© 2011 Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 10 (4): 363 - 368
ISSN 0717 7917
www.blacpma.usach.cl

Artículo Original | Original Article

Polyphenols Content, Cytotoxic, Membrane Stabilizing and Thrombolytic activities of *Sarcolobus globosus*: A Medicinal Plant from Sundarban Forest

[Contenido de polifenoles, citotoxicidad, estabilizante de membranas y actividades trombolíticas de *Sarcolobus globosus*. Una planta medicinal de la Foresta Sundarban]

Mohammad R. KUDDUS[1], Fahima AKTAR[2], Mohammad K. MIAH[2], Mohammed A. BAKI[2] & Mohammad A. RASHID[2]

[1]Department of Pharmacy, Manarat International University, Mirpur-1, Dhaka-1217, Bangladesh. [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Bangladesh, Dhaka-1000.
Contactos | Contacts: Mohammad A. RASHID E-mail address: rashidma@univdhaka.edu

Abstract
The crude methanolic extract of the bark of *Sarcolobus globosus* (Family-apocynaceae) and its different organic soluble Kupchan fractions were screened for total phenol content (TPC), cytotoxic, membrane stabilizing and thrombolytic activities. The polyphenol content was determined colorimetrically using Folin-Ciocalteu method and expressed in gallic acid equivalent. The chloroform soluble Kupchan fraction (CSF) exhibited higher level of Total Polyphenol Contents (TPC, 54.21 gm of GAE/100 gm of dried extract). In the brine shrimp lethality bioassay, the crude methanolic extract (MEBP) exhibited significant cytotoxicity. The membrane stabilizing activity was assessed by using erythrocyte in hypotonic solution and was compared with acetyl salicylic acid. The hexane soluble Kupchan fraction (HSF) produced 52.73 % inhibition of hemolysis of RBC as compared to 65.38 % revealed by acetyl salicylic acid (0.10 mg/mL). In thrombolytic study screening, the crude methanolic extract demonstrated significant thrombolytic activity in human blood specimen.

Keywords: *Sarcolobus globosus*, Apocynaceae, total phenolics, cytotoxicity, membrane stabilizing, thrombolytic activity

Resumen
El extracto crudo metanólico de la corteza de *Sarcolobus globosus* (Familia-apocynaceae) y sus diferentes fracciones solubles Kupchan fueron identificadas para contenido total de fenoles (CTF), actividades citotóxicas, estabilizantes de membrana y trombolíticas. El contenido de polifenoles fue determinado colorimétricamente usando el método Folin-Ciocalteu y expresados en equivalentes a ácido gálico. La fracción Kupchan soluble en cloroformo (FSC) exhibió los mayores niveles de Contenido Total de Polifenoles (CFT, 54,21 gm of GAE/100gm de extracto seco). En el bioensayo de letalidad (Artemia salina), el extracto metanólico crudo (EMC) exhibió una siginificativa citotoxicidad. La actividad estabilizadora de membrana fue estimada usando eritrocitos en un medio hipotónico y fue comparado con el ácido acetil salicílico. La fracción Kupchan soluble en hexano (FSH) produjo un 52,73% de inhibición de la hemólisis de los glóbulos rojos comparado con un 65,38% revelado por el ácido acetil salicílico (0,1 mg/mL). En las determinaciones trombolíticas, el extracto metanólico crudo demostró una significativa actividad trombolítica en una muestra de sangre humana.

Palabras Clave: *Sarcolobus globosa*, Apocynaceae, fenoles totales, citotoxicidad, estabilización de membrana, actividad trombolítica.

Recibido | Received: March 21, 2011.
Aceptado en versión corregida | Accepted in revised form: May 20, 2011.
Publicado en línea | Published online: July 30, 2011.
Declaración de intereses | Declaration of interests: The authors wish to thank the authority of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) for supplying test organism to perform these investigations.
Este artículo puede ser citado como / This article must be cited as: Mohammad R. KUDDUS, Fahima AKTAR, Mohammad K. MIAH, Mohammed A. BAKI & Mohammad A. RASHID. 2011. Polyphenols Content, Cytotoxic, Membrane Stabilizing and Thrombolytic activities of *Sarcolobus globosus*: A Medicinal Plant from Sundarban Forest. Bol Latinoam Caribe Plant Med Aromat 10(4): 363 – 368.

