 mg and aqueous (AQL) 1.0 mg soluble materials and from flowers: pet-ether (PEF) 1.0 mg, carbon tetrachloride (CTCF) 0.8 mg, dichloromethane (DCMF) 1.1 mg and aqueous (AQL) 0.9 mg soluble materials. The residues were then stored in a refrigerator for further experimental purposes.

Phosphomolybdenum antioxidant assay

The total antioxidant activity of the extract was evaluated by the phosphomolybdenum assay method (Prieto et al., 1999), which is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex in acedic condition. A 0.3 ml extract (2 mg/ml) was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and the reaction mixture was incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a UV–visible spectrophotometer against blank after cooling to room temperature. The antioxidant activity was expressed as the number of gram equivalents of ascorbic acid (mg of ascorbic acid/100 g of plant extract).

Free radical scavenging activity

The free radical scavenging activity (antioxidant capacity) of the plant extractives on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by the method established by Brand-Williams et al. (1995). Here 2.0 ml of a methanol solution of the sample (extractive/standard) at different concentration (500 to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min of reaction at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by a UV spectrophotometer. Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$$(I\%) = (1 - A_{sample}/A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material).

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted with inhibition percentage against extractive/standard concentration

Total phenolics analysis

The total phenolic content of the extractives was determined with the Folin-Ciocalteau reagent using the method developed by Harbertson and Spayd (Harbertson and Spayd 2006). To 0.50 ml of each sample (three replicates), 2.5 ml of 1/10 dilution of Folin-Ciocalteau reagent in water and 2 ml of Na_2CO_3 (7.5%, w/v) were added and incubated at 45°C for 15 min. The absorbance of all samples was measured at 765 nm using a SPECTRAmax-PLUS384 UV–vis spectrophotometer. The phenolic content was expressed as milligram of gallic acid equivalent per gram of dry weight (mg GAE/g) of extract.

Brine shrimp lethality bioassay

For pharmacological screening, we have applied the brine shrimp lethality bioassay method which developed a rapid general bioassay technique for the natural products. This method indicates cytotoxicity as well as a wide range of pharmacological activities for example; anticancer, antiviral and pesticidal etc (Meyer et al., 1982). Brine shrimp eggs were hatched in simulated sea water to get nauplii. Test samples of different concentrations (400 to 0.781 µg/ml) were prepared by dissolving in dimethylsulfoxide (DMSO). The nauplii were counted by visual inspection and were taken in vials containing 5 ml of simulated sea water. Test samples were added to the pre-marked vials through micropipette. After an incubation period of 24 h, the survivors were counted. The LC_{50} (lethal concentration to half of the test organism) values of the test samples were calculated from the regression equation, prepared from the logarithm of sample concentration and percentage mortality of the shrimp nauplii.

Statistical analysis

Three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD. Correlation analysis of free radical scavenging activity versus total phenolic content and reducing power were carried out using the correlation and regression program.

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the antioxidant activity of different organic soluble materials of the methanolic extract of leaves and flowers of *G. philippensis* chem.. The results are shown in Table 1. The total antioxidant capacity of the *G. philippensis* chem. extracts expressed as the mg of ascorbic acid/100 g of plant extract was determined by phosphomolybdenum assay where the highest value was found in carbon tetrachloride soluble fraction of leaves followed by dichloromethane soluble fraction of leaves and methanol extract of flower as evident from 0.175 mg, 0.149 mg and 0.145 mg equivalents of ascorbic acid, respectively.

The total phenolic content in the analyzed extractive were found in between 1.354 to 10.179 mg of GAE/100 gm of sample. The total phenolic content of MeOH extract of leaves and flowers were 4.25 mg and 8.75 mg of GAE/100 gm of sample, respectively and its sub-fractions including pet-ether, carbon tetrachloride, dichloromethane fraction and aqueous fraction of leaves and flowers were 2.57, 10.179, 9.253, 2.574,1.354, 8.179, 2.413, 4.282 mg GAE/100 gm of sample, respectively. This indicated that the carbon tetrachloride fraction of leaves had the highest amount of phenolic

Table 1. Total antioxidant capacity, total phenolic content and free radical scavenging activity of different partitionates of leaves and flowers of *G. philippensis* chem.

