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In vitro comparative evaluation of anti-inflammatory and thrombolytic activity of three *Mikania* species available in Bangladesh

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Abstract
The present study was designed to investigate comparative anti-inflammatory and thrombolytic activity of the ethanolic extract of *Mikania cordata* (MC), *Mikania micrantha* (MM) and *Mikania scandens* (MS) (Family: Asteraceae). Phytochemical screening of these three species was also evaluated. Anti-inflammatory activity was performed using hypotonic solution induced hemolysis and heat induced hemolysis at various concentration and the result was expressed as % of inhibition of haemolysis. Ethanolic extract of MC, MM, MS and standard aspirin at 500µg/ml showed 80.943±0.64, 71.465±0.16, 60.351±0.40 and 92.894±0.30 % inhibition for heat induced haemolysis and 87.098±0.05, 86.242±0.45, 77.709±0.43 and 97.520±0.38% inhibition for hypotonic solution induced haemolysis, respectively. The data showed MC possessed strong anti-inflammatory activity compared to standard aspirin. The activity order was Aspirin>MC>MM>MS. In thrombolytic activity experiment % of clot lysis in MC, MM, MS and standard streptokinase were found to be 40.36±0.11, 48.44±0.34, 55.80±0.54 and 70.00±0.43 %, respectively, indicating moderate thrombolytic activity of the extracts compared to standard. The activity order was streptokinase>MS>MM>MC. The phytochemical screening showed the extracts contains alkaloids, terpenoids, tannins and cardiac glycosides. From the observation it was concluded that *Mikania cordata* is a good candidate for anti-inflammatory activity whereas *Mikania scandens* for thrombolytic potential.

Keywords: *Mikania cordata*, *Mikania micrantha*, *Mikania scandens*, Anti-inflammatory activity, Thrombolytic, % of clot lysis.

1. Introduction
Inflammatory response is associated with a range of diseases such as rheumatoid arthritis, atherosclerosis and asthma, which all show a high prevalence globally and it is difficult to establish an effective therapy to control the inflammatory processes [1].
The attention of pharmacologists throughout the world has been focused on finding out safer and potent anti-inflammatory drug [2] especially those derived from plants. Nature is an excellent source of high phytochemical diversity, many of them possessing interesting biological activities and medicinal properties [3] due to the presence of pharmacologically active secondary metabolites such as alkaloids, phenolics, tannins and flavonoids [4-5]. The use of traditional medicine is increasing and getting popularity throughout the developed and developing world due to unaffordable cost and increased side effects of synthetic drugs [6]. It is well accepted that medicines derived from plant products are safer, more low cost, and more easily available than their synthetic counterparts [2].
Mikania species are found in the tropics of America and Asia and the plants from the genus are widely and combined known as guaco that are wide spread weeds. It comprises about 300 identified species [7] but only 15-20 of them have been studied. In folkloric medicine, the plants from the genus are used to treat rheumatism, inflammation, oxidative stress, spasmolytic, respiratory diseases as well as snake bites and scorpion stings [1, 7-9].
Various studies showed anti-inflammatory property of *Mikania* species including *M. laevigata* and *M. involucrata* [8]. *M. glomerata* was shown to inhibit inflammatory and oxidative stress caused by a single coal dust intratracheal instillation in rat [10] and through the antiedema activity test in the rat paw induced by carrageenan and quantified by plethysmography [11]. The extracts of *M. micrantha* was reported to inhibit mouse ear inflammation [12]. Significant anti-inflammatory activity of *M. scandens* [2] and *M. cordata* was reported both *in vitro* and *in vivo* [10]. *M. cordata* has been used in traditional herbal medicine of Bangladesh to treat various ailments including pain, inflammations and some other infectious diseases by folklore people [13]. In Bangladesh *M. cordata*, *M. micrantha* and *M. scandens* are widely available. Though various reports show anti-inflammatory activity of *Mikania* species but most of them are

limited to *M. cordata* and *M. scandens* and there is no clear explanation about thrombolytic activity. Also, comparative anti-inflammatory and thrombolytic evaluation were not reported elsewhere that might be interesting for pharmacologists as well as phytochemists to evaluate active principle relates to the comparative effectiveness of these plants making the use of active medicinal plants safe. So, the present study is designed to evaluate phytochemical screening and comparison of anti-inflammatory and thrombolytic activity of three commonly available *Mikenia* species in Bangladesh i.e. *Mikania cordata*, *Mikania micrantha* and *Mikania scandens*.

