Original Research

Bagcilar Medical Bulletin 2018;3(1):1-6
DOI: 10.5350/BMB20171220090230

Thrombolytic, Membrane Stabilizing, Analgesic activities along with Phytochemical Screening of the Methanolic Extract of Xanthium indicum Koenig Fruits

Sharmin Akter[1], Rubaiya Anjum[2], Md. Monir Hossain[3], Md. Saddam Hussain[1], Md. Mamun Or Rashid[1]

ABSTRACT

Objective: This study aimed to assess the thrombolytic, membrane stabilizing, and analgesic activities of the methanolic extract of the fruits of *Xanthium indicum* Koenig and undertook a phytochemical screening.

Material and Methods: A qualitative analysis method to evaluate phytochemicals was adopted. Analgesic efficacy was assessed *in vivo* using the acetic acid-induced writhing method in a mouse model. Membrane-stabilizing and thrombolytic tests were done in vitro measuring the percentage of inhibition of hypotonic solution-produced hemolysis and running a clot disruption assay, respectively.

Results: Crude methanolic extract was used for phytochemical screening, confirming the presence of reducing sugars, tannin, saponin, protein, phenol, and diterpenes. In the analgesic activity test, a 500 mg/kg dose of crude extract showed 40% inhibition of writhing, while 200mg/kg showed a reduction of 22.64%. In the membrane stabilizing activity test, 10 mg/ml extract resulted in the highest inhibition rate of hemolysis with a value of 51.79%, while for acetyl salicylic acid (0.10 mg/ml), this value was 71.35%. In the thrombolytic activity test, 10 mg/ml plant extract showed 27.11% clot-lysis, which was the maximum among our tested concentrations; however, 40.08% lysis was achieved by the standard drug streptokinase. In all cases, we found a dose-dependent response. Tannin and flavonoid are known to be responsible for analgesic and thrombolytic response.

Conclusion: As the methanolic extract of the fruits of *X. indicum* possesses potential pharmacological effects, the plant should be scrutinized comprehensively to detect the bioactive components present and the exact mechanism of their action in view of a drug development program.

Keywords: Xanthium, writhing, membrane stabilizing, thrombolytic agents

ÖZ

Xanthium indicum koenig meyvelerinin metanolik ekstraktının fitokimyasal tarama ve farmakolojik araştırması

Amaç: Bu çalışma, *Xanthium indicum* Koenig meyvelerinin metanolik özütünün trombolitik, membran stabilize edici ve analjezik aktivitelerini fitokimyasal taramayla değerlendirmeyi amaçlamaktadır.

Gereç ve Yöntem: Bitki kimliğini değerlendirmek için kalitatif analiz yöntemi benimsenmiştir. *In-vivo* analjezik etkinlik, fare modelinde asetik asitle indüklenen kıvrımlama yöntemi kullanılarak değerlendirildi. *In-vitro* membran stabilizasyonu ve trombolitik test, sırasıyla, hipotonik solüsyon tarafından üretilen hemoliz ve pıhtı bozunum deneyi inhibisyonu ile yapıldı.

Bulgular: Metanolik özüt, indirgeyici şekerler, tanin, saponin, protein, fenol ve diterpenlerin varlığını doğrulayan fitokimyasal taramada kullanıldı. Analjezik aktivite testinde, 500 mg/kg dozunda ham ekstrakt %40 oranında kıvrım inhibisyonu gösterirken, 200 mg/kg %22.64 oranında görülmüştür. 10 mg/ml ekstre membran stabilize etme aktivitesi testinde %51.79 değerinde en yüksek hemoliz oranını engeller; asetil salisilik asit (0.10 mg/ml) için bu değer %71.35'dir. Trombolitik aktivite testinde, 10 mg/ml bitki özütü, test edilen konsantrasyonumuz arasında maksimum olan pıhtı-lizizin %27.11'i; Bununla birlikte standart ilaç streptokinaz ile %40.08 lizis meydana geldi. Her vakada doza bağlı yanıtı bulduk. Tannin ve flavonoidin analjezik ve trombolitik cevaptan sorumlu olduğu bilinmektedir.