INTRODUCTION

Sarcolobus globosus is a medicinal plant growing in mangrove forests in Asia (Wangensteen *et al*., 2005). The plant is listed by the US Food and Drug Administration as a poisonous plant and the seeds are known to be highly toxic to mammals. Native people of Asia widely use it to kill dogs and wild animals. It was demonstrated that it effectively kills cats (Arokiasamy, 1968). The plant extracts cause inhibition of the neuro-muscular system (Mustafa and Hadi 1990). The plant has been used in traditional medicine for treatment of rheumatism, dengue and fecer. Previous phytochemical study led to the isolation of new rotenoid sarcolobin, isoflavone sarcolobone, 12α-hydroxydeguelin, 11-hydroxytephrosin (Wangensteen *et al*., 2005) and barbigerone from S. globosus which is reported to have significant antioxidant (Wangensteen *et al*., 2006), antimalarial (Yenesew *et al*., 2003) and anticancer potential (Li *et al*., 2009).

In the present study, we evaluated the antioxidant, cytotoxic, membrane stabilizing, and thrombolytic activities of Kupchan fractions of methanolic extract of bark *of S. globosus* to verify the traditional uses of the plant as antioxidant and anticancer agent.

MATERIALS AND METHODS

Plant Material

The bark of the plant was collected from mangrove forest, Khulna in June, 2009. A voucher specimen for this collection has been deposited in Bangladesh National Herbarium, Mirpur, Dhaka-1216. The samples were then cut into small pieces and sun dried for 7 days followed by oven drying for 24 hours at 40º C to facilitate proper grinding.

Extraction and Isolation

The powdered material (300 g) was soaked in 1.0 L of methanol in a large conical flask for 7 days with occasional shaking and stirring. The whole mixture was then filtered off through a cotton plug followed by Whatman filter paper no.1 and the filtrate thus obtained was concentrated by evaporation at room temperature. A portion (5.0 g) of the concentrated methanolic extract was fractionated by the modified Kupchan partitioning protocol (Van Wagenen *et al.*, 1993), which afforded n-hexane (650 mg), carbon tetrachloride (950 mg), chloroform (450 mg) and aqueous (2.05 g) soluble materials.

Total phenolics content

Total phenolic content (TPC) of *S. globosus* extractives was measured according to using Skerget *et al.*, (2005) using gallic acid as a standard. To 0.5 ml of extract solution (2 mg/ml) in water, 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of sodium carbonate (7.5% w/v) solution were added. The Folin-Ciocalteu method is a relatively straightforward procedure that is useful for determining the total phenolic content of an extract (Ricco *et al*., 2010; Cervantes-Cardoza *et al*., 2010; Chavez et al., 2011). Briefly after 20 minutes incubation at room temperature the absorbance was measured at 760 nm. Total phenolics were quantified by the gallic acid calibration curve. The phenol content of the sample was expressed as gm of GAE (gallic acid equivalent)/100 gm of the dried extract.

Cytotoxicity screening

DMSO solutions of all the extractives were applied against *Artemia salina* in a one-day in *vitro* assay (Meyer *et al.*, 1982; Martinez *et al*., 1997). For the experiment, 4 mg of each of the Kupchan fractions was dissolved in DMSO and serial dilutions from 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.78125 µg/ml were tested. Vincristine sulphate and DMSO were used as the positive and negative control, respectively. Table 1 shows the results of the brine shrimp lethality bioassay after 24 hr exposure of the shrimps to the test samples.