Materials and Methods

Collection and Identification of plant material

The whole part of *M. cordata* was collected from Rajshahi (University of Rajshahi campus) which is northern part of Bangladesh. *M. micrantha* and *M. scandens* were collected from Barisal (Southern region) and Kushtia (western region, Kumarkhali area), respectively, during the month of August 2015 and were identified by taxonomist Dr. AHM Mahbubur Rahman, Associate Professor, Department of Botany, University of Rajshahi, Bangladesh. The plants were labeled, air dried for several days and then oven dried at 45ºC for 24 hours to assist grinding. The dried plants were crushed separately into course powder.

Preparation of extract

About 170gm dried powdered plant materials of *M. cordata* (MC), *M. micrantha* (MM) and *M. scandens* (MS) was taken separately in an amber colored extraction bottle (2.5 L capacity) and the materials were soaked with 70% ethanol (90ml × 3 times). The sealed bottle was kept for 7 days with occasional shaking and stirring. The extracts were filtered through cotton and then Whatman No.1 filter papers and were concentrated with a rotary evaporator under reduced pressure at 45°C and was preserved in refrigerator for further assay. The obtained MC, MM and MS percentage were 11.00%, 9.5% and 7.3%, respectively.

Phytochemical screening of CEE

The crude ethanolic extracts of MC, MM and MS was tested for the presence of alkaloids, steroids, tannins, saponins and glycosides. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

Test for alkaloids: About 15 mg of each extract was separately stirred with 1% HCl (6 mL) on a water bath for 5 min and filtered. These filtrates were tested for the presence of alkaloid using Dragendorff's test, Mayer's test and Wagner's test described before [14-15].

Tests for terpenoids: About 100 mg sample was shaken with chloroform (2 mL) followed by the addition of concentrated H_2SO_4 (2 mL) along the side of the test tube, a reddish brown coloration of the interface indicates the presence of terpenoid [16].

Test for tannins: 0.5 g sample was stirred with distilled water (10 mL) and then filtered. A few drops of 5% ferric chloride were then added. Black or blue-green coloration or precipitate was taken as positive result for the presence of tannins [17].

Test for Saponins: Each of plant sample (0.5 g) was separately shaken with distilled water (10 mL) in a test tube. The formation of frothing, which persists on warming in a water bath for 5 min, shows the presence of saponins [17].

Tests for glycosides: (a) Borntrager's test for anthraquinone glycoside: To the extract solution (1 mL), 5% H_2SO_4 (1 mL) was added. The mixture was boiled in a water bath and then filtered. Filtrate was then shaken with equal volume of chloroform and kept to stand for 5 min. Then lower layer of chloroform was shaken with half of its volume with dilute ammonia. The formation of rose pink to red color of the ammoniacal layer gives indication of anthraquinone glycosides [14]. (b) Keller-Killiani test for Cardiac glycoside: Extract (0.5 g) was shaken with distilled water (5 mL). To this, glacial acetic acid (2 mL) containing a few drops of ferric chloride was added, followed byH_2SO_4 (1 mL) along the side of the test tube. The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring [16].

In-vitro Anti-inflammatory assay

In-vitro anti-inflammatory activity of extracts were assessed by evaluating their ability to inhibit hypotonic solution and heat-induced haemolysis of human erythrocytes [18]. Red blood cells (RBCs) suspension was prepared by taking fresh human blood (10 ml) from healthy human volunteers (n = 03) without a history of oral contraceptive or anticoagulant therapy and was transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline.

Heat induced hemolysis

Potion 5ml of the isotonic buffer containing 31.25, 62.50, 125, 250, 500 µg/ml of ethanol solution of plants extracts were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension 50µl was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54º C for 20 min in a water bath. The other pair was maintained at 0-5 ^{0}C in an ice bath.

The reaction mixture was centrifuged for 5 min at 5000 rpm and the absorbance of the supernatant was measured at 540 nm using spectrophotometer. Aspirin 31.25, 62.50, 125,250, 500µg/ml was used as a reference standard [19-20]. The percentage inhibition of haemolysis was calculated according to the equation:

% inhibition of haemolysis=

$$100 \times \left[1 - \frac{OD_2 - OD_1}{OD_3 - OD_1} \right]$$

Where OD_1 and OD_2 are test sample unheated and heated, respectively, and OD_3 is control sample heated

Hypotonic solution induced hemolysis

31.25, 62.50, 125, 250, 500 µg/ml of sample extracts were taken and 2ml hyposaline (0.25 % NaCl) was added. Then1 ml (.15M) sodium phosphate buffer was added in each test tube and 0.5 ml 10 % RBC solution was added in each test tube. Mixtures were incubated for 10 min at room temperature. Then the mixtures were centrifuged at 5000 rpm for 5 min. Absorbance was taken at 540 nm. Aspirin 31.25, 62.50,125,250, 500 µg/ml was used as standard [19-20]. The percentage inhibition of haemolysis was calculated according

to the equation:
% inhibition of haemolysis =100× {(OD_1-OD_2)/OD_1} where, OD_1= Optical Density of hypotonic buffered saline solution (control) and OD_2= Optical density of the test sample in hypotonic solution.