Sonuç: *X. indicum*'un meyvelerinin metanolik ekstraktı potansiyel farmakolojik etkilere sahip olduğundan bitki, ilaç geliştirme programı için kesin etki mekanizmaları ile doğal biyoaktif bileşenlerin ortaya çıkarılması için kapsamlı bir şekilde incelenmelidir.

Anahtar kelimeler: Xanthium, kıvranma, membran stabilizasyonu, trombolitik ajanlar

[1]Noakhali Science and Technology University, Department of Pharmacy, Sonapur, Noakhali-3814, Bangladesh
[2]Rajshahi University, Department of Applied Chemistry and Chemical Engineering, Rajshahi, Bangladesh
[3]Jagannath University, Department of Pharmacy, Chittaranjan Avenue, Dhaka, Bangladesh

Corresponding author:
Md. Mamun Or Rashid,
Noakhali Science and Technology University, Department of Pharmacy, Sonapur, Noakhali-3814, Bangladesh
E-mail address: mamun_nstu@yahoo.com

Date of submission: December 20, 2017
Date of acceptance: February 23, 2018

Citation: Akter S, Anjum R, Hossain MM, Hussain MS, Rashid MMO. Thrombolytic, membrane stabilizing, analgesic activities along with phytochemical screening of the methanolic extract of xanthium indicum koenig fruits. Bagcilar Medical Bulletin 2018;3(1):1-6.
https://doi.org/10.5350/BMB20171220090230

Introduction

The term Xanthium is derived from the Greek Xanthos, meaning yellow, from the ancient name of a certain plant, whose fruit was used to dye the hair (1). *Xanthum indicum* is a coarse annual herb growing about a meter or more tall. *X. indicum* fruits contain glucoside (strumaroside), β-sitosterol, stigmasterol, 3,5-di-O-caffeoylquinic, polyphenols, 1,3,5-tri-O-caffeoylquinic acid, etc. (2).

Pain is a complicated disagreeable phenomenon composed of sensory experiences that include time, space, intensity, cognition, emotion, and motivation, originating from damaged tissue or abnormal physiological conditions. Due to the adverse effects of conventional analgesic drugs (e.g. gastric irritation, suffering, and opiate-induced dependency), their use in treatments as analgesic agents may be unsuccessful and sometimes life-threatening. Hence, analgesic treatment without those side effects is being sought all around the globe as a replacement for NSAIDs and opiates. Therefore, plant-based traditional medicine has received a huge interest, given the availability, low cost, small side effects, etc. (3).

On the other hand, myocardial or cerebral infarction, atherothrombotic disease may generate thrombus in arteries. (4,5). Thrombolytic substances like tissue plasminogen activator and urokinase are used to dissolve clots within the blood vessels that have deposited already (6-8). However, all of these drugs have certain limitations, such as a risk of developing fatal disorders, anaphylactic reactions, hemorrhage, lack of specificity, etc. Moreover, nowadays it is found that immunogenicity (due to thrombolytic therapy with streptokinase) may restrict the healing attempts of diseases for some patients (9). Agents isolated from plant sources are likely to be inexpensive and less antigenic. Accurate studies have been designed concerning the search for and development of different plant and microorganism sources that possess anticoagulant, anti-platelet, thrombolytic activity, etc. (10-15).

Inflammation is one of the key outcomes of a number of pathological disorders. Physiologically functional arachidonate metabolites are generated from inflammatory cells alongside a complex mixture of growth factors (16,17). These mediators work collectively to increase vasodilation and blood vessel permeability, which further leads to an elevation of blood flow, plasma protein exudation, and relocation of neutrophils from the outer region of veins and arteries to the inner area of damage tissue. Some pathological data also demonstrate that inflammation is the main initiator for developing atherosclerosis (18-20). The erythrocyte membrane is similar to the lysosomal membrane. As a result, it could be hypothesized that agents for stabilizing the RBC could be used to stabilize the lysosomal membrane (21). Those agents affect the release and action of mediators such as prostaglandins, histamine, leukotrienes, serotonin, etc. (22).

