American Journal of Clinical and Experimental Medicine
2015; 3(5): 228-232
Published online October 7, 2015 (http://www.sciencepublishinggroup.com/j/ajcem)
doi: 10.11648/j.ajcem.20150305.15
ISSN: 2330-8125 (Print); ISSN: 2330-8133 (Online)

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Comparative Study of Potential Thrombolytic and Anti-arthritic Activities of *Pterospermum acerifolium* and *Sonneratia caseolaris* Leaves

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To cite this article:

Sudipta Chowdhury, Md. Irfan Amin Chowdury, Mohammad Nazmul Alam, Abu Hena Mustafa Kamal, S. M. Arif Bin Alam, Sharmin Sultana, Tamanna Jahan. Comparative Study of Potential Thrombolytic and Anti-arthritic Activities of *Pterospermum acerifolium* and *Sonneratia caseolaris* Leaves. *American Journal of Clinical and Experimental Medicine*. Vol. 3, No. 5, 2015, pp. 228-232.
doi: 10.11648/j.ajcem.20150305.15

Abstract: This comparative study was performed for the evaluation of the thrombolytic and anti-arthritic effects of methanolic leaf extracts of *P. acerifolium* and *S. caseolaris*. The thrombolytic activity was evaluated by using the in vitro clot lysis model and anti-denaturation method was performed by using bovine serum albumin (BSA) to evaluate the anti-arthritic potential. Here, the thrombolytic activity of *P. acerifolium* leaves showed (35.15 ± 1.77)% whereas *S. caseolaris leaves* exhibited (26.05 ± 0.92)% and standard streptokinase demonstrated (63.54 ± 2.61)%. In the case of anti-arthritic study, *P. acerifolium* showed (35.48 ± 0.98)% at lower concentration and (76.64 ± 1.29)% at higher concentration and *S. caseolaris* exhibited (27.42 ± 0.98)% and (59.68 ± 1.07)% at lower and higher concentration respectively whereas standard diclofenac sodium showed (52.31 ± 0.56)% at 31.25 μg/ml and (86.67 ± 0.92)% at 1000 μg/ml. The results of these experiments suggest that methanolic leaf extract of *P. acerifolium* showed higher thrombolytic and anti-arthritic activities than *S. caseolaris*.

Keywords: *P. acerifolium*, *S. caseolaris*, Thrombolytic, Streptokinase, Anti-arthritic, Protein Denaturation

1. Introduction

Pterospermum acerifolium (Sterculiaceae) is a tall evergreen tree. Locally it is known as Moochkunda, Mushkand, Madhurabura. Leaves are variable in size and shape, 20-35 cm long, orbicular or oblong, with a cordate base, entire or variously lobed, or remotely toothed, sometimes peltate. Flowers are tonic, laxative, anthelmintic; used in leucorrhoea, gastralgia, blood troubles, ulcers, tumours, leprosy, and inflammations. It is also used as an insecticide. Bark is used for impotence in Khagrachari [1]. It is extensively found in forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar and Sylhet. *Sonneratia caseolaris* is a small, glabrous, evergreen tree, reaching 4.5 m high; Leaves subfleshy, 5-9.5 cm long, elliptic-oblong or obovate. Flowers solitary at the end of branchlets, about 5 cm across. Fruits are used as a poultice in sprains and swellings. Fermented juice of the fruits is useful in arresting haemorrhage.Leaves are rich in carbohydrates, lipids and proteins [2]. It is widely distributed in tidal forest of Barisal, Chittagong, Sunderbans and Chakaria Sundarbans.

2. Materials and Methods

2.1. Plant Collection and Identification

Leaves of *Pterospermum acerifolium* and *Sonneratia caseolaris* were collected from different parts of the Chittagong region, Bangladesh. The plants were identified and authenticated by Dr. Shaikh Bokhtear Uddin, Taxonomist and Associate Professor, Department of Botany, University of

Chittagong, Bangladesh.

