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Pharmacological Activities of *Grevillea robusta*, a Medicinal Plant of Bangladesh

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Abstract

The objective of this study was to evaluate the crude methanol extract of leaf of *Grevillea robusta* as well as its hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates for cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities. In the brine shrimp lethality bioassay, the crude methanolic extract of *G. robusta* leaf revealed the highest cytotoxic activity with LC50 values as 1.50 ± 0.45 µg/ml as compared to 0.45 µg/ml for vincristine sulphate. Among the extractives of *G. robusta*, the carbon tetrachloride soluble fraction showed 69.95±0.11% clot lysis as compared to 70.77% clot lysis by standard streptokinase. At concentration of 1.0 mg/ml, the chloroform soluble fraction inhibited 40.31 ± 0.59% and 62.93 ± 0.73% of haemolysis of RBC induced by heat and hypotonic solution as compared to 42.12% and 71.90% by acetyl salicylic acid, respectively. The test samples also showed antimicrobial activity with zone of inhibition ranging from 7.0 to 17.0 mm in diameter. The chloroform soluble partitionate demonstrated the highest zone of inhibition (17.0 mm) against *Salmonella* Typhi.

Key words: *Grevillea robusta*, brine shrimp lethality, thrombolytic activity, membrane stabilizing activity, hypotonic solution, zone of inhibition.

Introduction

Grevillea robusta A. Cunn. ex R.Br. (Synonyms: *Grevillea venusta* A. Cunn. ex Meisn., *Stylurus robustus* A. Cunn. O. Deg.; Bengali name: rupasi), commonly known as the southern silky oak or Australian silver oak, is a fast growing evergreen tree belonging to the Proteaceae family. It is a native species of eastern coastal Australia and in other riverine, subtropical and rainforest environments that receive more than 1,000 mm per year of average rainfall. The flowers and fruits of the plant contain toxic hydrogen cyanide (Everist, 1974). Tridecylresorcinol of *G. robusta* is responsible for contact dermatitis (Menz *et al.,* 2006). Previous phytochemical investigation provided six new 5-alkyl resorcinols (gravicycle, dehydrogravicycle, bisgravillol, dehydro-bisgravillol, dehydrograviphane and methyldehydro-graviphane) and eight known compounds (Chuang and Wu, 2007). Leaves contain rutin (Philippine Medicinal plants).

As part of our ongoing investigations on medicinal plants of Bangladesh (Kaisar *et al.*, 2011 and Sharmin *et al.,* 2012 and 2013), the crude methanol extract of leaf of *G. robusta* growing in Bangladesh as well as its organic and aqueous soluble fractions were studied for cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities for the first time and we, here in, report the results of our preliminary investigations.

Materials and Methods

Collection of plant materials and extraction: The leaf of *G. robusta was* collected from National Botanical Garden of Bangladesh in June, 2012. A voucher specimen (DACB-38206) for this plant has been deposited in Bangladesh National Herbarium, Dhaka, Bangladesh for future reference.

The sun dried and powdered leaves (500 g) were macerated in 1.5 L of methanol for 7 days. The extract was filtered through a fresh cotton bed and finally with Whatman filter paper number 1. It was concentrated with a rotary evaporator at reduced temperature and pressure. An aliquot (5 g) of the concentrated methanol extract was fractionated by the modified Kupchan (VanWagenen *et al.,* 1993) partitioning protocol and the resultant partitionates were evaporated to dryness with a rotary evaporator to yield hexane (HXSF, 1.5 g), carbon tetrachloride (CTCSF, 1.5 g), chloroform (CSF, 1 g) and

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aqueous (AQSF, 0.5 g) soluble materials. The residues were then stored in a refrigerator until further use.

Brine shrimp lethality bioassay: This technique was applied for the determination of general toxic properties of the dimethylsulfoxide (DMSO) solutions of plant extractives against *Artemia salina* in a single day *in vivo* assay (Meyer *et al*., 1982). Vincristine sulphate was used as positive control.

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

Membrane stabilizing activity: The membrane stabilizing activity of the extractives was assessed by evaluating their ability to inhibit heat- and hypotonic solution- induced hemolysis of human erythrocytes following the method developed by Omale *et al*. (2008).

Antimicrobial screening: Antimicrobial activity was determined by disc diffusion method (Bauer *et al.,* 1966).

Statistical analysis: For all bioassays, sample data were compared with that of negative control by student's t-test

Results and Discussion

The present study was undertaken to evaluate the cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities of different organic and aqueous soluble materials of the crude methanol extract of *G. robusta*.

In brine shrimp lethality bioassay, all fractions demonstrated cytotoxic potential against *A. salina* with LC_{50} values ranging from 1.50 to 191.14 µg/ml. The crude methanol extract and the carbon tetrachloride soluble fraction revealed the presence of significant bioactive principles with LC_{50} values 1.50 ± 0.45 µg/ml and 3.85 ± 0.22 µg/ml, respectively as compared to 0.45 µg/ml for Vincristine sulphate (Table 1).

