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In vitro Bioactivities of Three Reputed Medicinal Plants of Bangladesh

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Abstract

The study was designed to evaluate the preliminary biological activities of crude methanol extracts of the leaf of *Justicia gendarussa*, *Sansevieria trifasciata* and *Hydnocarpus kurzii* and their organic soluble partitionates. The polyphenol content was determined spectrophotometrically and expressed as gallic acid equivalents. The total phenolic content was found to vary for different test samples ranging from 1.45 ± 0.25 mg to 40.73 ± 0.22 mg of GAE/ gm of dried extract. The antioxidant potential was evaluated by DPPH free radical scavenging assay using butylated hydroxytolune (BHT) and ascorbic acid as standards. In the assay, the CSF of *H. kurzii* and the PESF of *J. gendarussa* revealed the highest free radical scavenging activity with IC_{50} values 3.25 ± 0.05 µg/ml and 24.68 ± 0.26 µg/ml, respectively. The brine shrimp lethality bioassay was utilized to evaluate the cytotoxicity. The chloroform soluble fraction (CSF) and methanol extract (ME) of *J. gendarussa* exhibited strong cytotoxicity with LC_{50} values of 0.002 µg/ml and 0.06 µg/ml, respectively. The membrane stabilizing activity was assessed by evaluating hemolysis of RBC in hypotonic solution and was compared with acetyl salicylic acid. On the other hand, the chloroform soluble fraction of *J. gendarussa* and aqueous soluble materials of *S. trifasciata* produced 75.60% and 75.0% inhibition of hemolysis of RBC, respectively as compared to 77.9% inhibited by acetyl salicylic acid (0.10 mg/ml).

Key words: Membrane stabilization, TPC, Free radical scavenging, cytotoxicity

Introduction

Justicia gendarussa Burm.f. (Bengali name: Bishjaron, Family: Acanthaceae) is a shade loving, quick growing, evergreen scented shrub found throughout India and all Asian countries like Malaysia, Indonesia, Srilanka. The plant is used in traditional medicine for chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases and fever. *Justicia* species has been found to contain lignans, naturally occurring phenolic dimmers and triterpenoids (Uddin *et al*., 2011). Lignans have been used as lead compounds for the development of anti rheumatic agents (Sikder *et al.,* 2011a).

Sansevieria trifasciata prain (Bangali name- Ghorchhokor, English- Powstring hemp, Mother-in-law's tongue, Family- Agavaceae) is a perennial seamless herb with erect leaves arising from an underground rhizome. Leaves are thick, flat, fibrous, and smooth in texture, up to 1 m long, with thin pointed apices, the blade of light green colour with small white lines running perpendicular to the growth of the leaf. Phytochemical screening of this plant has shown the presence of *N*-butyl-4-ol-*N*-propylphthalate, pregnane glycosides, and steroidal sapogenins (Yoshihrio *et al*., 1996). The leaf sap is applied directly to infected sores, cuts and grazes. It is also used to treat fungal infection and scabies (Traditional Medicine Database- 2002).

Hydnocarpus kurzii (King) Warb (Bangali name- Chaulmoogra, Family- Achariaceae) is a tree attaining the height of 40-50 feet. Hydnocarpus oil and the crushed seed have long been used in Southeast Asia to treat various skin diseases like scabies, eczema, psoriasis, scrofula, ringworm, and intestinal worms and it has been shown that the active principles of the oil (hydnocarpic and chaulmoogric acids) are strongly antibacterial in nature. For this reason Chaulmoogra is employed in Indian medicine to treat leprosy (Oommen *et al.,* 1999). The bark contains principles capable of reducing fevers. Seeds are usually applied externally as a dressing for skin diseases combined with walnut oil and pork lard for

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ringworm; with calomel and sesame oil for leprosy; and with sulfur and camphor for scabies (Sikder *et al*., 2011b).

