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Research Article

Preliminary antioxidant and thrombolytic potential of leaves of *Axonopus compressus*

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Abstract:

The goal of this research was to find out the phytochemical constituents as well as the antioxidant and thrombolytic potential of Axonopus compressus, a traditional medicinal herb. Qualitative analysis was used to screen the phytochemical content of the plant's ethanolic extract. The total phenolic content was measured using Folin-reagent Ciocalteu's reagent and total flavonoid content was determined using the AlCl3 colorimetric method to measure antioxidant activity. A clot lysis experiment was used to test the extract's thrombolytic effects. In phytochemical screening, carbohydrates, saponin, tannin, terpenoid, alkaloids, and flavonoids were found. The extract contained total phenolic compounds as 25.74 mg Gallic acid equivalent (GAE)/g of dried extract & total flavonoid compounds as 21.3 mg Quercetin equivalent (QE)/g dried extract. The extract exerted 2.74%, 13% & 15.26% lysis of the blood clot at a concentration of 1 mg/ml, 2 mg/ml and 3 mg/ml respectively in the thrombolytic activity test while 65% lysis was obtained for positive control (streptokinase) at (100000 I.U/ml) and 3.03% for negative control. Axonopus compressus possesses some phytochemicals, significant antioxidant activity and showed a dose-dependent increase in clot lysis in an in-vitro thrombolytic assay that could be promising to study its potential with high-performance research work.

Keywords: *Axonopus compressus*, Antioxidant, Thrombolytic, Streptokinase

1. Introduction:

Nature has bestowed on us an abundance of amazing medicinal herbs (Krishnaiah *et al.* 2009). In the last few decades, plant phytochemicals and herbal items have gained appeal as alternative therapy methods (Hostettmann, 1998 & Pattanayak *et al.* 2009). Medicinal plants have historically been a rich source of novel drugs, and many of the drugs that are currently in use were discovered in plants or produced based on their chemical composition as a lead component (Ajaiyeoba *et al.* 2006).

Plant-based products that are eco-friendly and bio-friendly are extremely beneficial in the prevention and treatment of a variety of human ailments (Afolabi *et al.*2013). A. compressus can be found growing wild along roadsides, in gardens, waste areas, and plantations (Manidool, 1992). Carpet grass (with the symbol AXCO), a perennial, terrestrial, stem compressed grass with bristly or hairy nodes, is widely used in the Southern part of

Nigeria to treat diabetes mellitus (Dweck *et al.* 2002). (Edeoga *et al.* 2005). This herb, on the other hand, is thought to be non-toxic (Khandare *et al.* 2012). A paste made from the leaves is applied to shattered bones as a bandage, while juice extract is used to reduce breast swelling and malaria fever. The goal of this study was to identify and compare the phytochemical contents of this plant to provide relevant information for the successful use of these plants as well as to demonstrate the scientific foundation for their use in traditional medicine. As no thrombolytic activity test was done before on this plant, so this was taken for this test in this research.

Fig. 1: ***Axonopus compressus***

2. Materials and methods:

2.1 Collection of plant material:

Axonopus compressus was obtained from Enayethpur, Sirajgonj. 26 November 2019. The Department of Pharmacy at Khwaja Yunus Ali University then assisted in the accurate identification of the plant sample.

The plant material was dried in the sun for a few days. The dried plant was subsequently pulverized into a coarse powder in the Phytochemical Research Laboratory, Department of Pharmacy, Khwaja Yunus Ali University, using a high-capacity grinding machine.

2.2 Plant Extract preparations:

A.compressus plants were shade dried and roughly powdered before being exposed to ethanol solvent extraction in a Soxhlet extractor. To obtain ethanol extract, the extract was dried under a fan (Chowdhury *et al.* 2017).

9 gm crude extract was obtained from 155gm of dried powder material

So, yield = $\frac{9\,gm}{155gm}$ ×100%

=5.80 % of dried plant material.