2.2. Preparation of Extracts

Leaves of *P. acerifolium* and *S. caseolaris* were dried and ground into powder (40-80 mesh, 500 gm) through Moulinex Blender AK-241. Then the powder was soaked in 2 L of methanol at room temperature (23.0 ± 0.5)°C for a week. By using Whatman filter paper No. 1 and cheesecloth, filtrate was obtained. Filtrated solution was concentrated under reduced pressure by using a rotary evaporator. Less than 50°C was maintained for this filtration process. Glass Petri dishes were used to keep the extract. Each of the extracts (100 mg) was suspended in 10 mL distilled water. By using a vortex mixer, the suspension was shaken vigorously. Suspension of extract and distilled water was kept overnight and gradually poured through a 0.22 µm syringe filter for the filtration. In this way, soluble supernatant was removed. Then 100 µL of this filtrated aqueous preparation was added to microcentrifuge tubes which contained the clots to check the ex-vivo thrombolytic activity. The same concentration (10 mg/mL) of both plant extracts was prepared for the ex-vivo screening of anti-arthritic effects. In this process the leaves were dried and ground. The ground leaves (300g) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring then filtered through a cotton plug followed by Whitman filter paper No. 1. The solvent was evaporated under vacuum at room temperature to yield semisolid. The extract was then stored in a refrigerator till further use.

2.3. Chemicals and Drugs

Lyophilized streptokinase vial (1 500 000 IU) which was imported from Durakinase, Dongkook Phama. Co. Ltd, South Korea and sterile distilled water (5 mL) were added and mixed properly. Mixture of distilled water and lyophilized streptokinase was used as a stock. From this mixture, 100 µL (30 000 IU) was used for determination of thrombolytic activity. The chemicals used were Bovine serum albumin (BSA), Diclofenac Sodium, Sodium Dihydrogen phosphate, Disodium hydrogen phosphate, Sodium Chloride, Dextrose, sodium citrate, citric acid were purchased from Sigma-Aldrich. All chemicals in this experiment were of analytical reagent grade.

2.4. Thrombolytic Activity

2.4.1. Blood Sample

About 2 mL of blood was drawn from three healthy humans. They had no history of taking any types of contraceptive or anticoagulant therapy. A total of 500 µL of blood was transferred to each of the three previously weighed microcentrifuge tubes to form clots.

2.4.2. Clot Lysis

At first, three different sterile microcentrifuge tubes (0.5 mL/tube) were taken and weighed. Then 2 mL venous blood from human volunteers was added in pre-weighed sterile microcentrifuge tubes. The tubes were incubated at 37°C for 45 min. In this process, serum was totally eliminated after the formation of clots without disturbing the clots. Each tube containing clot was again weighed to know the weight of clot. For the determination of clot weight, weight of tube alone were excluded from the weight of clot and tube. For each microcentrifuge tube containing pre-weighed clot, 100µL of methanol extracts of both plants (*Pterospermum acerifolium* and *Sonneratia caseolaris*) were added separately. About 100µL of streptokinase was used as a positive control and 100 µL of distilled water was used as a negative control. At last, all the tubes were incubated at 37°C for 90 min. In this way, clot lysis was observed. After incubation, released fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference in weight before and after clot lysis was expressed as percentage of clot lysis [3]. Evaluation of thrombolytic effects of both methanolic extracts were performed by the formula below:

Percentage of clot lysis = (Weight of released clot/clot weight) ×100

The experiment was repeated three times with the blood samples from the three healthy volunteers.

2.5. In Vitro Anti-arthritic Activity

For the evaluation in vitro anti-arthritic activity of *P. acerifolium* and *S. caseolaris,* the method used was "inhibition of protein denaturation" with diclofenac sodium as a standard [4-7]. The test solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution (methanolic leaf extract of *P. acerifolium* and *S. caseolaris*). The test control solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water. Product control (0.5 ml) consists of 0.45 ml of distilled water and 0.05 ml of test solution. Standard solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of diclofenac sodium. Various concentrations (31.25, 62.5, 125, 250, 500, 1000 µg/ml) of methanolic leaf extract of sodium (standard) were taken respectively. All the solutions were adjusted to pH 6.3 using 1 N HCl. The samples were incubated at 37 °C for 20 min and the temperature was increased to keep the samples at 57 °C for 3 min. After cooling, 2.5 ml of phosphate buffer was added to the previous solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm. The control represents 100% protein denaturation. The results were compared with diclofenac sodium. The percentage inhibition of protein denaturation of different concentrations is tabulated in table 2. The percentage inhibition of protein denaturation can be calculated as:

Percentage of inhibition = [100 − (OD of test solution − OD of product control)]×100

Where OD = optical density.

