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Study of thrombolytic and neuropharmacological activities of crude extract of *Achyranthes aspera* leaves and *Piper nigrum* fruits

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Abstract
This study aimed to evaluate the *in vitro* thrombolytic potential and *in-vivo* neuropharmacological activities of methanol extract of *Achyranthes aspera* leaves (MEAAL) and *Piper nigrum* fruits (MEPNF). *The in-vitro* thrombolytic activity was determined by the clot lysis method, and in vivo, neuropharmacological activity (antidepressant) was determined by the tail suspension test. The *in-vitro* thrombolytic activity study revealed that methanol extract of *Achyranthes aspera* leaves (MEAAL) *Piper nigrum* fruits (MEPNF), and combination of these two plant extract showed 29.73±1.21%, 35.4±0.39%, and 24.58±1.37% clot lysis respectively. In tail suspension test the immobile times for methanol extract of *Achyranthes aspera* leaves were observed to be 114.33±4.66 seconds and 97.66±6.06 seconds for dose 200 mg/kg and 400 mg/kg respectively when compared with control at 204.33±1.76seconds of immobile time. And the immobile times for methanol extract of *Piper nigrum* fruits were observed to be 118.66±6.11 seconds and 90.66±6.33seconds for dose 200 mg/kg and 400 mg/kg, respectively when compared with control at 204.33±1.76 seconds of immobile time. The outcome demonstrates that methanol extract of *Achyranthes aspera* leaves and *Piper nigrum* fruits have moderate thrombolytic activity, it also shows good antidepressant activity.

Keywords: *Achyranthes aspera*, *Piper nigrum*, thrombolytic, neuropharmacological activity

1. Introduction
Medicinal plants are a vital segment of non-timber forest products that are traditionally used in healthcare and source of livelihood all over the world. It has global recognition in health heed apart from sourcing monetary benefits to the local people and forest users. It has a rich history and traditional cultures that have exhibited the primary health care of the local communities based on medicinal plants and related knowledge (Caniago and Stephen, 1998) [4]. Medicinal plants may be termed as 'a group of plants that possess few special properties or virtues that qualify them as articles of therapeutic and drug agents, and are used for medicinal purposes' (Sharmin, 2004) [20]. According to FAO 2007, an estimated 121 505 tonnes of medicinal plants and aromatic products extracted globally, out of which 90 181 tones are from Asia, and according to (Subrat 2002). Among the 422 000 plant species documented worldwide 12.5 percent are reported to have medicinal value (Rasool Hassan, 2012) [19].

Atherothrombosis is the hardening and narrowing (medically known as 'stenosis') of the body's arteries. It is caused by a slow and progressive build-up of plaque under the lining of the arterial wall, which may gradually narrow the artery and restrict blood flow to the target organ. Thrombolysis, also known as thrombolytic therapy, is a treatment to dissolve dangerous clots in blood vessels, improve blood flow, and prevent damage to tissues and organs. It works by stimulating fibrinolysis by plasmin through an infusion of analogs of tissue Plasminogen Activator (tPA), the protein that normally activates plasmin. Thrombolytic agents that include tissue Plasminogen Activator (t PA), Urokinase (UK), Streptokinase (SK), etc., are used all over the world for the treatment of atherothrombotic diseases such as myocardial or cerebral infarction, at times leading to death (Mucklow, 1995) [13].

Thrombus formation inside the blood vessels obstructs blood flow through the circulatory system leading hypertension, stroke to the heart, anoxia, and so on. Thrombolytic drugs are widely used for the management of cerebral venous sinus thrombosis patients, but they have certain limitations. Medicinal plants and their components possessing antithrombotic activity have been reported before. However, plants that could be used for thrombolysis has not been reported so far (Hussain *et al*., 2014) [8].

