Bangladesh Pharmaceutical Journal ***15****(2): 141-43, 2012*

In vitro Antioxidant, Membrane Stabilizing and Thrombolytic Activities of *Glycosmis arborea*

Sayed Mamur Hossain[1], Farhana Islam[2], Tasnuva Sharmin[1], Hasib Sheikh[1], A.M. Rashedul Hasan[1] and Mohammad A. Rashid[2]

[1]Department of Pharmacy, State University of Bangladesh, Dhaka-1205, Bangladesh
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

Abstract

The methanol extract of leaves and stems of *Glycosmis arborea* and their pet-ether, carbon tetrachloride, chloroform and aqueous soluble partitionates were subjected to assays for antioxidant activity by Folin-Ciocalteau reagent, membrane stabilizing and thrombolytic activities. The carbon tetrachloride soluble fraction of stems of *G. arborea* demonstrated the presence of significant amount of phenolic compounds (36.95 ± 0.54 mg of GAE/g of extract). The extractives inhibited heat as well as hypotonic solution-induced haemolysis of rat erythrocytes *in vitro*. The pet-ether soluble fraction of leaves and stems of *G. arborea* showed 20.46 and 38.24% and 22.50 and 48.25%. inhibition of hemolysis of RBC caused by heat and hypotonic solution, as compared to 30.55 and 72.91% inhibition of hemolysis of RBC caused by the standard Acetyl salicylic acid at 0.01 mg/ml concentration, respectively. Among the four fractions, the chloroform soluble materials of the stems of *G. arborea* revealed highest thrombolytic activity with clot lysis value of 36.50% while standard streptokinase and water used as positive and negative controls, showed 64.25±0.26 and 2.35±0.35 % lysis of clot respectively

Key words: *Glycosmis arborea*, total phenolic content, antioxidant, membrane stabilization and thrombolysis

Introduction

Glycomis arborea Roxb. DC (Synonym- *Glycosmis pentaphylla* Retz. DC, Bengali name- Ashsaora, Kawatuti, Matmati, Ban Jamir) belongs to the family Rutaceae. The genus *Glycosmis* is represented by nearly 11 species. It is a shrub or small (1.5–5.0 m) tree widely distributed from India, Malaysia and Southern China to the Philippine Islands where it occurs in tropical forests at low altitudes (Wang *et al.*, 2006). Traditionally it is used for the treatment of fever, liver complaints and certain other diseases. The stems are widely used as a brush for cleaning teeth (Quader *et al.*, 1999).The plant is also used for cough, rheumatism, anemia and jaundice. Leaf juice is given with sugar in empty stomach in the morning to eradicate ascariasis, while the leaf paste mixed with ginger is used in eczema and skin infections. The leaf extract and crude alkaloids possess antibacterial and antifungal properties (Medicinal plants database of Bangladesh). As a part of our continuing studies of medicinal plants of Bangladesh (Kaisar *et al*., 2011; Kabir *et al*., 2010) the methanol extracts of leaves and stem of *G. arborea* growing in Bangladesh were screened for antioxidant activity in terms of total phenolic content as well as membrane stabilizing and thrombolytic activities for the first time and we, here in, report the results of our preliminary investigations.

Materials and Methods

Plant materials: The leaves and stems of *G. arborea* were collected from Dhaka and a voucher specimen (DUSH-5638) of the plant sample has been deposited in Department of Botany, University of Dhaka for future reference.

Extraction and fractionation: The collected plant parts were sun dried for several days and then oven dried for 24 hours at 40^{o}C to facilitate grinding. The powdered leaves (450 gm) and stem (300 gm) were separately macerated in 1.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extracts were concentrated with a rotary evaporator at low temperature (40-45ºC) and reduced pressure. The concentrated methanol extracts were fractionated by the modified Kupchan partitioning protocol (Van Wagenen *et al.*, 1993) and the resultant partitionates i.e., pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble fractions (Table 1) were used for the biological screenings.