X. indicum is included in the list of potent medicinal plants of Bangladesh. Its fruit is used against sores of the lips and mucous membranes. Seeds are used for healing inflammatory swelling, bladder infections, herpes, erysipelas, etc. In this study, we evaluated the thrombolytic, membrane-stabilizing, and analgesic actions of the methanolic extract of fruits from *X. indicum* and undertaken phytochemical screening.

Material and Methods

Material

Lyophilized alteplase/streptokinase (Genentech Inc., USA), acetyl salicylic acid or aspirin (Sigma Aldrich Corporation, USA), and other chemicals used in the study were bought from the laboratory of the Department of Pharmacy, NSTU, Bangladesh.

Human subjects and experimental animals

For the thrombolytic and membrane-stabilizing activity test, blood samples were taken from young healthy persons who did not have a record of using birth-control pills or anticoagulant therapy. Twenty swiss albino mice (weight: 25-30 g, age: 30-35 days) were obtained from JU, Bangladesh (from the animal house of the university). They were kept under controlled temperature (25±2°C) and RH (60-70%) for 7 days with rodent food and water to adapt before starting the experiment.

Preparation of the methanolic extract

Mature fruits of *X. indicum* (Family: *Asteraceae*) were collected in various areas of Noakhali district, Bangladesh. The plant material was prepared by taxonomist Naimur Rahman of the National Herbarium, Mirpur, Dhaka, and a specimen was kept there for future reference. For preparing the methanolic extracts, 500 gm ground powder material was filled into an opaque colored glass vessel and macerated with methanol (approx. 1800 ml). The container was sealed tightly and stored for 15 days with occasional shaking. Then the substance

macerated in methanol was first passed through a cotton filter and subsequently through filter paper.

Phytochemical screening

Qualitative tests were carried out to evaluate the presence of phytoconstituents in the *X. indicum* extract. By using standard phytochemical procedures, the presence of these constituents in the extract was identified (23).

Study of analgesic activity

To evaluate the analgesic activity of *X. indicum*, the acetic acid-induced writhing method was used in a mouse model (24). Reflexive writhing was induced in the mice. At first, 1.0% acetic acid solution was injected intraperitoneally and the animals' specific contraction (writhing) observed. Comparisons of writhing were made against a standard (Indomethacin), control, and test samples. To prepare the test material at a dose of 250 and 500 mg/kg body weight, 62.5 and 125 mg plant extract, respectively, were triturated with a small amount of DMSO. Proper mixing was done and distilled water was added slowly for adjusting the suspension volume to 2.5 ml. The dose of crude sample extracts was administered orally 30 minutes before acetic acid injection. Distilled water was given as control. Analgesic activity was assessed by counting the number of writhing movements. Experimental mice were selected arbitrarily and divided into four groups (n=5 for each group) designated G-I (control/vehicle only), G-II and G-III for plant extract, and G-IV (standard/indomethacin). Five minutes after injecting acetic acid, a count of the abdominal contractions was done (10 minutes duration for each mouse). The percentage of writhing inhibition was quantified according to the following formula:

$$\text{Inhibition} = \frac{\text{Mean no. of writhing movements (control)} - \text{Mean no. of writhing (test)}}{\text{Mean no. of writhing movements (control)}} \times 100$$

Study of thrombolytic activity

Clot lysis activity (*in vitro*) of *X. indicum* was measured by using the methodology described in Prasad et al. with slight modifications (25). Blood (approx. 7 ml) was taken from healthy people (n=5) and kept in different sterilized micro-centrifuge tubes followed by incubation (37°C, 45 minutes). After the formation of clots, serum was discarded totally from the tubes (without disturbing the clots formed already) and the weight of the clots determined (with a Cubis analytical balance: Model: Sartorious, MSE225S-100-DA, Germany). Proper labeling of the all tubes containing clot was done and various concentrations of the extract were added accordingly. We used 10 mg, 20 mg, 30 mg, 40 mg, 50 mg plant extract in 5 ml distilled water, stirring thoroughly, to prepare 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml plant extract, respectively. Distilled water was used as a control, and streptokinase (30,000 IU) was used as a standard. The mixtures were incubated once again (37°C, 90 minutes). Then the fluid was discarded from the tubes and the tubes weighed again to determine the differences. Finally, the weight differences were estimated and results were calculated as clot lysis percentage using the equation below:

