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Phytochemical and Antioxidant Investigation of *Barringtonia acutangula* (*L.*)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SMRH, RI and ND performed the whole research work. Author MA wrote the first draft of the paper. Authors MA and MSR read and approved the final manuscript.

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ABSTRACT

Aims: Different crude extracts of *Barringtonia acutangula* (L.) Gaertn were subjected to vigorous phytochemical and pharmacological investigations to validate the traditional use and to find out any other therapeutic activities.
Study Design: The present study was dedicated to investigate phytochemical and pharmacological properties of different parts of the plant extracted with various solvents (petroleum ether, methanol).
Place and Duration of Study: Department of Pharmacy, Jahangirnagar University, between June 2010 and July 2011.
Methodology: Phytochemical study was done through conducting preliminary phytochemical group tests of the crude extracts of leaf and bark. Antioxidant potential was evaluated using DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay, NO radical scavenging assay.
Results: In DPPH radical and NO radical scavenging methods, a dose dependent scavenging different phytoconstituents including carbohydrate, glycoside, saponin, steroids was found distributed in all parts of the plant where as alkaloid and tannin was found only in the leaf

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extractives. Highest DPPH radical scavenging was demonstrated by pet. Ether extract of bark with IC_{50} value of 8037.5 μg/ml whereas IC_{50} value of standard ascorbic acid was noted as 16.88 μg/ml. In case of NO radical scavenging method, highest NO radical scavenging was demonstrated by pet ether extract of bark with IC_{50} values of 295.27 μg/ml whereas IC_{50} value of standard ascorbic acid was 35.52 μg/ml.
Conclusion: The phytochemical screening revealed chemical constituents that form the foundation of their pharmacological activity. Leaf and Bark of the plant has good efficacy against many oxidation processes in our body.

Keywords: Phytoconstituents; scavenging; IC_{50} value; ascorbic acid.

1. INTRODUCTION

Barringtonia acutangula (L) *geartn* is a smooth tree growing to a height of 12 meters. Bark is dark brown, rough, 10 to 12 millimeters thick. Leaves are somewhat crowded at the end of the branches, oblong-obovate, 6 to 14 centimeters long, pointed at the ends, the young leaves finely toothed at the margins. Flowers are numerous, axillary and pendulous racemes, 10 to 40 centimeters long. Fruit is oblong-ovoid, 3 to 4 centimeters long, about 1.5 centimeters thick, bluntly quadrangular, pointed at the ends, and crowed by persistent calyx lobes.

The plant is widely distributed in tropical and subtropical area. It is found from Nepal to Southeast China, North Malaysia and Sri Lanka. It is also introduced to Philippines, West Borneo, Singapore, West Africa (Ivory Coast) and the Neotropics [1].

Barringtonia acutangula (L) geartn is distributed throughout the country. But it is mainly found in Chittagong, Chittagong Hill Tracts, Tangail, Sylhet and Dhaka [2]. *Barringtonia acutangula* (L) *geartn* the plant selected for the current study, has various ethnopharmacological use and a tuberous herb from (Family: Barringtoniaceae). The family of (Family: Barringtoniaceae) has some medicinally valuable species. *Barringtonia acutangula* is a neglected species on which very few scientific investigations have been conducted. There remains a possibility that the extract of the plant may possess some bioactive compounds. The work described in this article is dedicated to phytochemically and pharmacologically characterize different parts of the specified plant to: (1) Identify the groups of chemical constituents present in the plant parts (2) Rationalize the traditional uses of the selected plant (3) Explore the possible newer medicinal activities of the same plant those are not traditionally claimed.

The present study was designed to identify the groups of chemical constituents that are present in the crude extracts of *Barringtonia acutangula* (L) *gearth* as well as to observe the pharmacological activities of the extracts of the plant.