Control represents almost 100% protein denaturation. The results were compared with diclofenac sodium [8].

2.6. Statistical Analysis

In the case of thrombolytic study, statistical significance between percentage of clot lysis by streptokinase and both of the plant extracts were evaluated by paired t-test analysis. Expression of data was expressed as mean ± SD. The mean difference between positive and negative controls was considered significant at P<0.05. In the case of anti-arthritic study, the results were expressed as mean of the three repetitions and standard deviations were calculated. Statistical comparisons were made using the Independent t-test and P<0.01 was considered as significant. The tests were performed by using the software SPSS version 20.0 (SPSS for Windows, IBM Corporation, New York, USA).

3. Results

3.1. Thrombolytic Activity

At first, 100 µL of streptokinase (positive control) was added to the clots and kept in incubator at 37 °C for 90 min, which showed significant lysis of clot (63.54 ± 2.61)%. When distilled water (negative control) was used to treat clots, it showed negligible clot lysis (4.21 ± 0.73)%. A significant value (probability value of P<0.05) was obtained by calculating the mean difference in the clot lysis (%) between streptokinase (positive control) and distilled water (negative control). When methanolic leaf extracts of *P. acerifolium* and *S. caseolaris* were used to treat clots, the lysis of clot was (35.15±1.77)% and (26.05±0.92)% respectively. A statistically significant value (probability value of P<0.05) was obtained from the mean percentage of clot lysis of *P. acerifolium* and *S. caseolaris. P. acerifolium* exhibited a relatively higher percentage of clot lysis than *S. caseolaris*. However, both plant extracts showed statistically significant values when compared with streptokinase (positive control) and distilled water (negative control) (probability value of P<0.05).

Table 1. *Effect of methanolic leaf extracts of P. acerifolium and S. caseolaris on in-vitro clot lysis.*

Treatment	% of clot lysis (Mean ± S. D.)
Streptokinase(Positive Control)	63.54 ± 2.61**
Distillled water (Negative Control)	4.21 ± 0.73**
PA	35.15 ± 1.77**
SC	26.05 ± 0.92**

Statistical representation of the effective clot lysis percentage by herbal preparations, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) done by paired t-test analysis; clot lysis percentage is represented as mean ± S.D. **P<0.05 compared to control.

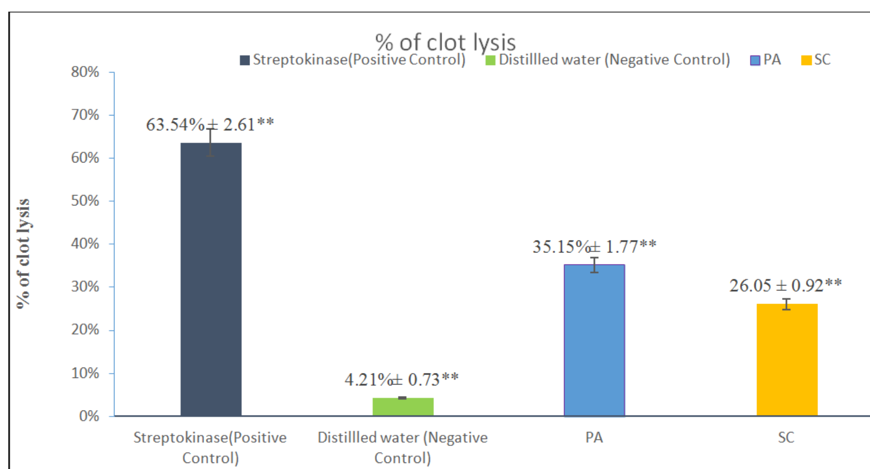

Figure 1. *Clot lysis by streptokinase, water and both of methanolic leaf extracts. *PA =P. acerifolium and SC= S. caseolaris.*

3.2. Anti-arthritic Study

Different concentrations of methanolic extract of *P. acerifolium*, *S. caseolaris* and diclofenac sodium was tested for anti-arthritic activity and found significant percentage inhibition in protein denaturation. Here, in lower concentration (31.25 µg/ml) the extract of *P. acerifolium* and *S. caseolaris* showed 35.48±0.98% (P<0.01) and 27.42±0.98% (P<0.01), where the standard drug diclofenac sodium showed 52.31±0.56% of inhibition. In the case of higher concentration (1000 µg/ml), methanolic extract of *P. acerifolium* and *S. caseolaris* exhibited the 76.64±1.29% (P<0.01) and 59.68±1.07% (P<0.01) of inhibition, whereas the diclofenac sodium showed 86.67±0.92% of inhibition of protein denaturation.