2.3 Phytochemical Screening (Qualitative analysis) :

Qualitative analysis was carried out on ethanolic extract of A. compressus to determine the presence of phytochemical constituents Alkaloids, Flavonoids, Saponins, Gums, Tannins, Carbohydrate, Terpenoids (Prabhavathi, 2016)

2.4 Total phenolic content determination:

Folin-Ciocalteu reaction was used to assess the total quantity of phenolic content in A. compressus crude extracts. In a nutshell, 0.5 mL of stock solution extracts were combined with 7.5 mL distilled water and 1 ml Folin-Ciocalteu reagent. Then 10 mL of a 7% Na2CO3 solution was added, and the volume was adjusted with distilled water to make a final volume of 25 ml. After that, they were left for 30 minutes at room temperature.

To measure the absorbance, a UV-Vis spectrophotometer was used. The wavelength was fixed at 750 nm against a blank sample. The experiment was done in triplicate and the results were represented as gallic acid equivalents (percent GAE) of dry weight (Nisa K, 2017).

2.5 Total flavonoid content determination:

A colorimetric test was used to determine the total flavonoid content of the A. compressus preparations. By combining a 5 ml sample of each extract (stock solution, 1 mg/ml) with AlCl3 reagent (133 mg

crystalline Aluminum Chloride (AlCl3) and 400 mg crystalline Sodium Acetate diluted in 100 ml distilled water). The solution was vortexed and left to sit at room temperature for 40 minutes to allow for reaction. The absorbance was measured at 510 nm after 40 minutes. The total flavonoid was calculated as a percentage of quercetin equivalents (% QE) dry weight. The experiment was carried out in triplicate (Jiao & Wang, 2000).

2.6 Assay of thrombolytic activity:

Instruments & Chemicals:

The chemical used for this work was ethanol (Analytical grade), the test required Eppendorf tubes and Syringe and the standard drug Streptokinase (15, 00,000 I.U) was purchased from a local pharmacy which was of - Beacon pharmaceutical Ltd, Bangladesh. The laboratory of the Department of Pharmacy provided additional required chemicals (analytical grade) for this study.

Collection of blood samples:

From healthy human volunteers (n = 08 both male and female; age=20-25years), 3ml of blood was collected who had never used an oral contraceptive or anticoagulant for two weeks before this study.

Negative Control and Positive Control (Standard) preparation:

Streptokinase (15, 00,000 I.U) was purchased from Beacon Pharmaceutical Ltd in Bangladesh as a standard. The streptokinase vial was prepared by adding 5mL normal saline into it, appropriately mixed, and labeled as a positive control. In the thrombolytic assay, distilled water was employed as a negative control.

Extract preparation for thrombolytic Test:

The extract was prepared at a concentration of 1 mg/ml. Similarly, 20 mg and 30 mg of crude extract were suspended in 10 mL distilled water and vortexes thoroughly to prepare 2 and 3 mg/ml concentrations respectively. The suspension was then maintained overnight before being decanted and filtered using filter paper to remove the soluble supernatant. The solution was then suitable for in-vitro clot lysis activity testing.

Assay of in-vitro thrombolytic action:

An in-vitro clot lysis model was used to test Axonopus compressus extract for thrombolytic activity described by Prasad et al. 2006.

were weighed in empty condition. The plasma fluid or serum was entirely extracted by the syringe, devoid of disrupting the clot, From the obtained blood sample, 500µl of fresh blood was shifted to reweighed Eppendorf tubes (500µl/tube) and incubated at 37°C for 40-50 minutes for clot creation. The tube containing the clot from the extracted serum was weighed once more.

Clot weight WC = Weight tube with clot– Weight of blank tube

Eppendorf tubes were appropriately labeled and in the labeled tubes, 100 µl of ethanolic extract solution, 100 µl of streptokinase (For this, Streptokinase (Plasmin activator) was used as a reference to test the extract. Eppendorf tubes. 1500000 I.U/ml) as the positive control, and 100 µl of distilled water as negative control were added. After a 90-minute incubation period at 37°C, check for clot lysis.