Sample	Sample description	Total phenolic content (mg of GAE/100 gm of dried extract)	Total antioxidant capacity (mg of ascorbic acid/100g of plant extract)	Free radical scavenging activity (IC_{50} µg/ml)
BHT	Butylated hydroxytoluene	-	-	27.5 ± 0.54
ASA	Ascorbic acid	-	-	5.8 ± 0.21
MEL	Methanol extract of leaves	4.254 ± 0.87	0.089 ± 0.38	27.02 ± 1.02
PEL	Pet ether soluble fraction of leaves	2.574 ± 0.55	0.062 ± 0.35	30.20 ± 1.08
CTCL	Carbon tetrachloride soluble fraction of leaves	10.1790 ± 1.08	0.175 ± 1.98	9.75 ± 0.89
DCML	Dichloro Methane soluble fraction of leaves	9.2536 ± 0.89	0.149 ± 1.37	10.52 ± 0.88
AQL	Aqueous soluble fraction of leaves	2.574 ± 0.25	0.070 ± 0.75	34.25 ± 1.08
MEF	Methanol extract of flowers	8.754 ± 1.47	0.145 ± 0.22	11.02 ± 1.15
PEF	Pet ether soluble fraction of flowers	1.354 ± 0.25	0.060 ± 0.75	35.25 ± 1.08
CTCF	Carbon tetrachloride soluble fraction of flowers	8.1790 ± 1.78	0.121 ± 0.98	10.02 ± 0.59
DCMF	Dichloro Methane soluble fraction of flowers	2.4136 ± 0.47	0.045 ± 0.47	33.52 ± 0.57
AQF	Aqueous soluble fraction of flowers	4.282 ± 0.87	0.087 ± 1.02	25.87 ± 1.05

The average values of three calculations are presented as mean ± S.D. (standard deviation).

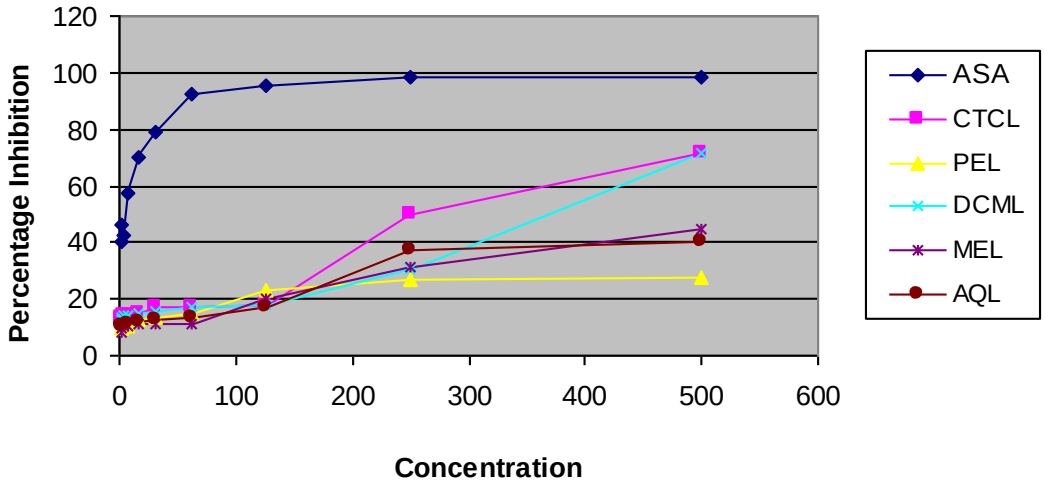

Figure 1. DPPH free radical scavenging activity of different extractives of leaves of *G. philippensis* chem.

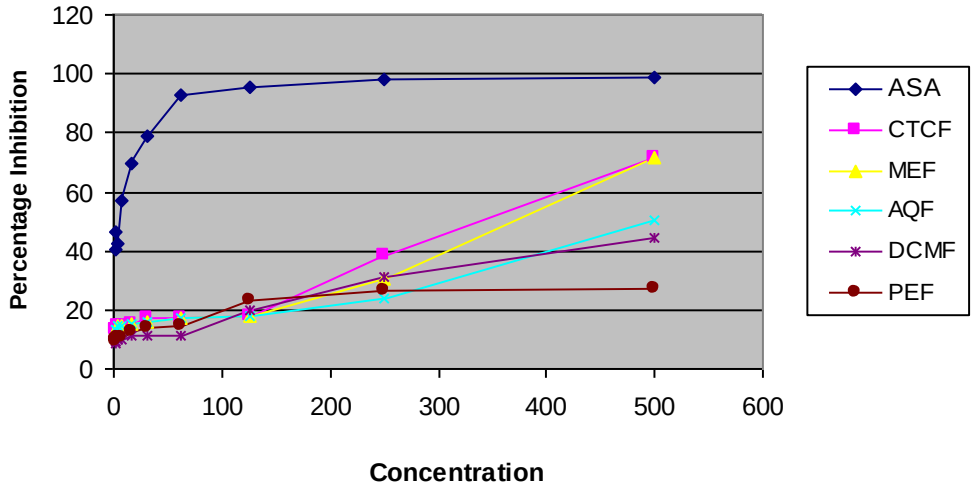

Figure 2. DPPH free radical scavenging activity of different extractives of flower of *G. philippensis* chem.