Table 2. *Percent inhibition of protein denaturation of P. acerifolium and S. caseolaris.*

Concentration(µg/ml)	Percent Inhibition of protein denaturation		
	PA(Test Solution)	SC(Test Solution)	Diclofenac sodium
31.25	35.48±0.98**	27.42±0.98**	52.31±0.56
62.5	40.32±1.03**	35.48±1.03**	62.29±0.59
125	48.48±1.37**	37.10±1.03**	65.59±0.66
250	56.65±1.89**	45.48±0.64**	75.52±0.93
500	67.74±2.06**	56.65±0.61**	80.58±0.95
1000	76.64±1.29**	59.68±1.07**	86.67±0.92

Values are expressed as mean ± SEM of three replicate (n=3), **P<0.01

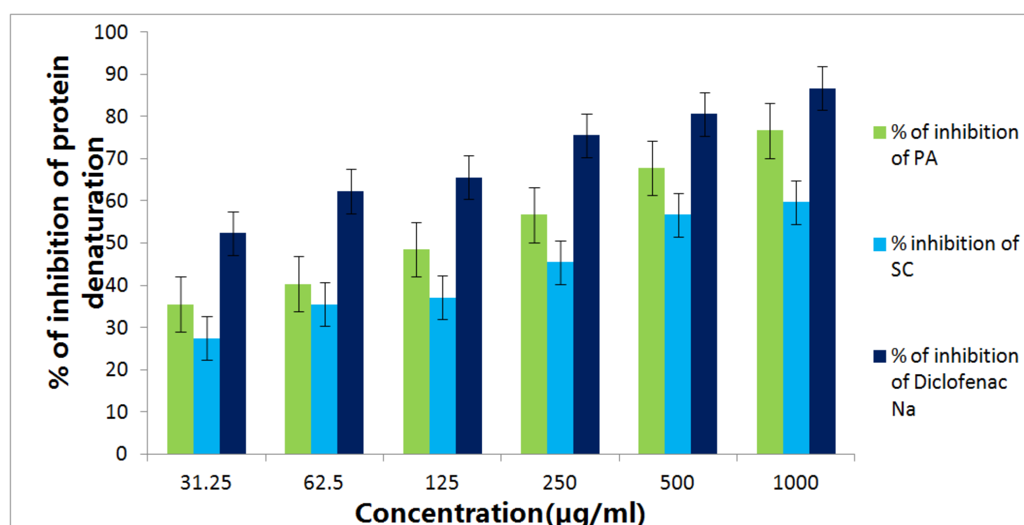

Figure 2. *Percent Inhibition of protein denaturation of P. acerifolium, S. caseolaris and Diclofenac sodium.*

4. Discussion

In the case of thrombolytic study, methanolic leaf extracts of *P. acerifolium*, *S. caseolaris* and positive control (streptokinase) truly demonstrated the effect on clot lysis. By comparing the clot lysis percentage obtained through streptokinase and distilled water, a promisingly significant ($P<0.05$) thrombolytic effect was observed after the clots were treated with both extracts. It is established from the previous experiment that there are some bacterial pollutants of plants that have plasminogen receptors which are specific for plasminogen. Certain plasminogen on cell surface is rapidly activated to plasmin that could lead to fibrinolysis [9]. Bacterial plasminogen activator which also acts as cofactor molecules, such as staphylokinase, and streptokinase, can cause formation of exosite and increase the substrate activity towards the enzyme. Staphylokinase activates plasminogen to be in a position to break down clots, and also damages the extracellular molecules secreted by cells and fibrin particles that keep cells organized [10-12]. From the above experiment, it would be interesting to examine both of the mechanisms correlated to clot lytic effects showed by *P. acerifolium* and *S. caseolaris* extracts. However, these activities might be due to the presence of biologically active or inhibitory compounds or synergism by the existence of some compounds. A different type of constituents, such as saponins, polyphenols, alkaloids and flavonoids, may be present in the extracts, so further vast investigations are required to determine the specific thrombolytic effects of the leaf extracts. In this comparative study, *P. acerifolium* demonstrated higher thrombolytic activity than *S. caseolaris*. Additional investigations are required to be performed because phytochemicals derived from these plant could be incorporated as a thrombolytic agent for the improvement of the patients suffering from cardiac diseases. Arthritis is a form of joint disorder that involves inflammation of one or more joints, responsible for painful swelling, stiffness, loss of function in the joint. Denaturation of protein is one of the causes of arthritis as documented. Production of autoantigen in certain arthritic disease may be due to denaturation of protein. The mechanism of denaturation probably involves alteration I electrostatic hydrogen, hydrophobic and di-sulphide bonding [13]. In this comparative study, methanolic leaf extract of *P. acerifolium* showed higher anti-arthritic activity than *S. caseolaris* in both lower and higher concentration. According to the result of this comparative, it can be stated that methanolic leaf extract *P. acerifolium* has the capability of controlling the production of autoantigen to inhibit the denaturation of protein significantly.