The discharged fluid was removed from each tube and weighed to determine the weight difference after the clot was disrupted. As demonstrated below, the difference in weight acquired before and after clot lysis was expressed as a percentage of clot lysis.

% of clot lysis = (Weight of released clot RC /Clot weight WC) × 100

3. Results:

3.1 screening of phytochemicals:

Table 1: Chemical groups present in the Ethanolic extract of *Axonopus compressus*

Name of the group	Ethanol Extract
Alkaloids	+
Flavonoids	+
Saponins	+
Gums	-
Tanins	+
Carbohydrate	+
Terpenoids	+

Here, (+) = Presence, (-) = Absence

3.2 Quantitative determination of phytochemical contents

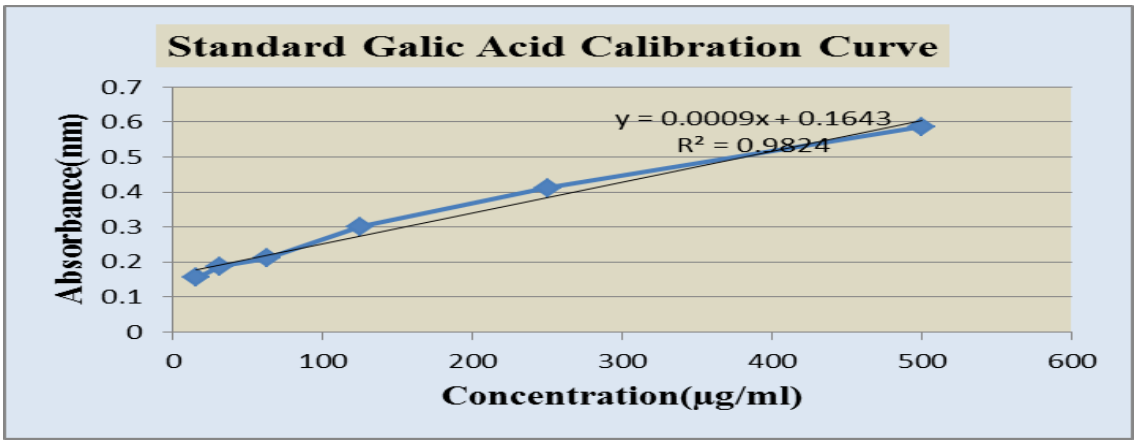

Fig. 2: Concentration Vs Absorbance of Galic acid for total phenolic content determination at 750 nm.

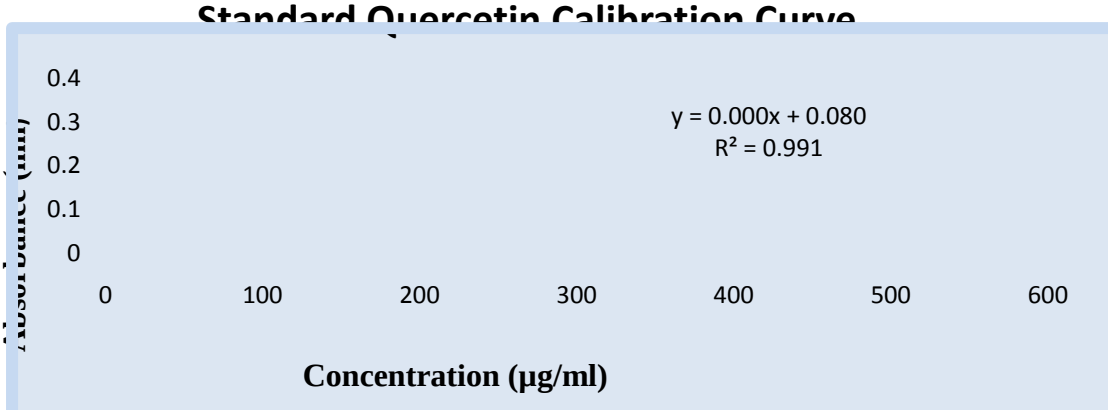

Fig. 3: Concentration Vs Absorbance of Quercetin for total flavonoid content determination at 510 nm.