Depression is a mood disorder characterized by persistently low mood and a feeling of sadness and loss of interest. A depressive disorder is a prevalent mental issue, which influences 21% of the total population. As indicated by the World Health Organization report, depression is the subsequent driving reason worldwide of disability-adjusted life years and the leading cause of years lived with disability in all ages. Each medication used to treat this issue has a success rate of about 60%. Likewise, most therapies require several weeks of treatment before the progress of signs and indications is watched, and there are various symptoms brought about by antidepressants (Kumar *et al*., 2014) [10]. Studies have demonstrated that depressive disorders lead to critical brokenness, disability, and low quality of life in sufferers and represent a significant burden on caregivers. Antidepressants are medications utilized for the treatment of depression and different conditions, including dysthymia, anxiety disorders, obsessive-compulsive disorder, eating

disorders, chronic pain, neuropathic pain and, in some cases, dysmenorrhea, snoring, migraine, attention-deficit hyperactivity disorder (ADHD), addiction, dependence, and sleep disorders. They can be utilized alone or in combination with different drugs, yet just when prescribed. The most significant classes of antidepressants are the particular serotonin reuptake inhibitors ,serotonin–norepinephrine reuptake inhibitors, tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), reversible monoamine oxidase An inhibitors (rMAO-An inhibitors), etc. (Preskorn *et al*., 2012) [17]. Depression is a regular mental condition generally experienced, yet to date, the adequacy of antidepressant medications is exceptionally constrained. In this way, there is a requirement for more up to date, better tolerated, and more efficacious useful treatment. In this manner, herbal therapies treatments ought to be considered as alternative/reciprocal drug. (Barua *et al*., 2009) [3]. *Achyranthesaspera* is a species of plant in the family Amaranthaceae. It is distributed throughout the tropical world (Committee, 1993) [5]. It can be found in many places growing as an introduced species and a common weed. It is an invasive species in some areas, including many Pacific Islands environments (Daehler *et al*., 2004) [6]. The herb is administered in India in cases of dropsy. The seeds are given in hydrophobia, and in cases of snake-bites, as well as in ophthalmia and cutaneous diseases. The flowering spikes rubbed with a little sugar, are made into pills, and given internally to people bitten by mad dogs. The leaves, taken fresh and reduced to a pulp, are considered a good remedy when applied externally to the bites of scorpions. The ashes of the plant yield a considerable quantity of potash, which is used in washing clothes. The flowering spike has a reputation in India (Oude) of being a safeguard against scorpions, which is believed to paralyze. (Maiden, 1889) [12]

Piper nigrum is a flowering vine in the family Piperaceae, cultivated for its fruit, known as a peppercorn, which is usually dried and used as a spice and seasoning. It is valued for its characteristic sharp and stinging qualities attributed to the alkaloid piperine. While it is used primarily as a food adjunct, black pepper is also used as a food preservative and as an essential component in traditional medicines in India and China. Since the discovery of black pepper's active ingredient, piperine, the use of black pepper has caught the interest of modern medical researchers(Srinivasan, 2009).

The goal of this study was to assess the impact of thrombolytic and neuropharmacological activities of methanolic extract of *Achyranthes aspera* leaves and *Piper nigrum* fruits.

2. Materials and method

2.1 Plant collection and identification

Leafs of *Achyranthes aspera* were collected from Kaptai, Rangamati, Chittagong, fruits of *Piper nigrum* were collected from the local market of Bangladesh, in April 2019. The plant was authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, and Bangladesh.

2.2 Preparation of Extract

Normally the plant materials are gathered in crisp condition. Then these are cut into little pieces if important to make it appropriate for granulating reason. The leaves and fruits were dried for a time of 15 days under shade and ground. The materials are crushed into a coarse powder with the assistance of a processor and soaked for seven days with 2–3 days interval in 2.0 L of methanol at room temperature. Filtered through a cotton plug followed by Whitman channel paper number. The solvent was evaporated with water bath to yield viscous mass. The viscous mass was kept at room temperature under a ceiling fan to get a dried extract. The prepared extract kept for further pharmacological screening

2.3 Streptokinase (SK) Solution Preparation

To the commercially available lyophilized SK vial (Polamin Werk GmbH, Herdecke, Germany) of 15, 00,000 I.U., 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for in vitro thrombolysis. (Prasad *et al*., 2007) [16]

