Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 1166-1169
Received: 20-05-2017
Accepted: 21-06-2017

Fatema Tabassum
Department of Pharmacy, Northern University Bangladesh (NUB), Dhaka, Bangladesh

Somaia Haque Chadni
Department of Pharmacy, Comilla University, Comilla, Bangladesh

Kamrun Nahar Mou
Department of Pharmacy, Northern University Bangladesh (NUB), Dhaka, Bangladesh

KM Imrul Hasif
Department of Pharmacy, Northern University Bangladesh (NUB), Dhaka, Bangladesh

Tareque Ahmed
Department of Pharmacy, Northern University Bangladesh (NUB), Dhaka, Bangladesh

Mahbuba Akter
Department of Pharmacy, Northern University Bangladesh (NUB), Dhaka, Bangladesh

Correspondence
Fatema Tabassum
Department of Pharmacy, Northern University Bangladesh (NUB), Dhaka, Bangladesh

In-vitro thrombolytic activity and phytochemical evaluation of leaf extracts of four medicinal plants of Asteraceae family

Fatema Tabassum, Somaia Haque Chadni, Kamrun Nahar Mou, KM Imrul Hasif, Tareque Ahmed and Mahbuba Akter

Abstract
The present study was designed to investigate the comparative study of the thrombolytic activity and phytochemical analysis of the methanolic extract of leaves of *Wedelia chinensis* Osbeck. Merr., *Eclipta alba* (L) Hassk., *Emilia sonchifolia* (L.) DC. and *Spilanthes paniculata* Wall. (All of which have the same family-asteraceae) were determined. From our study we also found that *Wedelia chinensis* Osbeck. Merr., *Emilia sonchifolia* (L.) DC., *Eclipta alba* (L) Hassk. and *Spilanthes paniculata* Wall. Showed 24.48%, 28.71%, 15.19% and 42.77% clot lysis activity respectively and they showed significant % of clot lysis effect with reference of Streptokinase (71.43%) and water (2.96%). The phytochemical analysis showed the presence of different phytochemicals of different fractions of solvents. It is concluded that the *in-vitro* thrombolytic activity showed by the plants were due to the presence of these phytochemicals. Further studies are highly needed for further drug development.

Keywords: *Wedelia chinensis, Eclipta alba, Emilia sonchifolia, Spilanthes paniculata*, Thrombolytic, % of clot lysis

1. Introduction
Thrombus (blood clot) developed in the circulatory system due to failure of hemostasis causes vascular blockage and leads to serious consequences in thrombolytic diseases such as acute myocardial or cerebral infarction which may cause death [1]. Thrombolytic drugs are used to dissolve blood clots in a procedure termed thrombolysis [2]. Alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (tPA) are commonly used thrombolytic agents to dissolve clots [3]. Heparin and Aspirin are only moderately efficient for acceleration of lysis and prevention of reocclusion, but are safe. Continued investigation in this area will provide new insights and promote progress towards the development of the ideal thrombolytic activity which are characterized by maximal coronary arteral thrombolysis with minimal bleeding [4]. Selective third generation thrombolytic activity such as monoteplase, tenecteplase, reteplase etc. result in a greater angiographic potency in patients with acute myocardial infarction, although so far, mortality rates have been similar to those few drugs that have been studied in large-scale trials [3]. In recent years, it is observed that the heart diseases are increasing to a great extent and side effects of synthetic drugs are becoming an ever-increasing therapeutic problem. Almost all the available thrombolytic agents still have significant shortcomings [5]. According to one of the reports, approximately, 30% of the pharmaceuticals are prepared from plants worldwide [6] and are considered to be less toxic and freer from side effects than the synthetic one [7]. Hence, it is needed to find out the safe, less or no side effective herbal drugs, because natural products of higher plants may give a new source of thrombolytic agents, as well as antimitotic agents [8].
Wedelia chinensis Osbeck. Merr. (Synonyms: *Solidago chinensis* Osbeck., *Verbesina calendulacea* L.; Bengali name: Kesraj, Bangra, Bhimraj, Bhimra, Mahavringaraj) is a yellow coloured perennial herb of sunflower family Asteraceae which is commonly known as Chinese Wedelia; Extract of *W. chinensis* has been reported to attenuate androgen receptor activity and orthotopic growth of prostate cancer [9]. The fruits, leaves and stems of this plant are traditionally used in child birth and used in the treatment of bites and stings, fever and infections. The leaves are used in the kidney dysfunction, cold, wound and amenorrhea. The plant has been used as astringent, bitter, acrid, anti-inflammatory and cardiotonic and treatment of wounds, seminal weakness and viral hepatitis [10]. *Eclipta alba* (L) Hassk. (Synonyms: Eclipta prostrate Roxb., Bengali name Bhringraj, Bhringraja) commonly known as false daisy in England is regarded as a weed of Ethnomedicinal significance.