Membrane stabilizing activity

The membrane stabilizing activity was assessed by using hypotonic solution induced hemolysis of mice erythrocyte (Shinde *et al.*, 1999). To prepare the erythrocyte suspension whole blood was obtained using a syringe (containing anticoagulant EDTA) from mice through cardiac puncture. The blood was centrifuged and blood cells were washed three times with 154 mM NaCl solution in 10 mM sodium phosphate buffer (pH 7.4) through centrifuge action for 10 min at 3000 g. The test sample consisting of stock erythrocyte (RBC) suspension (0.50 mL) was mixed with 5.0 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing either the extract (2.0 mg/mL) or indomethacin (0.1 mg/mL). The control sample consisted of 0.5 mL of RBCs mixed with hypotonic-buffered saline alone. The mixture was incubated for 10 min at room temperature, centrifuged for 10 min at

3000 *g* and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of either hemolysis or membrane stabilization was calculated using the following equation-% inhibition of hemolysis = 100 x (OD_1-OD_2/OD_1) where, OD_1 = optical density of hypotonic-buffered saline solution alone (control) and OD_2 = optical density of test sample in hypotonic solution.

Thrombolytic activity

The thombolytic activity of all extractives was evaluated by the method of Prasad and collaborators (2006) using streptokinase as standard. The dry crude extract (100 mg) was suspended in 10 ml of distilled water and it was kept overnight. Then the soluble supernatant was decanted and filtered. Aliquots (5 ml) of venous blood were drawn from healthy volunteers which were distributed in five different pre weighed sterile micro centrifuge tube (1 ml/tube) and incubated at 37° C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (Clot weight = weight of clot containing tube – weight of tube alone).

To each microcentrifuge tube containing pre-weighed clot, 100 µl aqueous solutions of different partitionates along with the crude extract was added separately. As a positive control, 100 µl of streptokinase (SK) and as a negative non thrombolytic control, 100 µl of distilled water were separately added to the control tubes. All the tubes were then incubated at 37° C for 90 minutes and observed for clot lysis. After incubation, the released of fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. The differences in weights taken before and after clot lysis were expressed as percentage of clot lysis as shown below:

% of clot lysis = (wt of released clot /clot wt) × 100

Statistical analysis

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

RESULTS

The present study was undertaken to evaluate the antioxidant, cytotoxic, membrane stabilizing and thrombolytic activities of the organic soluble materials of a methanol extract of *S. globosus* and the results have been summarized in Table 1.

DISCUSSION

The total phenolic content i.e. TPC varied for different Kupchan fractions of methanolic extract ranging from 26.15 gm to 54.21 gm of GAE/100 gm of dried extract (Table 1 and Figure 1). The highest total phenolic was found in CSF (54.21 gm of GAE/100 gm of dried extract) demonstrating the significant antioxidant potentials. The crude methanolic extract (MEBP) and hexane soluble Kupchan fraction (HSF) revealed the moderate antioxidant activity. The lowest phenolic was seen in CTSF (26.15 gm of GAE/100 gm of dried extract).

In the brine shrimp lethality bioassay, the crude methanolic extract (MEBP) showed strong cytotoxic activity with LC_{50} value of 6.8 µg/ml. The AQSF exhibited significant lethality having LC_{50} value of 10.4 µg/ml while the CTSF, CSF and HSF demonstrated moderate activity against shrimp nauplii with the LC_{50} of 17.5, 22.7 and 24.2 µg/ml, respectively (Table 1 and Figure 2).

The different extractives of *S. globosus* 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 1 and Figure 3). The hexane soluble Kupchan fraction (HSF) revealed 52.73% inhibition of hemolysis of RBC as compared to 65.38% produced by the standard, acetyl salicylic acid (0.10 mg/mL). The carbon tetrachloride and aqueous soluble Kupchan fraction also showed significant inhibition of hemolysis of RBC. Table 1 and figure 4 show the results of thrombolytic activity of the sample, the positive control (Streptokinase) and a negative non thrombolytic control (distilled water).

Table 1. Total phenolic content, Cytotoxic activity (LC_{50} µg/ml), membrane stabilizing activity (% inhibition of hemolysis) and thrombolytic activity of different Kupchan fractions of *S. globosus*

Sample	**Total Phenolic Content** (gm of GAE/100 gm of dried extract)	**Cytotoxic activity** (LC_{50} µg/ml)	**Membrane stabilizing activity** (% inhibition of hemolysis)	**% of Clot Lysis**
VS	-	0.451 ± 1.25	-	-
ASA	-	-	65.38	-
SK	-	-	-	77.39
MEBP	27.25 ± 1.07	6.8 ± 0.76	25.32	58.68
HSF	33.04 ± 1.19	24.2 ± 0.42	52.73	51.76
CTSF	26.15 ± 0.78	17.5 ± 0.58	41.63	40.47
CSF	54.21 ± 0.52	22.7 ± 1.3	23.54	48.27
AQSF	38.11 ± 1.52	10.4 ± 0.92	46.54	36.59

