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In vitro Membrane Stabilizing Activity, Total Phenolic Content, Cytotoxic, Thrombolytic and Antimicrobial Activities of *Calliandra surinamensis* (Wall.)

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Different extractives of leaf of *Calliandra surinamensis* were evaluated for antimicrobial activity along with membrane stabilizing, thrombolytic and cytotoxic activities. The total phenolic content was also determined and expressed in gallic acid equivalent. The membrane stabilizing activity was assessed by heat and hypotonic solution induced method. In the present studies, the crude methanolic extract (ME) of leaf of *C. surinamensis* demonstrated strong membrane stabilizing activity, whereas its chloroform (CSF) and pet ether (PESF) soluble fractions revealed moderate membrane stabilizing properties. In brine shrimp bioassay, the aqueous (AQSF) soluble fractions revealed the highest lethality. In antimicrobial screening, the chloroform (CSF) and aqueous (AQSF) soluble fraction demonstrated strong antimicrobial activity against 13 pathogenic microorganisms. The chloroform (CSF) soluble fraction exhibited the highest inhibition against microbial growth having zone of inhibition ranged from 13.0±0.82 mm to 18.3±1.25 mm and maximum zone of inhibition was found 18.3±1.25 mm against *Salmonella typhi*. In thrombolytic study, the chloroform (CSF) soluble fraction demonstrated mild thrombolytic activity in human blood specimen.

Keyword: *Calliandra surinamensis*, Membrane Stabilizing, Thrombolytic, Antimicrobial.

1. Introduction

Botanical medicine or phytomedicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes[1].

Even among prescription drugs, minimum 25% contain at least one compound derived from plants. The percentage might be higher if we include over-the-counter (OTC) drugs[2]. In developing countries, about 75% of the world populations rely on traditional medicine for their primary health care[3]. Worldwide, infectious disease is one of the main causes of death accounting for approximately one-half of all deaths in tropical countries. This is really alarming as it was once believed that we would eliminate infectious disease by the end of the millennium. This can be attributed to the increases in respiratory tract infections and HIV/AIDS. Other contributing factors are an increase in antibiotic resistance in nosocomial and community acquired infections[4]. The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various bacteria to

any agent. This test measures the ability of each test sample to inhibit the *In vitro* bacterial growth[5]. In anticoagulation therapy, thrombolytic drugs like tissue plasminogen activator (t-PA), urokinase, streptokinase etc. play a crucial role in the management of patients with cerebral venous sinus thrombosis (CVST), is a common disorder that is often accompanied by significant morbidity and mortality[6,7]. In the present study attempt has also been taken to develop a new model system to study clot lysis in a simplified way using a known thrombolytic drugs, streptokinase.

Calliandra surinamensis (Wall.) belongs to the family Fabaceae**,** is a large and economically important family of flowering plants. A low branching evergreen tropical shrub, *C. surinamensis* is named after Suriname, a country in Northern South America**.** Commonly it is known as pink powder puff or Suriname powder puff.

In the present study, the organic soluble materials of a methanol extract of the leaf and its different organic soluble partitionates were evaluated for the antioxidant activity in terms of total phenolic content, membrane stabilizing capability, anti-microbial, cytotoxic and thrombolytic activities for the first time.

2. Materials and Methods

2.1 Plant materials

The leaf of *C. surinamensis* was collected from Demra, Dhaka, Bangladesh, in November 2010. A voucher specimen for this plant has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh (Accession no. 35390). The sun dried and powdered leaf (500 gm) of *C. surinamensis* was macerated in 2.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator at low temperature (40-45 ºC) and reduced pressure. The concentrated methanol extract (ME) was partitioned by modified Kupchan method[8] and the resultant partitionates i.e., pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF), and aqueous (AQSF) soluble fractions were used for the experimental processes.

2.2 Membrane stabilizing activity: The membrane stabilizing activity of the extractives was assessed by using hypotonic solution and heat-induced hemolysis of mice erythrocyte by the method developed by Shinde *et al*. (1999)[9] and modified by Sikder *et al*. (2011)[10].

2.3 Total Phenolics Analysis: Total phenolic content of *C. surinamensis* extractives was measured by employing the method Skerget *et al*. (2005) [11] involving Folin-Ciocalteu reagent as an oxidizing agent and 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. After 20 minutes of incubation at room temperature the absorbance was measured at 760 nm using a UV-Visible spectrophotometer. Total phenolics were quantified by calibration curve obtained from the known concentrations of gallic acid (0-100 µg/ml) and were expressed as gm of GAE (gallic acid equivalent) / 100 gm of the dried extract.

2.4 Brine Shrimp Lethality Bioassay: This technique was applied for the determination of general toxic properties of the DMSO solutions of plant extractives[12,13] against *Artemia salina* in a 1-day *in vivo* assay. Vincristine sulphate was used as positive control.