Acknowledgments

Authors wish to thank the botanist Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Bangladesh, who helped to identify these plants. Authors are also grateful to the Department of Pharmacy, International Islamic University Chittagong, Chittagong, Bangladesh, for providing support and research facilities.

References

[1] Yusuf M., Begum J., Hoque M. N. and Chowdhury J. U., 2009. *Medicinal Plants of Bangladesh* (revised and enlarged). Bangladesh Council of Scientific and Industrial Research Laboratories, Chittagong, Bangladesh. 794 p.

[2] Ghani A., 2003. *Medicinal Plants of Bangladesh* (Second edition). Asiatic Society of Bangladesh, Dhaka, Bangladesh. 603 p.

[3] Prasad S., Kashyap R.S., Deopujari J.Y., Purohit H.J., Taori G.M., Daginawala H.F., 2006. Development of an in vitro model to study clot lysis activity of thrombolytic drugs. Thromb J. 4:14.

[4] Mishra N.K., Bstia S., Mishra G., Chowdary K.A., Patra S., 2011. Anti-arthritic activity of *Glycyrrhiza glabra*, *Boswellia serrata* and their synergistic activity in combined formulation studied in freund's adjuvant induced arthritic rats. J Pharm Educ Res. 2(2): 92-8.

[5] Lavanya R., Maheshwari S.U., Harish G., Raj J.B., Kamali S., Hemamalani D., 2010. Investigation of In-vitro anti-Inflammatory, anti-platelet and anti-arthritic activities in the leaves of *Anisomeles malabarica* Linn. Res J Pharm Biol Chem Sci. 1(4): 746-752.

[6] Singh M., Soni P., Upmanyu N., Shivhare Y., 2011. In-vitro anti-arthritic activity of *Manilkara zapota* Linn. Asian J Pharm Technol. 1(4): 123-124.

[7] Chippada S.C., Vangalapati M., 2011. Antioxidant, an anti-inflammatory and anti-arthritic activity of *Centella asiatica* extracts. J Chem Biol Phys Sci. 1(2): 260-269.

[8] Alam M. N., Biozid M. S., Chowdhury A. I., Mazumdar M. M. U., Chowdhury S., & Chowdury M. I. A., 2015. Anti-arthritic and cytotoxic effects of methanolic extract of *Ixora nigricans* leaf. Journal of Medicinal Plants Research. 9(25): 719-723.

[9] Pantzar M., Ljungh A., Wadström T., 1998. Plasminogen binding and activation at the surface of *Helicobacter pylori* CCUG 17874. Infect Immun. 66(10): 4976-4980.

[10] Parry M.A.A., Zhang X.C., Bode W., 2000. Molecular mechanisms of plasminogen activation: bacterial cofactors provide clues. Trends Biochem Sci. 25(2): 53-59.

[11] Collen D., 1990. Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator? Ann Intern Med. 112(7): 529-538.

[12] Rahman M.A., Imran T., Islam S., 2013. Antioxidative, antimicrobial and cytotoxic effects of the phenolics of *Leea indica* leaf extract. Saudi J Biol Sci. 20(3): 213-225.

[13] Shravan K.N., Kishore G., Siva K.G., Sindhu P.E.S., 2011. *In vitro* anti-inflammatory and anti-arthritic activity of leaves of *Physalis Angulata* L. Int J Pharm Ind Res. 1(3): 211-213.