Where, VS = Vincristine sulphate, ASA = Acetyl Salicylic Acid, SK = Streptokinase, MEBP = Methanolic Extract of Bark of Plant, HSF= Hexane Soluble Kupchan Fractions, CTSF= Carbon tetrachloride Soluble Kupchan Fractions, CSF = Chloroform Soluble Kupchan Fractions and AQSF = Aqueous Soluble Kupchan Fractions of crude methanolic extract.

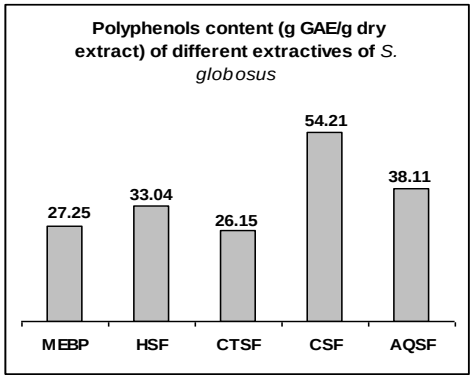

Figure 1. Polyphenols content (g GAE/100 g dried extract) of different extractives

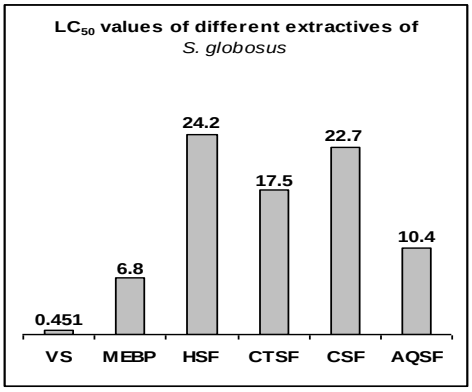

Figure 2. LC_{50} values of different extractives of *S. globosus*

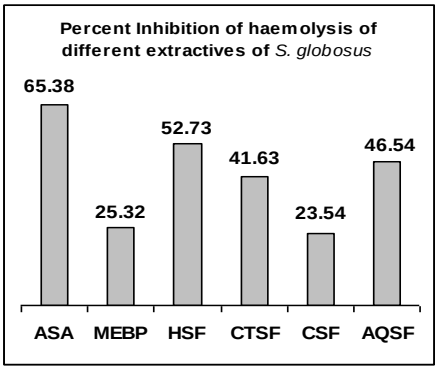

Figure 3. Membrane stablizing activity of different extractives of *S. globosus*

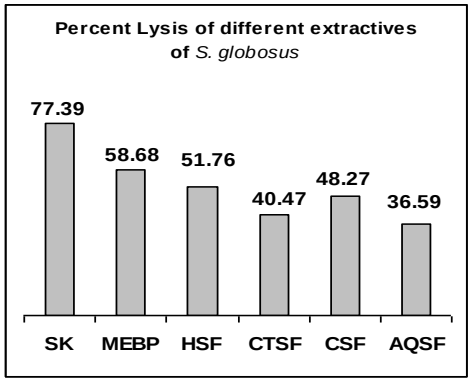

Figure 4. Thrombolytic ativity of different extractive of *S.globosus*

CONCLUSIONS

From the above results, it is evident that the methanolic crude extract and all of its Kupchan fractions showed significant cytotoxic activities which suggest the presence of bioactive metabolites with biological properties such as antimalarial, anticancer etc. The strong cytotoxic activity of the crude extract is probably due to the synergistic effects of the compounds present in the extract which correlates the traditional uses of the plant as anticancer, antimalarial agent etc. Moreover, the chloroform soluble Kupchan fraction (CSF) showed highest Total Polyphenol Contents (TPC, 54.21 gm of GAE/100 gm of dried extract) which is close agreement to the presence of barbigerone (Wangensteen *et al.*, 2006) and related antioxidant compounds in this plant. The hexane soluble Kupchan fraction (HSF) produced 52.73% inhibition of hemolysis of RBC as compared to 65.38% revealed by acetyl salicylic acid (0.10 mg/mL). In thrombolytic study screening, the crude methanolic extract demonstrated significant thrombolytic activity in human blood specimen. The plant could be subjected for extensive chromatographic separation and purification processes to isolate bioactive lead compounds for the discovery of novel therapeutic agents.