It is reported to have anthelmintic, antipyretic, anti-inflammatory, antihistaminic, hepatoprotective and expectorant properties [11]. *Eclipta alba* is one of the endangered plant and therefore its conservation is of great need and importance in the present scenario [12].

Emilia sonchifolia (L.) DC. (Synonyms: *Crassocephalum sonchifolium* (L.) Less., *Emilia javanica* (Burm. f.) C.B. Rob., Bangali Name: Sadimodi, Mechitra, Sadusi) belongs to the family Asteraceae is distributed in India, Ceylon and in most tropical and subtropical regions. Different parts of the plant have been used in the treatment of asthma, inflammation, intermittent fever, breast cancer, opthalmia, cuts and wounds [13]. The plant is astringent, depurative, diuretic, expectorant, febrifuge and sudorific [14].

Spilanthes paniculata Wall. (Synonym: Acmella paniculata, Splinthus acmella; Bengali Name: Marhati-tiga) is an important medicinal plant with rich source of therapeutic and medicinal constituents. This species is famous as a folklore remedy for toothache and for throat and gum infections, earning it the English nickname, the "toothache plant" [15]. It have traditionally been used for the treatment of a number of disease conditions such as malaria [16].

In the present investigation an attempt has been made enrich the knowledge of thrombolytic activity and phytochemical analysis of methanoic extract of the leaf of different plants of asteraceae family.

2. Materials and Methods

2.1 Collection and drying of plant materials

The plants *Wedelia chinensis* Osbeck. Merr., *Emilia sonchifolia* (L.) DC., *Eclipta alba* (L) Hassk. and *Spilanthes paniculata* Wall. were collected at their fully matured form, in August 2016, from local area of Narayangang, Bangladesh and the plant's were identified by (DACB- 43879, 43877,43878 and 43880 respectively) Dhaka-1216, Bangladesh. The leaves were collected, properly washed with water and air dried for one months and then kept in an oven at 45 °C for 24 hours and the powdered samples were kept in sealed containers for extraction purposes.

2.2 Preparation of plant extract

The air dried and powdered plant material 100 gm was extracted successfully with 1 liter of methanol, petroleum ether and chloroform by using a soxlet extractor until a complete extract were effected(10-12h) at a temperature not exceeding the boiling point. The extracts were evaporated to dryness under reduced pressure using a Rota vapor and the resulting extracts were stored in a refrigerator for phytochemical screening [17].

2.3 Preliminary Phytochemical screening

The extracts were subjected to preliminary Phytochemical testing to detect for the presence of different chemical groups of compounds. The plant extracts were carried out qualitatively for the presence of alkaloids, carbohydrates, glycosides, flavonoids, saponins and steroids by using the standard method given by [18].

2.4 Sample preparation

The crude extract was suspended in 10 ml distilled water and shaken vigorously on a vortex mixture. Then the suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a filter paper. The solution was then ready for *in vitro* evaluation of clot lysis activity [19].

2.5 Preparation of streptokinase (SK)

About 5 ml sterile distilled water was added to the commercially available lyophilized SK vial of 15,00,000 I.U. and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolysis study [19].

2.6 Collection of blood

Whole blood was drawn from healthy human volunteers without a history of oral contraceptives or anticoagulant therapy and 1 ml of blood was transferred to the previously weighted sterile eppendorf tubes and was allowed to form clots.

2.7 Determination of thrombolytic activity

The eppendorf tubes were incubated at 37 °C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each eppendorf tube having clot was again weighted to determine the clot weight (clot weight= weight of clot containing tube- weight of tube alone.) To each eppendorf tube containing pre-weighted clot, 100 µ aqueous solutions of different extracts along with the crude extract was added separately. As a positive control, 100µ of streptokinase and a negative non thrombolytic control, 100µ of distilled water were separately added to the control eppendorf tubes. All the eppendorf tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, the released fluid was removed and eppendorf tubes were again weighted to observe the difference weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis is shown below:

% clot lysis= (Weight of the lysis clot/ Weight of clot before lysis)*100

3. Results and Discussion

The results of the phytochemical screening showed the presence carbohydrates and alkaloids in all the extracts of *Emilia sonchifolia* (L.) DC. and *Spilanthes paniculata* Wall. All methanoic extract of the plants except *Spilanthes paniculata* Wall. Showed the presence of glycosides and flavonids. Methanoic extract of *Wedelia chinensis* Osbeck. is absent of saponins and steroid, but chloroform extract contains saponins and pertolium ether extract contains steroids. Methanoic and petroleum ether extract of *Emilia sonchifolia* (L.) DC. and *Eclipta alba* (L) Hassk. Showed positive result in case of saponins and steroids. Petroleum ether extract of *Spilanthes paniculata* Wall. Showed the presence of saponins and steroids, methanoic extract of this plant showed the presence of saponins and chloroform extract showed the presence of steroids. Phytochemical analysis of the four different plants of Asteraceae family of different solvent extracts have showed in the Table 1.

