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PHARMACOLOGICAL SCREENING OF *IN VITRO* ACTIVITY OF METHANOL EXTRACT OF NAGALINGA FLOWER

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Abstract

The aim of the present study was to investigate the presence of phytochemical constituents and presence of some potent pharmacological activity of *Couroupita guianensis*. The extract was prepared from the flowering part of the plant. Methanolic extract of the dry pulverized flowers of *C.guianensis* was obtained by the soxhlet cold extraction method. The flower extract was subjected to an initial phytochemical screening by standard methods. Phytochemical analysis revealed the presence of flower extracts. Antioxidant properties were evaluated of methanolic e alkaloids, Phenols, tannins, saponins, terpenes and gums extracted using DPPH free radical scanning models. Antioxidant effect was observed with IC50 (262.18 µg / ml), which is comparable to the ascorbic acid with IC50 value (170.54µg / ml). Ex-vivo Cardioprotective activity was determined by clot disruption method, and showed % clot lysis of methanol shows (25.58%), and standard streptokinase shows (87.58%) as positive control respectively.On the anthelmintic side there are paralyzed and dead worms through methanol extract where as standard albendazole showed paralyzed and dead worms.Anthelmintic activity test shows a potent activity.

Keywords: *Nagalinga Flower, Couroupita guianensis, antioxidant, anthelmintic,thrombolytic activity*.

Introduction

Traditional medicines are based on theories and experience the indigenous experience of different cultures used in the treatment of health, diagnosis, progress or physical and mental illness skills and practice beliefs. [1] Plants are an important source of medicine and play a key role in the world. [2] Throughout the ages, human depends on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavors and fragrances, and medicines.[3]Natural products and their derivatives mean more than 50% of all the drugs in clinical use in the world recently. Higher plants give no less than 25% of the total.[4] In the last 40 years, many potent drugs have been obtaining from flowering plants, including for example Dioscorea species (diosgenin), from which all an ovulatory contraceptive agents have been derived, reserpine and other antihypertensive and calmative alkaloids from Rauwolfia species, pilocarpine to recover glaucoma and a cardio refresher agent to treat heart failure from Digitalis species [5].Approximately half of the world's flowering plant species are originate in the tropical forests. Tropical rain forests pursue a vast reservoir of potential drug species. They pursue to provide natural product chemists with crucial compounds as starting points for the development of new drugs.[6] Traditional healing methods are not only included in the natural sources of medicinal materials, but also include magic (magic), skin, substances, religious verses, spiritual methods, tranquility, sacrifice, even aggressive physical and mental tortures.[7]

C.guianensis Aubl (Lecythidaceae) is commonly called the Cannonball tree.The tree also produces wonderful, almost human-shaped globular brown woody, unhealthy , amphisarca (double fleshy) fruit.[8] The flowers are strongly scented, and are especially fragrant at night and in the early morning.[9] It is widely planted in tropical and subtropical plant gardens as an ornamental, it does well under cultivations and it is used to feed animals. The leaves, flowers, and barks of *C.guianensis* to treat hypertension, tumours, pain, anti inflammatory processes. The trees are used to cure cold and stomach ache. [10] Juice made from the leaves is used to cure skin diseases. The inside of the fruit can disinfect wounds and young leaves cure toothache.[11] In present ,the research attempts were made to find consecutive methanolic extracts of *C.guianensis* flowers for their in phytochemical test, In vitro antioxidant activity, thrombolytic test , anthelmintic activity.[12]

Methods

Plant samples *C.guianensis* was collected from Share-Bangla-Agriculture University located in Dhaka in late November 2018. The plant was identified and confirmed by The National Herbarium, Government of Bangladesh located in Mirpur Area. The DACB Accession Number confirming the proper identification of the plants is as follows:DACB Association number:46759.

Preparation of the extract

The air dried 800 gm of the powdered flowers *C.guianensis* extract was taken in a clean, round bottomed flask (3 liters) and soaked in 2.5 liters of methanol. The container with its content was sealed by foil and kept for a period of seven days accompanying occasional shaking and stirring. The whole mixture was then filtered through a fresh cotton plug and finally with a What man filters paper. The volume of the filtrate was then reduced using a Buchi Rotavapor at low temperature and pressure(40°C). The weight of the crude extract was 36.5 gm.

Phytochemical screening

Phytochemical screening of methanol extract Couroupita guianensis flowers was subjected to qualitative phytochemical analysis for the presence of various classes of active chemical constituents such as alkaloids, Phenols, tannins, saponins, terpenes and gums etc. using standard procedures [13].

Reagents used for the different chemical group test

The following reagents were used for the different chemical group test: Mayer's reagent, Dragendroff's Reagent, Fehling's solution A, Fehling's solution B, Benedict's Reagent and Molish Reagent are tested for alkaloids.