Clot lysis (%) = (Weight of released clots /Total weight of the clots) × 100

Study of membrane-stabilizing activities

We followed the method of Rashid et al. for studying the membrane stabilizing effect of *X. indicum* (24). The blood collected from healthy volunteers (n=5) was rinsed by using isotonic solution (0.9%). In this aspect, NaCl solution (0.16 M) or nonelectrolyte solution (0.3 M) is almost isotonic with human erythrocytes (RBC). 4.505 g NaCl was dissolved in distilled water and mixed properly to prepare 500 ml isotonic solution (154 mM). 1.463 g NaCl was added and mixed properly to prepare 500 ml hypotonic solution (50 mM).

After collecting the blood, we added EDTA to prevent clotting. Ten mM sodium phosphate buffer (pH 7.4) was prepared by centrifuging at 3000 rpm for 10 minutes and used for washing the blood. Washing was done for three times with proper care. The suspension which we collected finally was a stock erythrocyte (RBC) suspension.

The solution we used in this experiment was hypotonic in nature. The sample consisted of RBC suspension with 5 ml of hypotonic solution in 10 mM Na_2PO_4 buffer containing either various concentrations of extract or standard drug. Before this, we prepared the concentration of 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, 10 mg/ml plant solutions by using 10 mg, 20 mg, 30 mg, 40 mg, and 50 mg plant extract, respectively, in 5 ml distilled water. Distilled water was used as control and acetyl salicylic acid was used as standard in this experiment. The concoctions were incubated at 25°C for 10 minutes, centrifuged

(3000 rpm, 10 minutes) and the absorbance (OD) was measured at 540 nm wavelength using UV-spectrophotometer (Shimadzu, Japan). Suppression of hemolysis was calculated by using the formula

Inhibition of hemolysis (%) = {(OD1- OD2)/ OD1} x 100

Where,

OD1 = Optical density of hypotonic-buffered solution (control)

OD2 = Optical density of tested samples in hypotonic solution

Statistical Analysis

One-way ANOVA followed by Dennett's t-tests were performed for analyzing the data of this study. *p≤0.05 was considered statistically significant.

Results

Evaluation of phytochemical screening

Qualitative chemical tests were carried out for identifying the phytochemicals present in *X. indicum* (methanolic extract). The outcomes of the different tests for detecting and ascertaining the chemical components are shown in Table 1.

Table 1: Phytochemical screening test results of *X. indicum* fruit extract.

Chemical group	Results
Saponin	-
Phytosterol	-
Reducing sugars	+
Alkaloid	-
Tannin	+
Carbohydrate	-
Protein	+
Phenol	+
Flavinoid	+
Diterpenes	+

(+) = present (-) = absent

Evaluation of analgesic activity

The effect of *X. indicum* against writhing in animals is summarized in Table 2. The plant extracts reduce writhing in a dose-dependent manner. The reduction is significant when compared with controls. Indomethacin is used as a standard in this experiment.

Evaluation of membrane stabilizing activity

In-vitro membrane-stability activity of the methanolic extract of *X. indicum* was assessed for confirming its activity. It is observed that methanolic extract of *X. indicum* has a dose-dependent membrane-stabilizing activity (Figure 1). The percentage of inhibition of RBC hemolysis is 35.54% for the crude methanolic extract of *X. indicum* (conc. 2 mg/ml). This effect increases proportionally with increasing extract

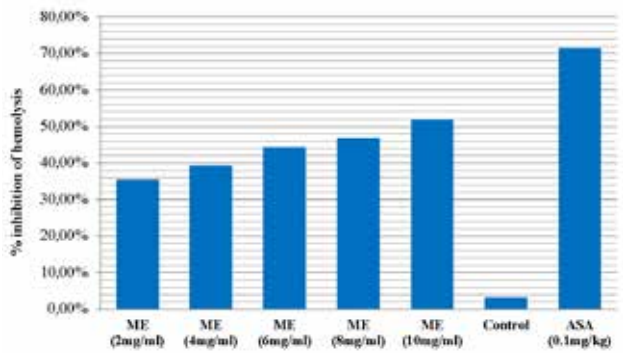

Figure 1: Percentage of inhibition of hemolysis of RBC.
ME= Methanolic Extract, ASA=Acetyl Salicylic Acid