The extract contains a considerable amount of total phenols and total flavonoids, with quantities of 25.74 mg Gallic acid per gram of dried extract and 21.30 mg Quercetin per gram of dried extract, respectively.

3.3 Thrombolytic activity test:

Table 3: Data presentation of clot lysis of *Axonopus compressus* extract in ethanol.

Sl .No	Concentration (µg/ml)	Weight of tube (gm)	Weight of tube with clot (gm)	Weight of Clot (gm)	Weight of tube clot after lysis (gm)	Weight of lysis	% of lysis
1	Control	0.8452	1.1092	0.2640	1.1012	0.0080	3.03
2.	1000	0.8333±0.0176	1.1316±0.0895	0.2983±0.0727	1.1237±0.0889	0.0079±0.0026	2.745±1.086
3.	2000	0.8447±0.0058	1.1671±0.0718	0.3224±0.0701	1.1256±0.0646	0.0415±0.0098	13±2.0224
4.	3000	0.8419±0.0217	1.1269±0.0669	0.2850±0.0715	1.0837±0.0630	0.0433±0.0116	15.65±4.724
5.	Standard (100000 I.U/ml)	0.7090	0.9945	0.2855	0.8089	0.1856	65

4. Discussion:

The study examined the prevalence of secondary metabolites such as alkaloids and flavonoids, as well as saponin, tannin, and terpenoids. These chemical components may be responsible for antioxidant activity, which may be linked to the traditional use's curative potential for a variety of diseases (Ibeh *et al*. 2013).

In quantitative measurement, A. Compressus extract exhibited a significant content of phenol and flavonoid compounds which play an important role in the plant's therapeutic effectiveness. It is also known that the secondary metabolites possessed by this plant (alkaloid, flavonoid) can reduce blood sugar level by inhibiting alpha-glucosidase activity in the blood (Geng *et.al.* 2007) which supports its traditional use in diabetes. Much research work has been carried out to discover thrombolytic drugs. Finding a drug with fewer side effects and more target specificity is desired in any naturally obtained thrombolytic agent. In this context, herbal drugs can be a promising source of this concern. (Hussain *et al*. 2014).

In this thrombolysis study, we explored a plant that has traditionally been used to treat a variety of ailments to see if it has any thrombolytic qualities. The majority of thrombolytic medicines work by activating the plasminogen enzyme, which dissolves the cross-linked fibrin mesh and allows blood to flow again through clogged blood arteries. Dissolution of clots is thus beneficial in the treatment of clot-related illnesses such as

myocardial infarction, deep vein thrombosis, thromboembolic strokes, and pulmonary embolism, as it allows a clogged artery to be cleared, avoiding stable tissue damage (Prasad *et al*. 2007).

A. Compressus' thrombolytic activity is a significant finding that could have implications for cardiovascular health, particularly in atherothrombotic individuals. This study is only preliminary work and further research should be carried out to finalize its potential and to identify the compound responsible for this activity and its exact mechanism of action.

5. Conclusion:

From the results of the analysis, it is important to note that the therapeutic benefit of this plant is derived from its bioactive phytochemical contents, which, when used together with nutrients and fibers, develop a unified component of the human defense mechanisms against diseases and stress. Phytochemical results showed that *Axonopus compressus* is rich in phenolic constituents and demonstrated good antioxidant activity. Because of its modest thrombolytic activity, the extract of Axonopus Compressus can be used to develop anti-thrombotic agents that will have a substantial impact on cardiovascular health. More research is required to isolate and define the chemicals is in charge of thrombolytic activity, as well as to investigate the activity in greater depth for more precise and accurate results.

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Authors contribution of the research:

Shahria N designed and directed the research work. Under the supervision of Shahria N , Basak RR, and Sarkar S carried out the experiment and analyzed the data. Shahria N wrote the manuscript with the support of Mahmud I. Ahmed FRS contributed to the discussion. All authors discussed the results and contributed to the final manuscript.

Conflict of interest:

The authors have no conflict of interest.

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