Materials and Methods

Plant materials: Leaves of *S. trifasciata*, and *H. kurzii* were collected from Dhaka while *J. gendarussa* leaves were collected from Gazipur in the month of January 2011. The voucher specimens for each of the collections (DACB 35490, 35491, and 35489, respectively) have been deposited in Bangladesh National Herbarium (BNH) for future references. The leaves were first separated from the plants, cleaned, cut into small pieces and air- dried for several days. The plant materials were then oven dried for 24 hours at 40°C and ground to a coarse powder. The powdered materials (300 gm each) were then soaked in methanol (1.5 liter each) and kept for 10 days at room temperature with occasional shaking. The crude extracts were then filtered through cotton plug followed by Whatman no. 1 filter paper and the extracts were concentrated with rotary evaporator. Each of concentrated methanol extracts were partitioned (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 experimental processes.

Table 1. Different partitionates of *J. gendarussa, S. trifasciata, H. kurzii,* obtained by Kupchan partitioning from 5 g of crude extract.

Partitionates	*J. gendarussa* (g)	*S. trifasciata* (g)	*H. kurzii* (g)
PTSF	1.2	1.4	1.0
CTSF	0.6	0.6	0.6
CSF	0.5	0.5	0.8
AQSF	1.2	1.1	1.4

PESF= Pet-ether soluble fraction of methanolic extract, CTSF= Carbon tetrachloride soluble fraction of methanolic extract, CSF= Chloroform soluble fraction of methanolic extract, AQSF= Aqueous soluble fraction of methanolic extract.

Membrane stabilizing activity: The membrane stabilizing activity of the extractives was determined by their ability to inhibit heat and hypotonic solution induced haemolysis of human erythrocytes following the method developed by Omale *et al*. in 2008.

Total phenolics analysis: Total phenolic content: The total phenolic content (TPC) of the extractives was determined with Folin Ciocalteau reagent by using the method developed by Harbertson and Spayd (2006).

Free radical scavenging activity: Following the method developed by Brand-Williams *et al*. (1995), the antioxidant activity of the test samples was assessed by evaluating the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical by using synthetic antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid as positive controls.

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

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

Results and Discussion

The present study was undertaken to evaluate the membrane stabilizing, free radical scavenging and cytotoxic properties of different organic soluble materials of the methanolic extract of *J. gendarussa, S. trifasciata* and *H. kurzii*.

The extractives of *J. gendarussa, S. trifasciata* and *H. kurzii* at 2.0 mg/ml significantly protected the lysis of mice erythrocyte membrane induced by hypotonic solution as compared to the standard acetyl salicylic acid (0.10 mg/ml) (Table 2). The chloroform soluble fraction of *J. gendarussa* inhibited 75.60% and aqueous soluble fraction of *S. trifasciata* produced 75.0% inhibition of hemolysis of RBC as compared to 77.9% revealed by acetyl salicylic acid (0.10 mg/ml). At the same time aqueous soluble fraction of *J. gendarussa,* chloroform soluble fraction of *S. trifasciata* and carbon tetrachloride, chloroform, and aqueous soluble fractions of *H. kurzii* revealed 71.93%, 72.7%, 70.56%, 70.24%, 69.45% inhibition, respectively.

The total phenolic content was found to vary for different test samples ranging from 1.45 ± 0.25 mg to 40.73 ± 0.22 mg of GAE/ g of dried extract (Table 3). In *S. trifacicata* the highest total phenolics was found in PESF (40.73 ± 0.22 mg of GAE/g of dried extract) and the

lowest in AQSF (1.45 ± 0.25 mg of GAE/g of dried extract), whereas, in *J. gendarussa* the highest total phenolics was found in PESF (38.89 ± 0.22 mg of GAE/ g of dried extract) and the lowest in CTSF (6.29 ± 0.16 mg of GAE/g of dried extract) and in case of *H. Kurzii* the highest phenolics was found in AQSF (12.69 ± 0.14 mg of GAE/g of dried extract). The antioxidant potential was evaluated by DPPH free radical scavenging assay using butylated hydroxytolune (BHT) and ascorbic acid as standards. In the assay, the CSF of *H. kurzii* and the PESF of *J. gendarussa* revealed the highest free radical scavenging activity with IC_{50} values 3.25 ± 0.05 µg/ml and 24.68 ± 0.26 µg/ml, respectively (Table 3).