2.4 Specimen for Thrombolytic Test

Whole blood (5 ml) was drawn from healthy human volunteers (n = 10) without a history of oral contraceptive or anticoagulant therapy (using a protocol approved by the Ethics Committee of Pharmacy Department, International Islamic University Chittagong (approval no. ECPDIIUC2013/03) 500 µl of blood was transferred to each of the ten previously weighed Eppendorf tubes to form clots. (Prasad *et al*., 2007) [16]

2.5 Test Procedure for Thrombolytic Test

Experiments for clot lysis were carried as reported earlier(Prasad *et al*., 2007) [16]. Venous blood drawn from healthy volunteers was transferred in different pre-weighed sterile Eppendorf tubes (500 µl/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed). Each tube has a clot was again weighed to determine the clot weight (Clot weight = weight of clot containing tube – the weight of tube alone). Each Eppendorf tube containing a clot was properly labeled, and 100 µl of plant extract was added to the tubes. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed, and tubes were again weighed to observe the difference in weight after clot disruption. The difference obtained in weight taken before and after clot lysis was expressed as the percentage of clot lysis. Streptokinase and water were used as positive and negative (nonthrombolytic) control respectively. The experiment was repeated several times with the blood samples of different volunteers.

% clot lysis = (Weight of the lysis clot / Weight of clot before lysis) × 10

2.6 Tail suspension test (TST)

Tail suspension test regularly utilized conduct model for screening antidepressant- like activity in mice was first given by (Steru *et al*., 1985) [22]. Treatment was given 60 min preceding investigation as portrayed by study structure. Mice were suspended on the edge of the table, 50 cm over the floor, with the assistance of sticky tape, set roughly 1 cm from the tip of the tail. The absolute duration of immobility incited by tail suspension was recorded during a 6 min of

the 10 min time frame. Animals were viewed as immobile when it did not show any movement of the body, hanged passively and completely motionless. (Ashok Kumar *et al*., 2014) [1]

2.7 Statistical Analysis

The significance between % clot lysis by herbal extract using weight difference was tested by the paired test analysis. Data are expressed as mean ± standard deviation

3. Results

3.1 Result of thrombolytic test:

The *in-vitro* thrombolytic activity was determined by the clot lysis method. The *in-vitro* thrombolytic activity study revealed that methanol extract of *Achyranthes aspera* leaves (MEAAL) *Piper nigrum* fruits (MEPNF), and combination of these two plant extract showed 29.73±1.21%, 35.4±0.39%, and 24.58±1.37% clot lysis respectively which shown in fig-1.

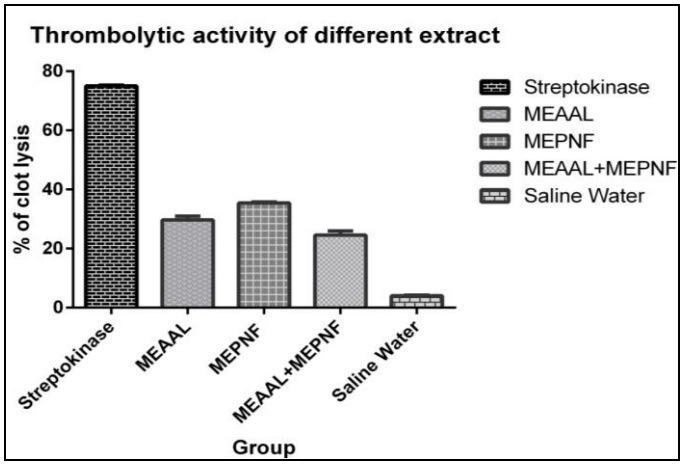

Fig 1: Thrombolytic test of MEAAL, MEPNF and MEAAL+MEPNF extract

3.2 Result of tail suspension test of MEAAL

The possible antidepressant impact of methanol extract of *Achyranthes aspera* leaves after oral administration was considered in the tail suspension test. In this test, animals treated with two doses of MEAAL at 200 mg/kg and 400 mg/kg, PO demonstrated lessening in their immobile times. The immobile times were observed to be 114.33±4.66 seconds and 97.66±6.06 seconds for dose 200 mg/kg and 400 mg/kg, respectively, when compared with control at 204.33±1.76seconds of immobile time. Correspondingly, animals treated with Imipramine 25mg/kg demonstrated a critical abatement in the immobile time of 85.33±1.76 seconds.