Correspondence to: Mohammad A. Rashid, E-mail address: rashidma@univdhaka.edu

Table 1. Kupchan partitionates of leaves and stem of *G. arborea* obtained from 5 gm of methanol extract

Leaf extracts	Amount (gm)	Stem extracts	Amount (gm)
ME	5.0	ME	5.0
PESF	1.2	PESF	1.0
CTCSF	1.0	CTCSF	0.8
CSF	0.7	CSF	0.5
AQSF	0.5	AQSF	0.5

Biological Investigation

(i) Total phenolic content: The total phenolic contents of the extractives were determined with Folin-Ciocalteau reagent by using the method developed by Harbertson and Spayd (2006). To 0.50 ml of each sample (three replicates), 2.5 ml of 1/10 dilution of Folin-Ciocalteau reagent and 2.0 ml of sodium carbonate (7.5%, w/v) in water were added and incubated for 15 min at 45 °C. The absorbance of all samples was measured at 765 nm with a visible spectrophotometer. The phenolic contents were expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of dry weight of extract.

(ii) Membrane stabilizing activity: The membrane stabilizing activity of the extractives was assessed by using hypotonic solution and heat-induced hemolysis of human erythrocyte by the method developed by Shindhe *et al.* 1999 with slight modification by Sikder *et al.* 2011 by using acetyl salicylic acid as standard.

(iii) Thrombolytic activity: The thrombolytic activity of all extractives was evaluated by the method developed by Prasad *et al.* 2006 using streptokinase (SK) as positive control.

Statistical Analysis: Three replicates of each sample were used for each assay to facilitate statistical analysis and the values are reported as mean ± SD.

Results and Discussion

The crude methanol extracts of leaves and stems of *G. arborea* as well as different Kupchan partitionates derived from these extracts were subjected to assays for total phenolic content, membrane stabilizing and thrombolytic activities by following standard protocols. The total phenolic content in the samples were found in the range of 8.0 ± 0.22to 36.95 ± 0.54 mg of GAE/g of sample. . In this study, the carbon tetrachloride soluble fraction of crude methanol extract of both the leaves and stems of *G. arborea* revealed the highest total phenolic content (18.08 ± 0.32 and 36.95 ± 0.54 mg of GAE/g of sample, respectively (Table 2).

The extractives of *G. arborea* at 2.0 mg/mL significantly protected the lysis of erythrocyte membrane induced by hypotonic solution and heat as compared to the standard, acetyl salicylic acid (0.10 mg/mL). In hypotonic solution induced conditions, the samples were found to inhibit lysis of erythrocyte membrane within the range of 21.42 ± 0.24 to 48.25 ± 0.75 %. Among the samples, the pet-ether soluble fraction (PESF) of leaves and stems of *G. arborea* displayed high inhibition (38.24 ± 0.26 and 48.25 ± 0.75 %) hemolysis of RBC as compared to 72.91 ± 0.21 % demonstrated by acetyl salicylic acid (Table 2).

Besides, in heat- induced conditions, the samples were found to inhibit lysis of erythrocyte membrane within the range of 12.55 ± 0.22 to 22.50 ± 0.44%. Here, the pet-ether soluble fraction (PESF) of leaves and stems of *G. arborea* inhibited maximum 20.46 ± 0.54 and 22.50 ± 0.44 % hemolysis of RBC as compared to 30.55 ± 0.55% demonstrated by acetyl salicylic acid (Table 2).

The extractives of *G. arborea* were also assessed for thrombolytic activity and the results are presented in Table 2. Addition of 100 µl SK, a positive control (30,000 I.U.) to the clots of human blood and subsequent incubation for 90 minutes at 37°C, showed 64.25% lysis of clot. On the other hand, distilled water when treated as negative control showed negligible percentages of lysis of clot (2.35 %). The mean difference in percentage of clot lysis between positive and negative control was found to be statistically significant. In this study, the chloroform soluble fraction of crude methanol extract of both the leaves and stems of *G. arborea* revealed highest thrombolytic activity (29.05 ± 0.45 and 36.50 ± 0.86%, respectively) whereas the carbon tetrachloride and pet-ether soluble materials from stems of *G. arborea* displayed moderate thrombolytic activities (31.68 ± 0.54 and 28.95 ± 0.33 %, respectively).