The present study was designed to identify the groups of chemical constituents that are present in the crude extracts of *Barringtonia acutangula* (L) *gearth* as well as to observe the pharmacological activities of extracts of the plant parts of leaves & bark with the solvent of Petroleum ether & Methanol. Phytochemical screening different qualitative tests to find out the presence of chemical constituents. Antioxidant activity tests to find out DPPH Scavenging Assay, Nitric Oxide Scavenging Assay.

2. MATERIALS

2.1 Preparation of the Plant Extracts

Collection: At first with the help of a comprehensive literature review *Barringtonia acutangula* from Barringtoniaceae family was selected for this investigation. The whole plants were collected from Noyarhat, Ashulia, Bangladesh.

Plant Material Preparation: The Plant was divided into two parts: “Aerial part (leaves)” and “bark”. The two parts of the plant were sun-dried separately and then, dried in a hot air oven (Size 1, Gallenkamp) at reduced temperature (not more than 50ºC) to make suitable for grinding purpose. After that plant parts were ground into coarse powders in the Department of Pharmacy using high capacity grinding mill which were then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

Extraction procedure: The powdered plant materials (200 g each of aerial part and the remaining part) were used for extraction by Soxhlet apparatus at elevated temperature (65°C) using Petroleum ether, Methanol consecutively (500 ml of each solvent). After each extraction the plant material was dried and used again for the next extraction. Extraction was considered to be complete when the plant materials become exhausted of their constituents that were confirmed from cycles of colorless liquid siphoning in the Soxhlet apparatus. After methanol extract was complete the plant materials were dried and soaked into distilled water (1L). The plant materials were kept in water for 7 days in sealed container accompanying occasional shaking and string. All four extracts of each part were filtered individually through fresh cotton bed. The filtrates obtained were dried at temperature of 40±2°C to have gummy concentrate of the crude extracts. Each extract was kept in suitable container with proper labeling and stored in cold and dry place.

3. METHODS

3.1 Phytochemical

The crude plant extracts were subjected to different qualitative tests to find out the presence of chemical constituents. These were identified by characteristic color changes using standard procedure. Test for glucosides (Glycosides with glucose as the glycone) Liebermann-Burchard's Test Frothing test (saponin), Lead acetate test (Tanin) General laboratory tests (Alkaloid).

3.2 Antioxidant

DPPH is a reactive free radical that acts as an electron acceptor (oxidant/ oxidizing agent) and causes oxidation other substances. On the other hand, antioxidants act as electron donors (reductant/ reducing agent). Antioxidants neutralize DPPH by being oxidized themselves. DPPH is found as dark-colored crystalline powder composed of stable free-radical molecules and forms deep violet color in solution. The scavenging of DPPH free radical (neutralization) is indicated by the deep violet color being turned into pale yellow or colorless.

3.2.1 DPPH solution

0.004 g (4 mg) DPPH is dissolved in 100 ml of solvent to make 0.004% solution.

3.2.2 Preparation of standard/ Extract solution

0.025 g ascorbic acid or extract was taken and dissolved into 5 ml of Absolute ethanol. The concentration of the solution was 5 mg/ml of ascorbic acid/ extact. The experimental concentrations from the stock solution were prepared by the following manner:

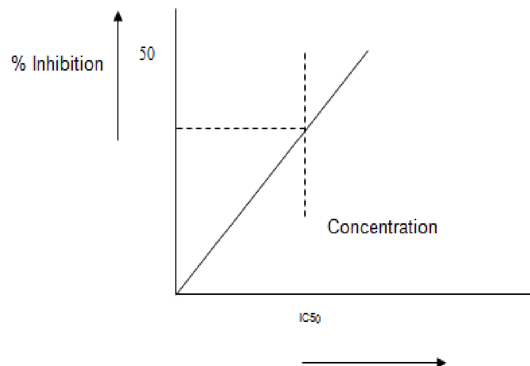
3.2.3 Procedure

The stock solution is serially diluted to achieve the concentrations of 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, Each test tube contains 1ml of each concentration and is properly marked, 2 ml of 0.004% DPPH solution in the solvent is added to each test tube to make the final volume 3 ml (caution: DPPH is light sensitive, so making the solution and adding it to the test tubes should be done in minimum light exposure), Incubate the mixture in room temperature for 30 minutes in a dark place, Then the absorbance is measured at 517 nm against dilute extract solution in the solvent.