Antioxidant activity

According to this reference, Several assays were used for measuring the antioxidant potential of the test samples. Free radical scavenging activity of the samples were determined by measuring the change of absorbance of DPPH (1, 1-Diphenyl-2-picrylhydrazyl radical) at 517nm by spectrophotometer method .[14]

At first volumetric flasks are taken to make different types of concentration 12.5, 25, 50, 100, 200 and 500 µg/ml. In the volumetric flasks serial dilution of extract is done and marked them respectively. The sample from each concentration of 0.004% DPPH solution is taken. Then the solution is kept in a dark place for 30 minutes .In another, a test tube of 0.004% DPPH & methanol is taken to prepare a blank solution. Then absorbance at 517nm is taken by UV Spectroscopy. Then the % of inhibition was calculated. The percentage of radical scavenging (%) was calculated by the following formula:

DPPH scavenging activity (%) = $[(A_0-A_1)/A_0] \times 100$

Where A0 is the absorbance of the control and A1 is the absorbance of the sample. The results are averages of measurements. Free radical scavenging activity is expressed as the percentage of DPPH Decrease [15].

Thrombolytic activity

Streptokinase (SK)

Streptokinase (15, 00,000 I.U.,) used as a standard which was collected from Beacon pharmaceutical Ltd, Bangladesh. 5 ml sterile distilled water was added to streptokinase vial and mixed properly. From this suspension100µl (30,000 I.U) was used for *in vitro* thrombolysis.[15]

Preparation of sample

The thrombolytic activities of the plant extracts were evaluated by a method using streptokinase (SK) as a reference standard. 10 mg of plants were dissolved respectively in 10 ml of methanol distilled water and was kept overnight. Then the soluble supernatant was decanted and filtered.

Blood sample

Blood samples were collected from healthy human volunteers (n=5) by maintaining aseptic condition without a history of oral contraceptive or anticoagulant therapy. 1ml of blood was transferred to the previously weighed microcentrifuge tubes to form clots.

Thrombolytic activity

The tube was then incubated at 37 °C for 45 minutes and observed for clot lysis. After incubation, the released fluid was discarded and tubes were again weighed to observe the difference in weight after clot disruption. Finally percentage of clot lysis was determined as followings:

% of clot lysis = (Weight of lysis clot/ initial clot weight) × 100

Anthelmintic activity

Earthworms were collected from wet fields. The average weight of each worm was 7-8cm long.

Preparation of sample

The extract was prepared as suspension the conc. was 25, 50, and 100mg/ml .0.25, 0.50 and 1 ml of extract were taken and titrated. In different Petri dishes 10 ml of control, standard and extract of each concentration were taken. In each different Petri dish, both types of 3 worms were taken. The Time taken for each worm for paralysis was recorded. The worm death was recorded when the worm did not move.

Results

The result of phytochemical screening of *Couroupita guianensis* are presenting in the table 1.Similarly DPPH (2,2-diphenyl-1-1 picrylhydrazyl) radical scavenging activity of *C. guianensis* and ascorbic activity is shown in table 1.1 and table 1.2 .In the % of inhibition at different concentration of extracts of ascorbic acid and % of inhibition at different concentration of the extracts of C. guianensis shows in fig1 and fig2.

The following thrombolytic test shows that the methanol extract possesses some amount of thrombolytic property.

Maximum for paralysis of worms was counted as 14 minutes for the concentration of 25 mg/ml and the death time as 59 minutes Table.1.4 Anthelmintic effect was found for the plant extract in this study.

Discussion

The result of preliminary phytochemical screening of *Couroupita guianensis* are presented in table 1.Result revealed that methanolic extracts of *C. guianensis* contains alkaloids, phenol, tannins, carbohydrates and terpinoid. Similarly DPPH (2,2-diphenyl-1-1 picrylhydrazyl) radical scavenging activity of *C. guianensis* and ascorbic activity is shown in table 1.1 and table 1.2**.** DPPH radical has the ability to donate hydrogen and electrons in order to remove reactive oxygen species and oxidative stress and to inhibit lipid peroxidation of the body. The plants are evaluated on the merit and potentiality of scavenging DPPH free radical and compared in respect to significant natural antioxidants named ascorbic acid to discover the effectiveness of the crude plant extract. I found that IC_{50} value of ascorbic acid 29.34µg/ml and IC_{50} value of methanoic *C. guianensis* extract of 60.67µg/ml.The result of thrombolytic activity of methanolic extracts and evaluated by a method using streptokinase standard with respect to each of the test presented in table 1.3.The following thrombolytic test shows that the methanol extract of possess some amount of thrombolytic property. Methanolic extract of the flowers shows slight anthelmintic activity in table 1.4 comparison to standard albendazole when tested against earthworms. Where all the worms become dead in 30 minutes when treated with albendazole whereas worms which are treated with the methanol extracts are dead in around 59minutes.