Table 1: Preliminary Phytochemical screening of some medicinal plants of asteraceae family

Name of the plant	Tests	Methanol extract	Chloroform extract	Petroleum ether extract
Wedelia chinensis Osbeck.	Carbohydrates	-	+	+
	Alkaloids	+	+	-
	Glycosides	+	-	-
	Flavonoids	+	-	-
	Saponins	-	+	-
	Steroids	-	-	+
Emilia sonchifolia (L.) DC.	Carbohydrates	+	+	+
	Alkaloids	+	+	+
	Glycosides	+	-	+
	Flavonoids	+	-	-
	Saponins	+	-	+
	Steroids	+	-	+
Eclipta alba (L) Hassk.	Carbohydrates	-	-	+
	Alkaloids	+	-	-
	Glycosides	+	-	-
	Flavonoids	+	+	-
	Saponins	+	-	+
	Steroid	+	+	+
Spilanthes paniculata Wall.	Carbohydrates	+	+	+
	Alkaloids	+	+	+
	Glycosides	-	+	+
	Flavonoids	+	-	+
	Saponins	+	-	+
	Steroids	-	+	+

Note: +=positive; -= negative.

In case of *in-vitro* thrombolytic activity, addition of 100 µl Streptokinase, a positive contol (30,000 I.U.) to the clots along with 90 minutes incubation at 37° C, showed 71.43% clot lysis. Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (2.96%). The *in-vitro* thrombolytic activity study revealed that methanoic extracts of *Wedelia chinensis* Osbeck., *Emilia sonchifolia* (L.) DC., *Eclipta alba* (L) Hassk. and *Spilanthes paniculata* Wall. Showed 24.48%, 28.71%, 15.19% and 42.77% clot lysis respectively. % Clot lysis obtained after treating clots with these two extracts and appropriate control is shown in Figure 1. Satistical representation of the effective clot lysis percentage by negative control (sterile distilled water), positive control (Streptokinase) and four herbal preparations has been shown in Table 2:

Table 2: Effect of herbal extracts on *in-vitro* clot lysis

Extracts/Drugs	Mean ±S.D. (% Clot Lysis)
Negative control	2.96±0.63%
Positive control	71.43±3.46%
Wedelia chinensis Osbeck.	24.48±5.23%
Emilia sonchifolia (L.) DC.	28.71±2.08%
Eclipta alba (L) Hassk.	15.19±4.29%
Spilanthes paniculata Wall.	42.77±6.14%

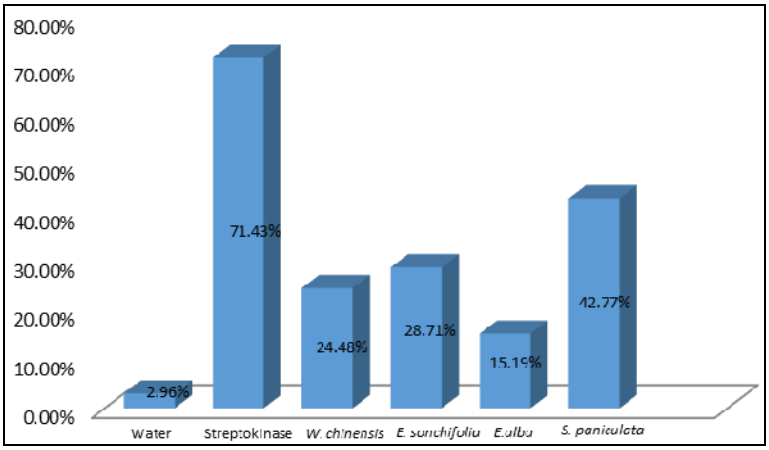

Fig 1: Clot lysis by Water, Streptokinase, *Wedelia chinensis* Osbeck. Merr., *Emilia sonchifolia* (L.) DC., *Eclipta alba* (L) Hassk. and *Spilanthes paniculata* Wall.

4. Conclusion

As from the research findings of the undertaken *in vitro* clot lysis study, we demonstrated that the leaf extracts of different plants of Asteraceae family showed mainly moderate thrombolytic activity. This may be due to the presence of different types of phytochemicals present on it. It may be assumed that these extracts can be considered as potential sources of natural thrombolytic agents. This is only a preliminary study and the extract should be thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potential.