2.5 Thrombolytic activity: The thrombolytic activity of all extracts was evaluated by the method developed by Daginawala (2006)[14] and modified by Kawsar *et al*. (2011)[15] using streptokinase (SK) as the standard.

2.6 Antimicrobial activity: The antimicrobial screening, which is the first stage of antimicrobial drug discovery, was performed by the disc diffusion method[16] against thirteen bacteria and three fungi (Table-6) collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. Standard disc of Ciprofloxacin (30 µg/disc) and

blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm.

3. Results and Discussion

The present study was undertaken to evaluate the membrane stabilizing, antimicrobial, cytotoxic, thrombolytic activities as well as total phenolic content determination of different organic soluble materials of the methanol extract of *C. surinamensis.*

The different extractives of *C. surinamensis* at concentration 2.0 mg/mL significantly protected the lysis of human erythrocyte membrane induced by hypotonic solution and heat induced haemolysis, as compared to the standard, acetyl salicylic acid (0.10 mg/mL) (Table-1). For hypotonic solution induced haemolysis, at a concentration of 2.0 mg/ mL, the methanolic extract (ME) inhibited 56.41% haemolysis of RBCs as compared to 71.9% produced by acetyl salicylic acid (0.10 mg/mL). The chloroform and pet ether soluble extractives also revealed significant inhibition of haemolysis of RBCs. In heat induced haemolysis, on the other hand, at same concentration, the aqueous soluble fraction (AQSF) produced 30.1% inhibition of haemolysis of RBCs as compared to 42.12% produced by acetyl salicylic acid (0.10 mg/mL).

The total phenolic content varied for different partitionates ranging from 20.87 gm to 243.25 gm of GAE/100 gm of dried extract (Table 2). The highest total phenolics were found in AQSF (243.25 gm of GAE/100 gm of dried extract) and the lowest in CTCSF (20.87 gm of GAE/100 gm of dried extract).

In case of brine shrimp lethality bioassay, the lethality of the methanol extract (ME) and its pet-ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble fractions were evaluated against *A. salina*. Table 2 shows the results of the brine shrimp lethality testing after 24 hours of exposure to the samples and the positive control, vincristine sulphate (VS). The aqueous soluble fraction (AQSF) showed potent cytotoxic activity having LC_{50} of 0.878 µg/ml as compared to 0.451 µg/ml for vincristine sulphate.

Table-2 shows the results of thrombolytic activity of the sample, the positive control (Streptokinase) and a negative non thrombolytic control (distilled water).

The crude methanolic extract, along with its pet-ether, carbon tetrachloride, chloroform and aqueous soluble partitionates of the crude extract were screened against thirteen bacteria and compared with standard antibiotic, Ciprofloxacin. The chloroform (CSF) soluble fraction exhibited the strong inhibition of microbial growth having zone of inhibition ranging from 13.0±0.82 mm to 18.3±1.25 mm and maximum zone of inhibition 18.3±1.25 mm was found against *S. typhi*. The aqueous (AQSF) soluble fraction demonstrated mild inhibitory activity against the tested microorganism with zone of inhibition of 7.6±0.47 mm to 11.6±2.35 mm. While other extractives like CTCSF showed mild activity against microbial growth having zone of inhibition ranged from 7.0±0.0 mm to 10.0±0.816 mm.

Table 1: Effect of extractives of *C. surinamensis* on hypotonic solution and heat induced haemolysis of erythrocyte membrane.

Sample code	Concentration (mg/mL)	Haemolysis inhibition (%)	
		Heat induced	Hypotonic solution induced
ME	2.0	17.96 ± 0.59	56.14 ± 0.24
PESF	2.0	19.77 ± 0.51	50.06 ±0.41
CTCSF	2.0	26.94 ± 0.43	38.26 ± 0.74
CSF	2.0	28.48 ± 1.0	51.606 ± 1.18
AQSF	2.0	30.1 ±0.16	36.4 ± 0.798

Acetyl salicylic acid	0.10	42.12 ± 0.0	71.9±0.0

Table 2: The total phenolic content, cytotoxic and thrombolytic activities of different extractives of *C. surinamensis*

Sample	Total Phenolic Content (gm of GAE/100 gm of dried extract)	Cytotoxic activity (LC_{50} µg/ml)	Thrombolytic activity (% of lysis)
VS	-	0.451±0	-
ME	141.20 ± .828	39.396 ± .740	15.78 ± 1.44
PESF	21.32 ± .964	18.706 ± 1.15	16.48 ± .490
CTCSF	19.55 ± 1.036	9.893 ± .609	17.38 ±3.01
CSF	25.91 ± 1.180	3.6 ± .670	20.94 ± .499
AQSF	243.77 ± .406	1.061 ± .188	19.94 ± 3.58
SK	-	-	77.39 ± 0
Water	-	-	2.5 ± 0