ACKNOWLEDGEMENTS

The authors wish to thank the authority of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) for supplying test organism to perform these investigations.

REFERENCES

Arokiasamy M. 1968.Toxicity of *Sarcolobus globosus* as observed in a cat. **Malaysian Veterin J** 4: 196 - 199.

Cervantes-Cardoza V, Rocha-Guzman NE, Gallegos-Infante JA, Rosales-Castro M, Medina-Torres L, González-Laredo RF. 2010. Actividad antioxidante de extractos de semilla de tres variedades de manzana (*Malus domestica* Borkh -Rosaceae-). **Bol Latinoam Caribe Plant Med Aromat** 9: 446 - 456.

Chavez F, Aranda M, García A, Pastene E. 2011. Los polifenoles antioxidants extraídos del epicarpio de palta (*Persea americana* var. Hass) inhiben la ureasa de *Helicobacter pilori*. **Bol Latinoam Caribe Plant Med Aromat** 10: 265 - 280.

Li ZG, Zhao YL, Wu X, Ye HY, Peng A, Cao ZX, Mao YQ, Zheng YZ, Jiang PD, Zhao X, Chen LJ, Wei YQ. 2009. Barbigerone, a natural isoflavone, induces apoptosis in murine lung-cancer cells via the mitochondrial apoptotic pathway. **Cell Physiol Biochem** 24: 95 - 104.

Martinez JL, Torres R, Morales MA. 1997. Hypotensive Effect of O-Methylisothalic-berine, a bisbenzylisoquinoline alkaloid isola-ted from *Berberis chilensis* on normotensive rats. **Phytother Res** 11: 246 - 248.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JB, Nicholsand DE, Mclaughlin JL. 1982. Brine shrimp, a convenient general bioassay for active plant constituents. **Planta Med** 45: 31 - 34.

Mustafa MR, Hadi AHA. 1990. Neuromuscular blocking activity of a glycosidic extract of the plant *Sarcolobus globosus*. **Toxicon** 28: 1237 - 1239.

Prasad S, Kashyap R S, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. 2006. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. **Thrombosis J** 4: 14.

Ricco RA, Wagner ML, Portmann E, Reides C, Llesuy S, Gurni AA, Carballo MA. 2010. Análisis de polifenoles, actividad antioxidante y genotoxicidad en especies argentinas de *Lippia* y *Aloysia* (Verbenaceae)**. Bol Latinoam Caribe Plant Med Aromat** 9: 388 - 396.

Shinde UA, Phadke AS, Nair A M, Mungantiwar AA, Dikshit VJ and Saraf VO. 1999. Membrane stabilizing activity–a possible mechanism of action for the anti-inflammatory activety of *Cedrus deodara* wood oil. **Fitoterapia** 70: 251 - 257.

Skerget M, Kotnik P, Hadolin M, Hras A, Simonic M, Knez Z. 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. **Food Chem** 89: 191 - 198.

Van Wagenen BC, Larsen R, Cardellina JH, Randazzo D, Lidert ZC, Swithenbank C. 1993. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. **J Org Chem** 58: 335 - 337.

Wangensteen H, Miron A, Alamgir M, Rajia S, Samuelsen AB, Malterud KE. 2006. Antioxidant and 15-lipoxygenase inhibitory activity of rotenoids, isoflavones and phenolic glycosides from *Sarcolobus globosus*. **Fitoterapia** 77: 290 - 295.

Yenesew A, Derese S, Midiwo JO, Oketch-Rabah HA, Lisgarten J, Palmer R, Heydenreich M, Peter MG, Akala H, Wangui J, Liyala P, Waters NC. 2003. Anti-plasmodial activities and X-ray crystal structures of rotenoids from *Millettia usaramensis* subspecies *usaramensis*. **Phytochemistry** 64: 773 - 779.