Table 2. Total phenolic content, membrane stabilizing and thrombolytic activities of leaves and stems of *G. arborea*

Test samples	Total phenolic content (mg of gallic acid/gm of extract)	Membrane stabilizing activity		Thrombolytic activity (% clot lysis)
		Heat induced inhibition (%)	Hypotonic solution induced inhibition (%)	
Leaf extractives				
ME	10.50 ± 0.25	16.25 ± 0.61	35.15 ± 0.15	20.55 ± 0.82
PESF	14.94 ± 0.65	20.46 ± 0.54	38.24 ± 0.26	24.22 ± 0.75
CTCSF	18.08 ± 0.32	19.58 ± 0.45	31.29 ± 0.61	27.65 ± 0.25
CSF	11.68 ± 0.22	17.59 ± 0.29	30.33 ± 0.68	29.05 ± 0.45
AQSF	8.0 ± 0.22	12.55 ± 0.22	21.42 ± 0.24	18.61 ± 0.34
Stem extractives				
ME	20.31 ± 0.35	18.20 ± 0.28	38.25 ± 0.56	24.26 ± 0.25
PESF	30.20 ± 0.61	22.50 ± 0.44	48.25 ± 0.75	28.95 ± 0.33
CTCSF	36.95 ± 0.54	21.65 ± 0.61	41.78 ± 0.11	31.68 ± 0.54
CSF	24.34 ± 0.26	18.31 ± 0.95	35.26 ± 0.38	36.50 ± 0.86
AQSF	10.15 ± 0.88	14.95 ± 0.16	30.55 ± 0.64	19.35 ± 0.12
ASA	-	30.55 ± 0.55	72.91 ± 0.21	-
SK	-	-	-	64.25 ± 0.26
Water	-	-	-	2.35 ± 0.35

ME = Methanolic crude extract; PESF = Pet-ether soluble fraction of methanol extract; CTCSF = Carbon tetrachloride soluble fraction of methanol extract; CSF = Chloroform soluble fraction of methanol extract; AQSF = Aqueous soluble fraction of methanol extract; ASA= Acetyl salicylic acid; SK = Streptokinase.

References

Harbertson, J. and Spayd, S. 2006. Measuring phenolics in the winery. *Am. J. Enol. Vitic*., **57**, 280-288.

Prasad, S., Kashyap, R.S., Deopujari, J.Y., Purohit, H.J., Taori, G.M. and Daginawala, H.F. 2006. Development of in vitro model to study clot lysis activity of thrombolytic drugs. *Throm. J*., **4**, 14

Quader, M.A., Nutan, M.T.H. and Rashid, M.A. 1999. Antitumor alkaloid from *Glycosmis pentaphylla. Fitoterapi*a, **70**, 305-307

Kabir, S., Rahman, M.S., Chowdhury, A.M., Hasan, C.M., and Rashid, M.A. 2010. An unusual bisnor-Clerodane diterpenoid from *Polygonum simiarum*. *Nat. Prod. Commun*.**5**, 1543-1546.

Kaisar, M.A., Rahman, M.S., Rahman, M.Z., Hasan, C.M. and Rashid, M.A. 2011. A review on phytochemicals from some medicinal plants of Bangladesh. *J. Phar. Nutri. Sci*., **1**, 87-95.

Medicinal plants database of Bangladesh; available online at: http://www.mpbd.info/plants/glycosmis-pentaphylla.php

Shindhe, U.A., Phadke, A.S., Nair, A.M., Mungantiwar, A.A., Dikshit, V.J. and Saraf, M.N. 1999. Membrane stabilizing activity – a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia*,**70**, 251-257.

Sikder, M.A., Rahman, M.A., Kaisar, M.A., Rahman, M.S., Hasan, C.M. and Rashid, M.A. 2011. *In vitro* Antioxidant, Reducing Power, Free Radical Scavenging and Membrane stabilizing activities of seeds of *Syzygium cumini* L. *Lat. Am. J. Pharm*., **30**, 781-785.

Van Wagenen, B.C., Larsen, R., Cardellina, J.H., Ran dazzo, D., Lidert, Z.C. and Swithenbank, C. 1993. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri. J. Org. Chem*., **58**, 335-337.

Wang, J., Di, Y., Yang, X., Li, S., Wang, Y. and Hao, X. 2006. Hydroquinone diglycoside acyl esters from the stems of *Glycosmis pentaphylla*. *Phytochemistry*, **67**, 486-491.