3.3 Calculation

% Inhibition = $(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ Control}) \times 100$

IC_{50} is the concentration at which 50% of the total DPPH free radical is scavenged/ neutralized and can be determined by linear regression method from plotting % inhibition against corresponding concentration.



3.3.1 Nitric oxide scavenging capacity assay

The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO•. Under aerobic conditions, NO^- reacts with oxygen to produce

stable products (nitrate and nitrite). The quantities of which can be determined using Griess reagent. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride was immediately read at 550 nm.

3.4 Preparation of 5 mM Solution of Sodium Nitroprusside

0.150 gm of Sodium nitroprusside was taken and placed into a 100 ml of a volumetric flask. The volume was adjusted by distilled water.

3.5 Preparation of 0.5% Solution of Griess Reagent

0.5 gm of griess reagent was taken and placed into a 100 ml of a volumetric flask. The volume was adjusted by distilled water.

3.6 Preparation of Standard/ Extract Solution

The stock solution was prepared by taking 0.025 g ascorbic acid/ plant extract and dissolved into 5 ml of ethanol whose concentration was 5 µg/µl. The experimental concentrations from the stock solution were prepared by the following manner:

3.6.1 Experimental procedure

4.0 ml of each plant extracts or standard of different concentration solutions were taken in different test tubes and 1.0 ml of Sodium nitroprusside, (5 mM) solution was added into the test tubes. Add the test tubes were incubated for 2 hours at 30ºC to complete the reaction. 2.0 ml solution was withdrawn from the mixture and mix with 1.2 ml of griess reagent and the absorbances of the solutions were measured at 550 nm using a spectrophotometer against blank. A typical blank solution contained the distilled water.

The percentage (%) inhibition activity was calculated from the following equation.

$$\{(A_0 - A_1)/A_0\} \times 100$$

Where, A_0 is the absorbance of the Control and

A_1 is the absorbance of the extract or standard.

IC_{50} was calculated by linear regression method.

4. RESULTS

Preliminary phytochemical screening of the crude extracts of different parts of *Barringtonia acutangula* (L) gaertn revealed the presence of different kind of chemical groups that are summarized in table.

4.1 DPPH Radical Scavenging Assay

4.1.1 Result

When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorised, which can be quantitatively measured from the changes in absorbance at 517 nm. The IC_{50} values of the different extracts of *B. acutangula are* presented in the Table 1. % scavenging of DPPH radical was found to rise with increasing concentration of the different extracts with highest scavenging displayed by Petroleum ether extract of bark of the plant (Fig. 1). DPPH radical scavenging capacity of the extracts was found to decrease in the following order: TBP> TLM>TBM>TLP.

Extract	Alkaloid			Steroids (Liebermann -Burchard's test)	Tannin (Lead acetate test)	Flavonoids	Saponin (Frothing test)
	Mayer's test	Hager's Test	Dragendorff's Test				
TLM	++	++	++	+	++	+	+
TLP	++	++	++	+	++	++	+
TBM	-	-	-	++	-	+	+
TBP	-	-	-	-	-	++	+

[TLM= Methanol extract of Leaf, TLP= Pet. ether extract of Leaf, TBM= Methanol extract of Bark, TBP= Pet. ether extract of Bark]
[+ = Presence, ++ = Strong presence, - = Absence]

4.2 Nitric Oxide (NO) Radical Scavenging Assay

4.2.1 Result

Scavenging of NO was determined by the decrease in its absorbance at 550 nm, induced by antioxidants. All the extractives showed a dose dependent scavenging of NO similar to the reference antioxidant ascorbic acid (Fig. 2). However, maximum scavenging of NO was found with pet ether extract of bark of *B. acutangula with* an IC_{50} value of 64.02 µg/ml; the result is comparable to ascorbic acid which was taken as the standard (35.52 µg/ml). NO scavenging activity decreased in the following order: TBP >TLP> TBM > TLM (Table 2).