In the brine shrimp lethality bioassay, chloroform extract of *J. gendarussa* showed strong cytotoxic activity with LC_{50} value of 0.002 µg/ml. The MEF exhibited significant lethality having LC_{50} value of 0.06 µg/ml while the PESF, CTSF and AQSF demonstrated cytotoxic activity against shrimp nauplii with LC_{50} values of 0.5,

Table 2. Effect of extractives of *H. Kurzii, S. trifacicata, J. gendarussa* on hypotonic solution-induced hemolysis of erythrocyte membrane

	% Inhibition of hemolysis		
Sample code	*J. gendarussa*	*S. trifacicata*	*H. Kurzii*
MEF	41.82 ± 0.22	14.05 ± 0.28	14.56 ± 0.33
PESF	41.78 ± 0.28	52.91 ± 0.28	40.04 ± 0.22
CTSF	53.17 ± 0.22	38.52 ± 0.23	70.56 ± 0.39
CSF	75.60 ± 0.45	72.71 ± 0.17	70.24 ± 0.23
AQSF	71.93 ± 0.17	75.00 ± 0.13	69.45 ± 0.17
ASA		77.9	

Table 3. Total phenolic content, free radical scavenging and cytotoxic activities of different partitionates of *H. Kurzii, S. trifacicata, J. gendarussa.*

Sample code	Total phenolic content (mg of GAE/ g of dried extract)	Free radical scavenging activity (IC_{50} µg/ml)	Cytotoxic activity (LC_{50} µg/ml)
		J. gendarussa	
MEF	11.41 ± 0.19	28.41 ± 0.21	0.06 ± 0.01
PESF	38.89 ± 0.22	24.68 ± 0.26	0.5 ± 0.01
CTSF	6.29 ± 0.16	41.21 ± 1.0	1.32 ± 0.01
AQSF	18.53 ± 0.23	59.84 ± 0.15	1.37 ± 0.32
CSF	7.60 ± 0.19	--	0.002 ± 0.00
		S. trifacicata	
MEF	12.50 ± 0.12	59.93 ± 0.30	0.89 ± 0.12
PESF	40.73 ± 0.22	48.77 ± 0.18	1.09 ± 0.11
CTSF	5.07 ± 0.16	80.53 ± 0.27	3.19 ± 0.72
AQSF	1.45 ± 0.25	138.35 ± 0.26	3.62 ± 1.56
CSF	11.08 ± 0.22	--	0.31 ± 0.01
		H. Kurzii	
MEF	9.33 ± 0.14	251.73 ± 1.90	1.90 ± 0.36
PESF	5.11 ± 0.13	64.41 ± 0.92	8.73 ± 2.12
CTSF	12.05 ± 0.12	30.91 ± 0.49	0.33 ± 0.05
CSF	8.57 ± 0.22	3.25 ± 0.05	0.25 ± 0.02
AQSF	12.69 ± 0.14	40.78 ± 0.44	5.87 ± 1.11
VS	--	--	0.451 ± 0.004
BHT	--	27.5 ± 0.54	--
ASA	--	5.8 ± 0.21	--

1.32 and 1.37µg/ml, respectively. The carbon tetrachloride and chloroform soluble partitionates of *H. Kurzii* showed potential cytotoxic activity with LC_{50} values of 0.25 and 0.33 µg/ml, respectively while the MEF, AQSF and PESF demonstrated moderate activity against shrimp nauplii with the LC_{50} values of 1.90, 5.87 and 8.73µg/ml, respectively. Methanol and chloroform extract of *S. trifacicata* revealed strong cytotoxic activity with LC_{50} value of 0.31 and 0.89µg/ml, respectively while the PESF, CTSF, and AQSF demonstrated moderate activity against

shrimp nauplii with the LC_{50} values of 1.09, 3.19 and 3.62 µg/ml, respectively (Table 3).

Conclusion

The results obtained in our studies indicate that extractives of three plants can be considered as the potential sources of bioactive compounds and natural antioxidants. Further studies are underway to isolate and characterize the compounds responsible for these activities.

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