Table 1: Tail suspension test of MEAAL

Groups	Treatments	Dose, Route	Immobile Time (Sec)
Control	1% tween 80 in water	10 mL/kg, p.o	204.33±1.76
Standard	Imipramine	10 mg/kg, i.p	85.33±1.76
Test	MEAAL	200 mg/kg, p.o	114.33±4.66
	MEAAL	400 mg/kg, p.o	97.66±6.06

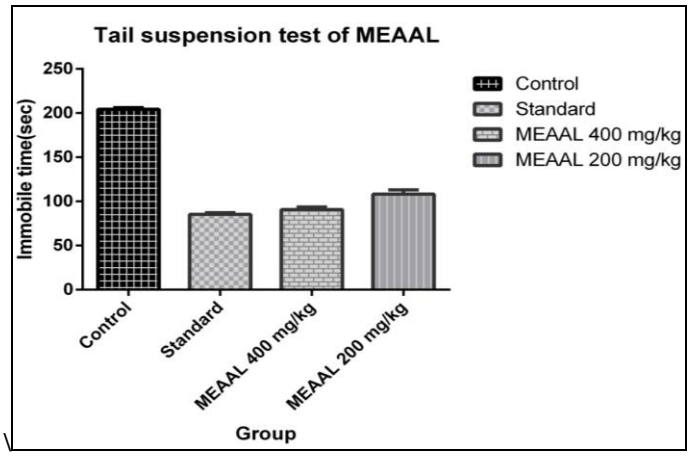

Fig 2: Tail suspension test of MEAAL

3.2 Result of tail suspension test of MEPNF

The possible antidepressant impact of methanol extract of *Piper nigrum* fruits after oral administration was considered in the tail suspension test. In this test, animals treated with two doses of MEPNF at 200 mg/kg and 400 mg/kg, PO demonstrated lessening in their immobile times. The immobile times were observed to be 118.66±6.11 seconds and 90.66±6.33 seconds for dose 200 mg/kg and 400 mg/kg, respectively, when compared with control at 204.33±1.76seconds of immobile time. Correspondingly, animals treated with Imipramine 25mg/kg demonstrated a critical abatement in the immobile time of 85.33±1.76 seconds.

Table 2: Tail suspension test of MEPNF

Groups	Treatments	Dose, Route	Immobile Time (Sec)
Control	1% tween 80 in water	10 mL/kg, p.o	204.33±1.76
Standard	Imipramine	10 mg/kg, i.p	85.33±1.76
Test	MEPNF	200 mg/kg, p.o	118.66±6.11
	MEPNF	400 mg/kg, p.o	90.66±6.33

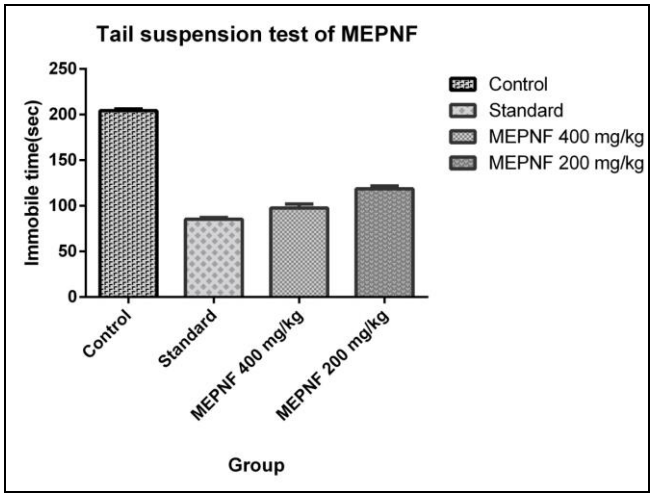

Fig 3: Tail suspension test of MEPNF

Discussion

From the beginning of civilization, for the treatment of many diseases human are dependable on plants, nowadays phytopharmacological investigation has created a new field of plant derivative drugs discovery which is effective in remedial of certain diseases and renewed the attention in herbal medicines. It is estimated that about 30% of the pharmaceuticals are prepared from plant derivative (Leta *et al*., 2002, Gillman *et al*., 1995) [11].