Thrombolytic assay
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 [21].

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 [21].

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.

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.

Statistical analysis
Statistical comparisons were performed using Microsoft Excel, 2016. Mean values ± SD. were calculated for the parameters where applicable.

Results
Phytochemical screening

Table 1: Phytochemical screening of CEE of MC, MM and MS

Test	CME
Alkaloids (a) Dragendorff's Test	+
(b) Mayer's Test	+
(c) Wagner's Test	+
Terpenoids	+
Tannins	+
Saponins	-
Glycosides (a) Anthraquinone Glycoside (Borntrager's Test)	-
(b) Cardiac Glycoside (Keller-Killiani Test)	+

+= presence, - = absence and CME= Crude ethanolic extracts

Anti-inflammatory activity
The MC, MM and MS showed a concentration dependent anti-inflammatory activity and the percent of protection increased with increase in the concentration of the samples. MC, MM, MS and aspirin showed 27.98±0.31, 12.588±0.316, 16.444±0.070 and 28.252±0.405 and 80.943±0.644, 71.465±0.166, 60.351±0.406 and 92.894±0.304 (Table 2, Fig. 1A) inhibition of heat induced RBC haemolysis at 31.25 and 500 µg/ml, respectively. Among three species, MC showed higher activity compared to the other species tested. The order of anti-inflammatory activity was: Aspirin > MC >MM > MS. Hypotonicity solution induced RBC membrane stabilization test result shown in Table 2 and Fig. 1B. At the concentration of 31.25 and 500µg/ml, MC, MM, and MS showed 61.766±0.184, 54.355±0.687, 55.152±0.445 and 87.098±0.050, 86.242±0.454, 77.709±0.436 % inhibition, respectively, whereas aspirin at above concentration showed 61.559±0.234 and 97.520±0.385 % inhibition of RBC haemolysis. Compared to standard Aspirin, MC showed remarkable activity compared to the other species. The order of anti-inflammatory activity was: Aspirin > MC >MM > MS.

Table 2: Effect of MC, MM and MS and Aspirin on Heat-induced and hypotonicity-induced haemolysis of RBC membrane

Sample	Concentration (µg/ml)	% of inhibition of Haemolysis	
		Heat Induced	Hypotonic solution Induced
MC	31.25	27.98±0.313	61.766±0.184
	62.5	45.085±0.288	79.451±0.493
	125	57.411±0.380	82.669±0.270
	250	79.447±0.518	86.744±0.204
	500	80.943±0.644	87.098±0.050
MM	31.25	12.588±0.316	54.355±0.687
	62.5	47.676±0.231	70.682±0.319
	125	63.297±0.127	78.181±0.135
	250	69.639±1.053	81.577±0.153
	500	71.465±0.166	86.242±0.454
MS	31.25	16.444±0.070	55.152±0.445
	62.5	20.566±0.204	57.278±0.134
	125	28.929±0.061	67.908±0.397
	250	41.631±0.396	75.849±0.184
	500	60.351±0.406	77.709±0.436
Aspirin	31.25	28.252±0.405	61.559±0.234
	62.5	64.036±0.558	79.215±0.568
	125	72.084±0.181	88.869±0.134
	250	86.896±0.447	94.420±0.265
	500	92.894±0.304	97.520±0.385