Table 1. IC_{50} values of the different extracts in DPPH scavenging assay

Sample/Standard	IC_{50} (µg/ml)
TLM	1459.125±1.724
TLP	687.51±1.021
TBM	1224.15±0.994
TBP	8037.5±1.574
Ascorbic acid	16.88±1.181

Table 2. IC_{50} values of the different extracts in Nitric oxide radical scavenging assay

Extract/Stanard	IC_{50} (µg/ml)
Ascorbic acid	35.52716±1.040
TLM	140.5556±2.286
TLP	180.5882±1.458
TBP	295.2795±1.040
TBM	164.2918±0.072

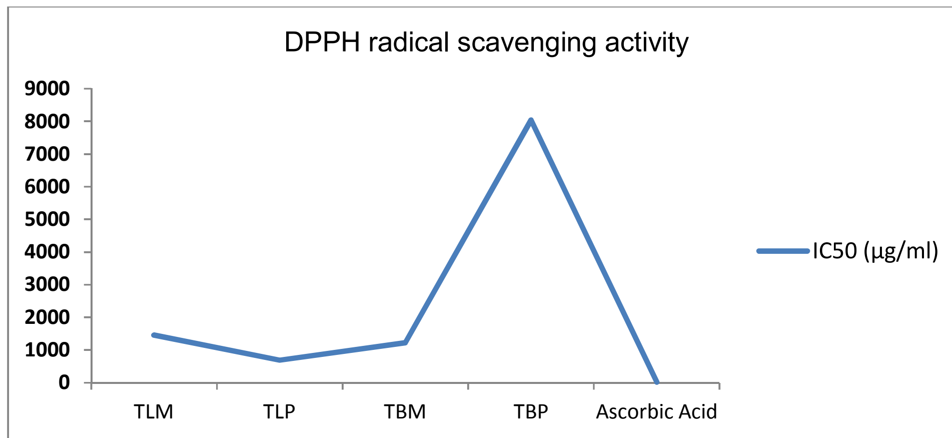


Fig. 1. DPPH radical scavenging activity of the different extracts of *B. acutangula*

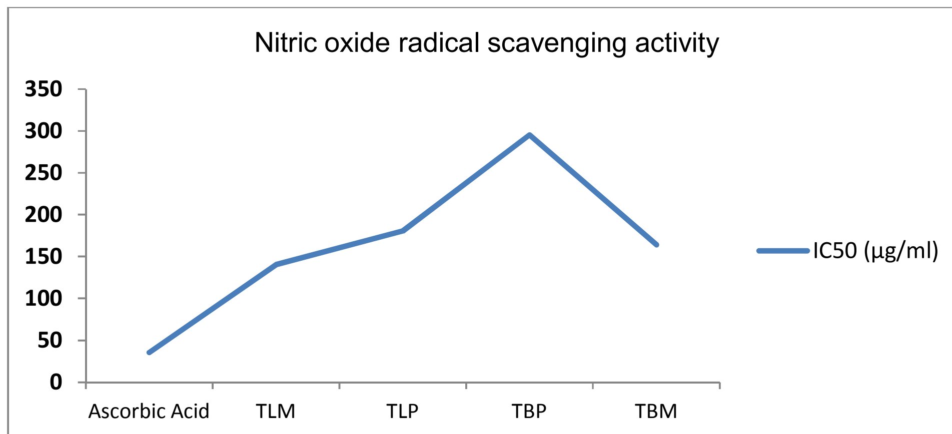


Fig. 2. Nitric oxide radical scavenging activity of the different extracts of *B. acutangula*