There are a few thrombolytic medications obtained from different sources. Some are adjusted further with the utilization of recombinant technology to make these thrombolytic medicate more site explicit and viable. Symptoms identified with these medications have been accounted for that lead to further complications. In some cases, the patients die because of bleeding and embolism (Komorowicz *et al*., 1998) [19]. Platelets play a significant role in the development of atherothrombosis as well as damage the regions of endothelial surface (produced by reactive oxygen species). The stimulated platelets form platelets to platelets bonds, also binds to leucocytes carrying them into an intricate process of plaque development and progression(Ramjan *et al*., 2014). Plasmin, a natural fibrinolytic agent, lyses clot by breaking down the fibrinogen and fibrin contained in a clot. Streptokinase forms a 1:1 stoichiometric complex with plasminogen that can convert additional plasminogen to plasmin (Banerjee *et al*., 2004) [2]. Moreover, phlorotannin, isolated from marine brown algae, have a unique property in the promotion of dissolution of intravascular blood clot via antiplasmin inhibition (Prasad *et al*., 2007) [16] The present study was done to research the thrombolytic action of two plants accessible in Bangladesh. Streptokinase (SK), a known thrombolytic drug, is utilized as a standard(Prasad *et al*., 2007) [16]. Water, then again, was assigned as a control. The comparison of positive control with negative control affirmed that coagulation disintegration didn't happen when water was added to the coagulation. By comparing with this standard & control, moderate thrombolytic activity was observed after treating the clots with *Achyranthes aspera*, *Piper nigrum,* and combination of these two plant extracts.

The reason for this investigation was to assess the antidepressant-like impact of MEAAL and MEPNF utilizing animal behavioral models. The tail suspension test is broadly acknowledged behavioral model for the evaluation of antidepressant-like effect. The characteristic behavior evaluated in this test, termed immobility, has been considered to reflect behavioral despair similar to that seen in human depression, and it is well known that antidepressant drugs can reduce the immobility time in rodents (Porsolt *et al*., 1977) [14]. It is interesting to note that the immobility shown by mice when subjected to unavoidable stress such as tail suspension test is thought to reflect a state of despair or lowered mood, which is thought to reflect depressive disorders in humans. Besides, the immobility time is reduced by treatment with antidepressant drugs (Thierry *et al*., 1986) [23]. Interestingly, our data indicate that higher doses of plant extracts were more effective than smaller doses in the tail suspension test.Based on our present study, the antidepressant-like effect of MEAAL and MEPNF in tail suspension test of depressant, where it was found to possess antidepressant-like activity comparable to the standard drug Imipramine. Imipramine acts by inhibiting norepinephrine (NE) reuptake and has been used as a standard drug in majority studies. (Pal and Dandiya, 1993) [14]

5. Conclusion

The present investigation utilizing that comparison among the extract of *Achyranthes aspera*, *Piper nigrum,* and combination of these two plant extracts in combination with Streptokinase, water (negative control) & streptokinase (positive control) concluded by the moderate thrombolytic activity, and methanol extract of *Achyranthes aspera* leaves and *Piper nigrum* fruits additionally show good antidepressant impact.

This is a significant finding, which may have suggestions in cardiovascular heath, particularly in atherothrombotic patients. This is just a primer report and to make the final explanation about the possibility of methanol extract of *Achyranthes aspera* leaves, and *Piper nigrum* fruits as thrombolytic and antidepressant medications may require further examination. Studies might be embraced to distinguish the synthetic structure of the dynamic elements of the extracts and to clarify the definite component of the activity.

6. Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper

7. Acknowledgment

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