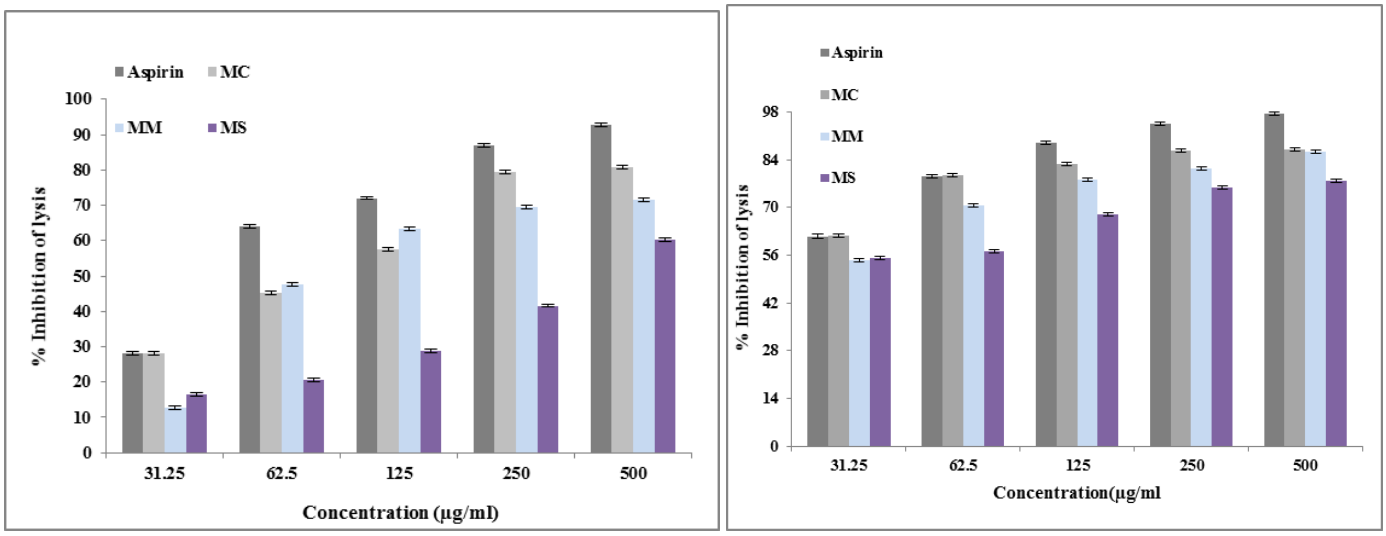


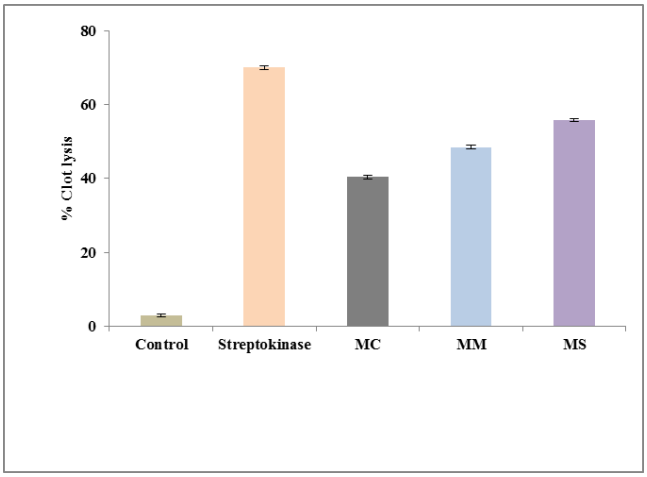
Fig 1: Comparative Percentage of inhibition of lysis (anti-inflammatory activity) of MC, MM, MS and Aspirin using A) Heat induced hemolysis and B) Hypotonicity induced hemolysis. The error bar represents the SD.

Thrombolytic assay

Percentage of clot lysis obtained after treating clots with different concentrations of sample was shown in Table 3 and Fig. 2. The *in vitro* thrombolytic activity study revealed that three species showed mild clot lysis activity. The percentage of weight loss of clot after application of extract solution was taken as the functional indication of thrombolytic activity.

Table 3: Effect of herbal extracts on in-vitro clot lysis

Sample	Mean±SD (% Clot Lysis)
Control	2.94±0.523
Streptokinase	70.00±0.432
MC	40.36±0.113
MM	48.44±0.342
MS	55.80±0.543



Discussion

Now a day, the search for phytochemicals possessing anti-inflammatory and thrombolytic properties have been on the rise due to their potential use in the therapy of various chronic and infectious diseases [11-12,14-15]. Plant phenolic compounds have been found to possess anti-inflammatory activity [22-23]. Our results corroborate that 70% ethanolic extract of *Mikania cordata* showed highest percentage of inhibition of haemolysis and *Mikania scandens* showed highest percentage blood clot lysis among the three extracts. These activities may be due to strong occurrence of polyphenolic compounds such as flavonoids, tannins, terpenoids, phenols and saponins. The anti-inflammatory activity was comparable with standard aspirin, however, thrombolytic activity was not significant compared to Streptokinase. Results of our findings confirmed the use of MC, MM and MS as traditional medicine [1, 7, 13]. The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. These findings provide scientific evidence to support traditional medicinal uses and indicate a promising potential for the development of an anti-inflammatory and thrombolytic agent from *Mikania cordata*, *Mikania micrantha* and *Mikania scandens.*