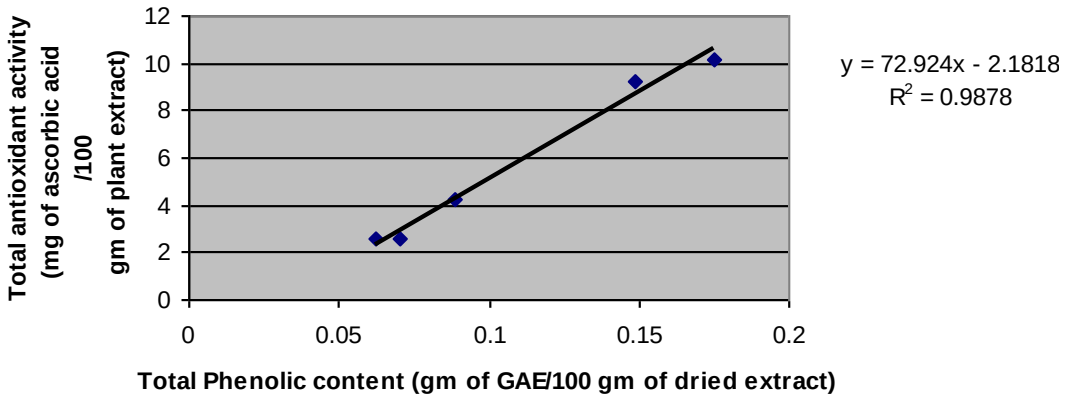

Figure 3. Correlation between the total phenolic content and total antioxidant activity of leaves extract of *G. philippensis* chem.

compounds (10.179 mg GAE/100 gm of sample).

In the free radical scavenging (DPPH) assay, the IC_{50} values of the analyzed fractions were found to be 9.75 to 35.25 µg/ml. Where the lower IC_{50} value is indicative of higher free radical scavenging activity. The free radical scavenging activity of carbon tetrachloride fraction of leaves (IC_{50} = 9.75 µg/ml) was higher among all the extractives and its activity was found to be even better than the used synthetic antioxidant butylated hydroxyl toluene (IC_{50} = 27.5 µg/ml) (Figures 1 and 2). The strongest activity of dichloromethane fraction is most probably due to its high phenolic content (10.179 mg of GAE/g of sample) (Figures 3 and 4).

In the brine shrimp lethality bioassay, different mortality rate of the nauplii in all samples and unchanged nauplii (no lethality/mortality) of the control group suggest that the methanol crude extract of the leaves and flowers and the 3 fractions (pet-ether, carbon tetrachloride, dichloromethane fraction) and aqueous fraction have moderate toxic activities. The results are shown in Table 2. The obtained LC_{50} values of the tested fractions were found in the range of 0.487 to 0.902 µg/ml where the lowest LC_{50} value (0.487 µg/ml) was obtained from the carbon tetrachloride fraction of leaves and the highest (0.902 µg/ml) from the aqueous soluble fraction of flowers extract, whereas the used standard Vincristine sulphate showed the LC_{50} value of 0.451 µg/ml.

Conclusion

After analyzing all the results it may be concluded that the leaves and flowers of *G. philippensis* chem. has moderate antioxidant activity, which needed the bioactivity

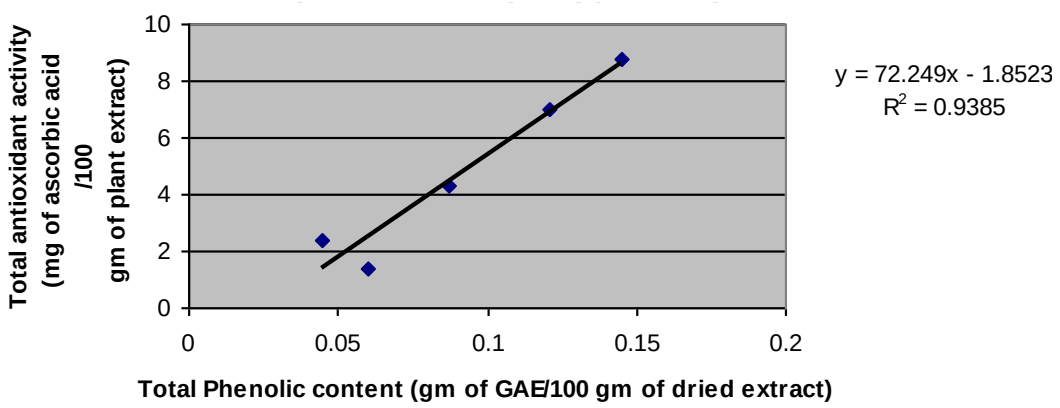

Figure 4. Correlation between the total phenolic content and total antioxidant activity of flowers extract of *G. philippensis* chem.

Table 2. LC_{50} values in brine shrimp lethality bioassay of different fractions of leaves and flowers of *G. philippensis* chem.

Samples	LD_{50} (µg/ml)
MEL	0.542
PEL	0.567
CTCL	0.487
DCML	0.503
AQL	0.608
MEF	0.498
PEF	0.760
CTCF	0.498
DCMF	0.890
AQF	0.902
Vincristine sulfate	0.451

guided purification to separate the active compound.

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