5. References

1. Sherwani SK, Bashir A, Haider SS, Shah MA, Kazmi SU. Thrombolytic Potential of Aqueous and Methanolic Crude Extracts of Camellia sinensis (Green Tea): *In vitro* study. Journal of Pharmacognosy and Phytochemistry. 2013; 2(1):125-129.
2. Fathima SN, Ahmad SV, Kumar BR. Evaluation of *In Vitro* Thrombolytic Activity of Ethanolic Extract of CURCUMA CAESIA Rhizomes, International Journal of Pharma Research & Review. 2015; 4(11):50-54.
3. Sayeed MA, Kabir H, Rashid MMU, Bhuiyan MFA, Ahamad FM, Rashid MA. Thrombolytic Activity of Methanolic Extracts of *Desmodium paniculatum* (L.) and Sarcochlamys pulcherrima (Roxb.), Bangladesh Pharmaceutical Journal. 2014; 17(1):67-69.
4. Collen D. Coronary thrombolysis: streptokinase or recombinant tissue-type plasminogen activator. Ann Intern Med. 1990; 112(7): 529-538.
5. Nicolini FA, Nichols WW, Mehta JL, Saldeen TG, Schofield R, Ross M *et al*. Sustained reflow in dogs with coronary thrombosis with K2P, a novel mutant of tissue plasminogen activator. J Am Coll Cardiol. 1992; 20:228-235.
6. Anwar AK, Ashfaq M, Nasveen MA. Pakistan Forest Institute, Peshawar NWFP. Pakistan. 1979:15-35.
7. Annapurna A, Kanaka, Mahalakshmi D, Murali KK. Antidiabetic activity of a polyherbal preparation (Tincture of Punchparna) in normal and diabetic rats. Indian J Exp Biol. 2001; 39:500-502.
8. Wagnor H, Hikino H, Farnsworth R, London NR. The economic significance of plants and their constituents as drug, in Economic and Medicinal Plant Research. Academic Press. 1989; 3:1-17.
9. Tsai CH, Lin Yang FM, Lee YC, Cha MT, TL, Wu GJ *et al*. Herbal extract of Wedelia chinensis attenuates androgen receptor activity and orthotopic growth of prostate cancer in nude mice. Clin. Cancer. Res. 2009; 15(17):5435-5444.
10. Banu HR, Nagrajam N. Antimicrobial activity of Wedelia chinensis leaves. Journal of Pharmacy Research, 2012; 5(1):407-412.
11. Swati M, Monalisa J. Abhisek P. Evaluation of antidepressant activity of *Eclipta alba* using animal models, Asian Journal of Pharmaceutical and Clinical Research. 2013; 6(3):118-120.
12. Dodake P, Pal M. Pharmacological and tissue culture studies on *Eclipta alba*: A review, Journal of Environmental Science, Toxicology and Food Technology. 2016; 10(11):07-09.
13. Thenmozhi K, Saradha M. Manian S. Paulsamy. *In-vitro* antimicrobial potential of root extracts of the medicinal plant species, *Emilia Sonchifolia* (Linn.) DC, Asian Journal of pharmaceutical and clinical research. 2013; 6(3):149-151.
14. Essien GE, Nwidu LL, Nwafor PA. Anti-Inflammatory and analgesic potential of methanolic extract of Emilia sonchifolia (Compositae) leaves in rodents. African Journal of Biomedical Research. 2009; 12(9):199-207.
15. Hossain H, Shahid-Ud-Daula, Jahan AFM, Hasan NK, Haq MM. Evaluation of antinociceptive and antioxidant potential from the leaves of Spilanthes paniculata growing in Bangladesh, International Journal of Pharmaceutical and Phytopharmacological Research. 2012; 1(4):178-186.
16. Haque S, Lopa SD, Das BK. Evaluation of antioxidant potential of *Spilanthes paniculata*, International Journal of Pharmacy and Pharmaceutical Sciences, 20157(8), 390-392.
17. Shankara BER, Ramachandra YL, Rajan SS, Preetham J, Ganapathy PSS. *In vitro* antibacterial activity of *Terminalia chebula* leaf gall extracts against some human pathogenic strains; International Current Pharmaceutical Journal. 2012; 1(8):217-220.
18. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 2nd Edition, J. B. Harborne Publishers, London, Chappman & Hall. 1998, 1-32.
19. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Effect of *Fagonia arabica* (Dhamasa) on *in-vitro* thrombolysis. BMC Complementary Altern. Med. 2007; 7(1):36.