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References

1. Hajra S, Mehta A, Pandey P, John J, Mehta P. Antibacterial property of crude ethanolic extract of Mikania micrantha. Asian J Exp Biol Sci. 2010; 1:166-168.
2. Banerjee S, Chanda A, Adhikari A, Das AK, Biswas S. Evaluation of Phytochemical Screening and Anti Inflammatory Activity of Leaves and Stem of *Mikania scandens* (L.) Wild. Ann Med Health Sci Res. 2014; 4: 532-536.
3. Hannah MAC, Krishnakumari S. Quantitative estimation of plant metabolites in the hot aqueous seed extract of watermelon (*Citrullus vulgaris* Schrad.). JMPS. 2015; 3:107-111.
4. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnol Mol Biol Rev. 2007; 1:97-104.
5. Farnsworth NR. Biological and phytochemical screening of plants. J Pharm Sci. 1966; 55:225-276.
6. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2013; 4:177.
7. Ittiyavirah SP, Sajid KP. Anti-stress activity of *Mikania*

micrantha Kunth roots in Wistar albino rats. JSIR. 2013; 2:999-1005.
8. Suyenaga ES, Reche E, Farias FM, Schapoval EE, Chaves CG, Henriques AT. Anti-inflammatory investigation of some species of Mikania. Phytother Res. 2002; 16:519-23.
9. Alves CF, Alves VB, de Assis IP, Clemente-Napimoga JT, Uber-Bucek E, Dal-Secco D *et al*. Anti-inflammatory activity and possible mechanism of extract from *Mikania laevigata* in carrageenan-induced peritonitis. J Pharm Pharmacol. 2009; 61:1097-1104.
10. Ysrael MC, Croft KD. Inhibition of leukotriene and platelet activating factor synthesis in leukocytes by the sesquiterpene lactone scandenolide. Planta Med. 1990; 56:268-270.
11. Freitas TP, Silveira PC, Rocha LG, Rezin GT, Rocha J, Citadini-Zanette V *et al*. Effects of *Mikania glomerata* Spreng. and *Mikania laevigata* Schultz Bip. ex Baker (Asteraceae) extracts on pulmonary inflammation and oxidative stress caused by acute coal dust exposure. J Med Food. 2008; 11:761-766.
12. Pérez-Amador MC, Ocotero VM, Balcazar RI, Jiménez FG. Phytochemical and pharmacological studies on *Mikania micrantha* HBK (Asteraceae). Fyton. 2010; 79:77-80.
13. Nayeem AA, Khatun A, Rahman MS, Rahman M. Evaluation of phytochemical and pharmacological properties of Mikania cordata (Asteraceae) leaves. J Pharmacog Phytother, 2011; 3:118-123
14. Joshi A, Bhobe M, Saatarkar A. Phytochemical investigation of the roots of *Grewia microcos* Linn. J Chem. Pharm. Res. 2013; 5:80-87.
15. Abdullahi MN, Ilyas N, Ibrahim H. Evaluation of phytochemical screening and analgesic activity of aqueous extract of the leaves of *Microtrichia perotitii* dc (Asteraceae) in mice using hotplate method. Med. Plant Res. 2013; 3:37-43.
16. Ayoola GA, Coker HB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC *et al*. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. Trop. J. Pharm. Res. 2008; 7:1019-1024.
17. Banso A, Adeyemo S. Phytochemical screening and antimalarial assessment of *Abutilon mauritianum, Bacopa monnifera* and *Datura stramonium*. Biokemistri. 2006; 18:39-44.
18. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf VO. Membrane stabilizing activity–a possible mechanism of action for the anti-inflammatory activity of Cedrusdeodara wood oil. Fitoterapia. 1999; 70:251-57.
19. Sadique J, Al-Rqobahs WA, Bughaith EI-Gindi Ar. The bioactivity of certain medicinal plants on the stabilization of RBS membrane system. Fitoterapia. 1989; 60:525-532.
20. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. I. J. Pharm. Pharm. Sci. 2010; 2:146-155.
21. Daginawala HF, Prasad S, Kashyap RS, Deopujari JW, Purothi HJ. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. Thrombosis J. 2006; 2:1-4.
22. Halliwell B. Antioxidants in human health and disease. Annu Rev Nutr.1996; 6:33-50.
23. Garg VKR, Jain M, Sharma PKR, Garg G. Anti-inflammatory activity of Spinacia oleracea. IJPPR. 2010; 1:1-4.