5. DISCUSSION

Different crude extracts of *B. acutangula* have been shown to possess phytoconstituents including carbohydrates (monosaccharides, reducing and mixed-reducing sugars), alkaloid, glycosides, steroids, tannins and saponin. flavonoid was detected in the pet ether extract both of leaf and bark. These phytoconstituents present in the extracts may account for their various pharmacological activities shown in other investigations [2]. In DPPH radical scavenging assays, the crude extracts of *B. acutangula showed* dose dependent scavenging of DPPH radicals in a way similar to that of the reference antioxidant ascorbic acid (Fig. 1). DPPH radical scavenging is a popular and reliable method for screening the free radical scavenging activity of compounds or antioxidant capacity of plant extracts [3,4,5,6]. The DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants [7]. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. So extracts of *B. acutangula has* the ability to donate electron among which Pet. ether extract of bark showed maximum potency (Table 2).

Nitric oxide (NO) is a physiologically important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various biochemical processes. Excess generation and accumulation of nitric oxide are implicated in the cytotoxic effects observed in various disorders like AIDS, cancer, Alzheimer’s, and arthritis [8]. Overproduction of NO can mediate toxic effects such as DNA fragmentation, cell damage and neuronal cell death [9]. NO does not interact with the bioorganic macromolecules such as the DNA or proteins directly. However, in the aerobic conditions, the NO molecule is very unstable and reacts with the oxygen to produce, intermediates such as NO_2, N2O4, N_3O_4; the stable products nitrate and nitrite [10] and peroxynitrite when reacts with superoxide [11]. These products progenitors are highly genotoxic, the deamination of guanine, cytosine and adenine is mediated primarily by the N_2O_3. In addition to the formation of nitrosoamines and deamination of the DNA bases, recent studies indicate that the NO may also act by affecting the enzymatic activities of several thiol rich DNA repair proteins like DNA alkyl transferase, formamopyrimidine-DNA glycosalase and the DNA ligase that play a critical role in the maintenance of the genetic integrity [12]. There is now increasing evidence to suggest that NO and its derivatives produced by the activated phagocytes may have a genotoxic effect and may contribute in the multistage carcinogenesis process [12].

Moreover, both nitric oxide and superoxide anion cause ischemic renal injury. The toxicity and damage caused by NO^- and O_2^- is multiplied as they react to produce reactive peroxynitrite ($ONOO^-$), which leads to serious toxic reactions with biomolecules as mentioned above [13,11,14]. The scavenging of the NO generated from sodium nitroprusside *in vitro* indicates the possibility of preventing the peroxynitrite formation in the cell *in vivo* [15]. Reducing the nitric oxide generation in the digestive tract was reported to be effective in preventing the reactions of nitrate with amines and amides to form carcinogenic nitrosamines and nitrosamides [16,15]. Different extracts of *B. acutangula was* able to scavenge NO produced from nitroprusside at a considerable level. Several reports have pointed out the role of flavonoids and phenolic compounds (the presence of which has been ascertained in the present study) in NO scavenging [17,18,19]. Based on these we speculate that nitric oxide scavenging activity of *B. acutangula* may have great relevance in the prevention and control of disorders where NO is thought to play a key role.

6. CONCLUSION

All the conducted experiments in the present study are based on crude extract and are considered to be preliminary and more sophisticate research is necessary to reach a concrete conclusion about the findings of the present study. Elaborate phytochemical investigation must be arranged that might lead to isolation and characterization of chemical constituents present in the crude extracts. Antioxidant testing methods undertaken were all *in vitro*. *In vivo* antioxidant testing methods like TBARS (Thiobarbituric acid reactive substance), erythrocyte membrane stabilization assay, measurement of NO and antioxidant enzyme levels in brain, heart and liver samples may confirm the antioxidant activity of the plant parts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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