Acknowledgments

The project work presented here deals with different studies on *Couroupita guianensis* To get preliminary idea about the active constituents present in extract, different chemical tests were performed showed the presence of alkaloids, phenol, tannins, carbohydrates and terpinoid.The extract showed antioxidant action compared to the standard ascorbic acid. The thrombolytic test shows that extract of *Couroupita guianensis* possesses some amount of thrombolytic property. When the earth worm was treated with extract the anthelmintic properties of the plant was also confirmed.

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Table 1. Different chemical group test results

Different Chemical Group	Result
Carbohydrate	-
Tannin	+
Flavonoids	-
Saponin	+
Alkaloid	+
Glycosides	-
Steroids	-
Gums	+
Phenols	+

Terpenoids	**+**

Sl.No.	Absorbance of control	Concentration **(µg/ml)**	Absorbance of Ascorbic acid	% of Inhibitions	IC 50
1		12.5	1.05	16	170.54
2		25	0.98	21.6	
3		50	0.88	29.6	
4		100	0.55	56	
5		200	0.42	66.4	
6		500	0.31	75.2	
7		blank	1.25		

Table1.2:Antioxidant activity of *Couroupita guianensis* extracts

Sl.No.	Absorbance of control	Concentration **(µg/ml)**	Absorbance of *Couroupita guianensis* Extracts	% of Inhibitions	IC 50
1		12.5	0.86	30.96	262.18
2		25	0.75	39.76	
3		50	0.72	42.16	
4		100	0.69	44.64	
5		200	0.64	49.2	
6		500	0.57	54.8	
7		blank	1.25		

Table1.3: Thrombolytic activity of *C. guianensis* extracts.

Sample	1st clot + tube weight (gm)	1st clot weight (gm)	2nd clot + tube weight (gm)	2nd clot weight (gm)	% of Lysis
Standard (Streptokinase)	1.652	0.822	0.9321	0.1021	87.58
Control (Distilled water)	1.452	0.622	1.4261	0.596	4.18
*C. guianensis*Extract	1.43	0.76	1.35	1.18	25.37

Table 1.4: Result of anthelmintic activity test

Result of anthelmintic activity test			
Group	Concentration mg/ml	Paralyzing time (min)	Death time
Distilled Water	-	NA	NA
Methanolic Extract of Leaves	25	28	59mins
	50	17	42mins
	100	9	28mins
Albendazole	25	22	30mins
	50	15	24mins
	100	7	20mins

Figure 1.

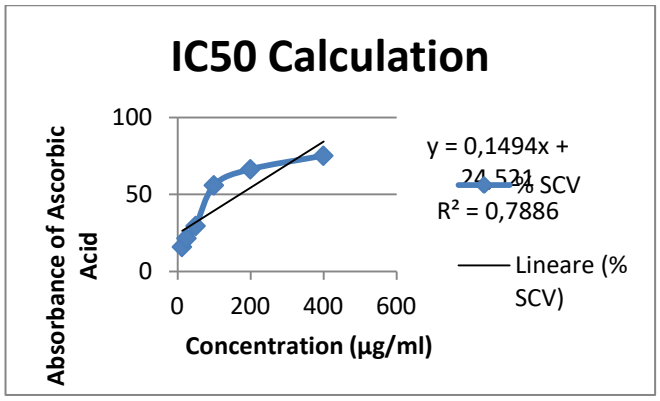

Fig.1: % of inhibition at different concentration of extracts of ascorbic acid

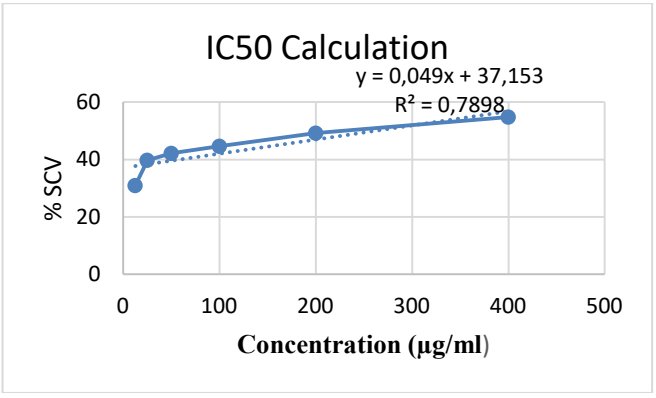

Fig. 2: % of inhibition at different concentration of the extracts of *C. guianensis*.