The extractives of *G. robusta* demonstrated mild to moderate thrombolytic activity. The carbon tetrachloride and aqueous soluble fractions displayed 69.95±0.11 % and 67.50±0.49% clot lysis, respectively as compared to 70.77% clot lysis revealed by standard streptokinase (Table 1).

At concentration 1.0 mg/ml, the extractives of *G. robusta* protected the hemolysis of RBCs induced by heat and hypotonic solution as compared to the standard acetyl salicylic acid (0.10 mg/ml). The chloroform soluble fraction inhibited 40.31 ± 0.59% and 62.93 ± 0.73% of hemolysis of RBCs induced by heat and hypotonic solution as compared to 42.12 % and 71.90% by acetyl salicylic acid, respectively (Table 1).

The antimicrobial activity of *G. robusta* test samples was evaluated against five gram positive and eight gram negative bacteria and three fungi and the results were compared with a broad spectrum antimicrobial agent, ciprofloxacin. Among the test samples of *G. robusta*, only the carbon tetrachloride and chloroform soluble fractions demonstrated antimicrobial activity with zone of inhibition ranging from 7.0 to 17.0 mm. The highest zone of inhibition (17.0 mm) was displayed by the chloroform soluble fraction against *Salmonella* Typhi (Table 2).

Table 1. Cytotoxic, thrombolytic and membrane stabilizing activities of *G. robusta.*

Samples/ standard	Brine shrimp lethality bioassay LC_{50} (µg/ml)	% Clot lysis of RBCs	% Inhibition of hemolysis	
			Heat- induced	Hypotonic solution-induced
ME	1.50 ± 0.45	64.94 ± 0.56	39.39 ± 0.60	37.56 ± 0.42
HXSF	7.84 ± 0.74	29.76 ± 0.34	37.59 ± 0.17	26.91 ± 0.82
CTCSF	3.85 ± 0.22	69.95 ± 0.11	35.55 ± 0.14	11.89 ± 0.44
CSF	10.87 ± 0.70	47.67 ± 0.63	40.31 ± 0.59	62.93 ± 0.73
AQSF	191.14 ± 0.19	67.50 ± 0.49	10.82 ± 0.05	17.89 ± 0.52
VS	0.45 ± 0.04	-	-	-
Water	-	3.791 ± 0.55	-	-
SK	-	70.77 ± 0.36	-	-
ASA	-	-	42.12 ± 0.38	71.90 ± 0.78
Hypotonic medium	-	-	-	-

ME = Methanolic crude extract; HXSF = Hexane soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction; AQSF = Aqueous soluble fraction; VS = Vincristine sulfate; SK = Streptokinase; ASA = Acetyl salicylic acid.

Table 2. Antimicrobial activity of carbon tetrachloride and chloroform soluble materials of *G. robusta.*

Test microorganisms	Diameter of zone of inhibition (mm)		
	CTCSF	CSF	Ciprofloxacin
Bacillus cereus	9.0 ± 0.93	10.0 ± 0.22	45.0 ± 2.01
B. megaterium	8.0 ± 1.12	9.0 ± 0.61	42.0 ± 1.17
B. subtilis	8.0 ± 0.83	15.0 ± 0.26	42.0 ± 0.73
Staphylococcus aureus	9.0 ± 1.05	12.0 ± 0.64	42.0 ± 0.23
Sarcina lutea	9.0 ± 0.31	13.0 ± 0.57	42.0 ± 0.56
Escherichia coli	7.0 ± 0.66	13.0 ± 0.65	42.0 ± 0.43
Pseudomonas aeruginosa	10.0 ±0.39	9.0 ± 0.33	42.0 ± 1.11
Salmonella Typhi	11.0 ± 0.58	17.0 ± 0.42	45.0 ± 0.73
S. Paratyphi	8.0 ± 0.71	9.0 ± 0.58	47.0 ± 2.33
Shigella boydii	7.0 ± 0.56	11.0 ± 0.44	34.0 ± 0.58
Sh. dysenteriae	7.0 ± 0.47	11.0 ± 0.68	42.0 ± 0.22
Vibrio mimicus	9.0 ± 0.22	10.0 ± 0.31	40.0 ± 0.45
V. parahaemolyticus	8.0 ± 0.32	10.0 ± 0.06	35.0 ± 0.44
Sacharomyces cerevisiae	8.0 ± 0.17	10.0 ± 0.83	38.0 ± 0.49
Candida albicans	7.0 ± 0.47	8.0 ± 0.90	37.0 ± 0.33
Aspergillus niger	9.0 ± 0.66	12.0 ± 0.24	38.0 ± 0.11

CTCSF = Carbon tetrachloride soluble fraction; CSF = Chloroform soluble fraction

Conclusion

It is clearly evident from the above findings that *G. robusta* possesses significant cytotoxic, thrombolytic and membrane stabilizing activities. The plant is a good candidate for further systematic, chemical and biological studies to isolate the active principles.

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