VS = Vincristine sulphate; ME = Methanolic extract; PESF = Pet-ether soluble fraction; CTCSF = Carbon tetrachloride soluble fraction; CSF= chloroform soluble fraction; AQSF = Aqueous soluble fraction of the methanolic extract of *C. surinamensis.* SK = Streptokinase

Table 3: Antimicrobial activity of different partitionates of *C. surinamensis*

Test microorganisms	Diameter of zone of inhibition (mm)			
	CTCSF	CSF	AQSF	Ciprofloxacin
Bacillus cereus	10.0±.816	14.0 ±0.82	9.0±.81	46.3±4.49
Bacillus megaterium	7.6±1.25	17.0±1.63	10.3±2.6	40.3±5.55
Bacillus subtilis	7.0±3.74	13.0±.82	7.0 ±.81	41.6±2.35
Staphylococcus aureus	7.0±0.00	17.0±.82	10±1.63	46.3±1.24
Sarcina lutea	7.0±0.00	15.0±4.08	9.3±2.05	50.0±0.0
Escherichia coli	7.0±0.0	14.0±2.94	9.6±1.24	46.3±.94
Pseudomonas aeruginosa	7.3 ±0.47	17.3±1.69	7.6±.47	44.0±2.8
Salmonella paratyphi	-	15.6±3.29	-	56.6±6.23
Salmonella typhi	8.0±0.82	18.3±1.25	9.3±2.49	45.6±3.29
Shigella boydii	9.3±0.25	15.6. ±94	9.3±2.05	51.0±1.41
Shigella dysenteriae	7.6 ±0.94	16.0±1.63	9.0±2.4	48.0±2.82
Vibrio mimicus	7.3 ±0.47	16.31±0.69	8.0±1.41	48.3±5.32
Vibrio parahaemolyticus	7.3±0.47	13.6±0.94	8.0±0.81	42.3±3.68

The average values of three calculations are presented as mean ± S.D. (standard deviation) ME= Methanolic extract; PESF= Pet ether soluble fraction; CTSF= Carbon tetrachloride soluble fraction; CFSF= Chloroform soluble fraction; AQSF =Aqueous soluble fraction of the methanolic extract of *C. surinamensis.*

4. Reference

1. Barrett B, Kiefer D, Rabago D. Assessing the risks and benefits of herbal medicine: an overview of scientific evidence. Altern Ther Health Med 1999; 5:40-49.
2. Duke JA. Promising phytomedicinals. In: Janick J and Simon JE (eds.), Advances in new crops. Timber Press, Portland, 1990, 491-498.
3. Matu EN, Staden JV. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. J Ethno pharmacol 2003; 87:35-41.
4. Pinner R, Teutsch S, Simonsen L, Klug L, Graber J, Clarke M, Berkelman R. Trends in infectious diseases mortality in the United States. J Am Med Assoc 1996; 275:189–193.
5. Ayafor JF. Limonoids and phyto derivatives from *Cedrela sinensis.* Tetrahedron 1972; 28:9343.

6. Allroggen H, Abbott RJ. Cerebral venous sinus thrombosis. Postgrad Med Journal 2000; 76:12-15.
7. Watson RD, Chin BS, Lip GY. Antithrombotic therapy in acute coronary syndrome. BMJ 2002; 352:1348-1351.
8. Wagenen BC V, Larsen R, Cardellina JH, Ran dazzo D, Lidert ZC, Swithenbank C. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. J Org Chem 1993; 58:335-337.
9. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Membrane stabilizing activity- a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. Fitoterapia 1999; 70:251-257.
10. Sikder MA, Rahman MA, Kaisar MA, Rahman MS, Hasan CM, Rashid MA. *In vitro* Antioxidant, reducing power, free radical scavenging and membrane stabilizing activities of seeds of *Syzygium cumini* L. Lat Am J Pharm 2011; 30:781-785.
11. Skerget M, Kotnik P, Hadolin M, Hras A, Simonic M, Knez Z. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food. Chem 2005; 89:191-198.
12. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JB, Nicholsand DE, Mclaughlin JL. Brine shrimp, a convenient general bioassay for active plant constituents. Planta Med 1982; 45: 31-34.
13. Maclaughlin JL, Anderson JE, Rogers LL. The use of biological assays to evaluate botanicals. Drug Info. Journal 1998; 32: 513-524.
14. Daginawala HF, Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM. Development of an *In vitro* model to study clot lysis activity of thrombolytic drugs. Thrombosis Journal 2006; 4: 14.
15. Kawsar MH, Sikder MA, Rana MS, Nimmi I, Rashid MA. Studies of thrombolytic and cytotoxic properties of two asteraceous plants of Bangladesh. Bang. Pharm Journal 2011; 14(2): 103-106.
16. Bauer AW, Kirby WMM, Sherries JC, Tuck M. Antibiotic susceptibility testing by a standardized disc diffusion method. Ame J Clin Pathol 1966; 45:493-496.