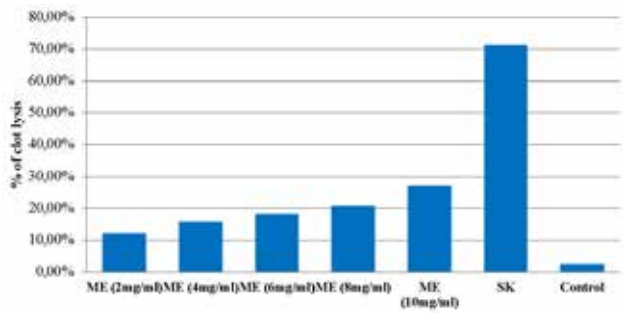

Figure 2: Clot lysis activity of the methanolic extract of *X. indicum* fruits.
ME= Methanolic Extract, SK= Streptokinase

Table 2: Assessment of analgesic activity of methanolic *X. indicum* fruit extract in mice.

Treatment group	Average number of writhing (after treatment)	Percentage of inhibition
G-I (control)	11.33 ± 0.58	-
G-I (250 mg/kg b.w.)	9.83 ± 0.76*	22.64
G-II (500 mg/kg b.w.)	6.50 ± 0.50*	40.00
G-IV (Indomethacin, 25 mg/kg)	5.33 ± 0.58*	46.64

Values indicated as Mean ± STD (n=5); p*<0.05 is significant.

concentration, and the highest inhibition of 51.79% was found for 10 mg/ml of extract. The standard (acetyl salicylic acid; concentration: 0.1 mg/kg) decreased hemolysis of RBC by 71.35%.

Assessment of thrombolytic efficacy

Thrombolytic efficacy of the crude extract in our study is shown in Figure 2. We found that *X. indicum* possesses a dose-dependent thrombolytic activity. Highest clot lysis (27.11%) was found for the plant extract at a 10 mg/ml concentration. Streptokinase (an enzyme used as standard) induced 71.35% inhibition and negative control (water) provided only 2.55% clot lysis.

Discussion

In the phytochemical screening test, we found that *X. indicum* (methanolic extract) contains reducing sugar, tannin, protein, phenol, flavonoid, diterpenes, etc. So, there is a great chance to find bioactive components in this plant extract. Both peripheral and central analgesia can be identified by the test of acetic acid-induced abdominal pain, followed by counting the number of writhing movements of mice. The sensation of pain is triggered by a local reaction starting with the release of arachidonic acid from the tissue phospholipid (26) via cyclooxygenase (COX) producing prostaglandin (PG). Writhing induced by acetic acid injection may be linked with an increment of PGE2 and PGF2α and lipoxygenase products followed by the rise of inflammatory pain by accelerating capillary permeability. This test was effective for achieving locally active analgesia (27,28). Our results showed that methanolic extract of the fruits of *X. indicum* exhibit a significant inhibitory effect on acetic acid-induced writhing. This inhibition is also dose-dependent. The COX inhibitory and antioxidant potential may be decreased either by the synthesis of free arachidonic acid or by inhibiting the enzymatic activities responsible for the production of PG. According to Das et al., flavonoids and tannins might be accountable for the analgesic activity (29). Phytochemical screening in this study also found that methanolic extract of *X. indicum* contains flavonoid and tannins. Perhaps these constituents are responsible for the analgesic activity of *X. indicum*.

Many studies (both *in vitro* and *in vivo*) show that flavonoids exert a stabilizing effect on lysosomes. In addition, tannins, and saponins are also capable of improving the stabilizing effect of erythrocytes because of having a binding capability with cations and other biomolecules (26). Our tested extract also contains flavonoids, saponin, and tannin. So there is a great possibility for *X. indicum* to have a membrane-stabilizing effect due to the presence of these phytoconstituents.

A number of studies have been done to research the anti-thrombolytic effects of natural sources in order to prevent cardiopulmonary disorders and strokes. In order to find better cardio-protective agents from plant sources, we have investigated the thrombolytic activity of *X. indicum* fruits. It was noticed that crude methanolic extract of *X. indicum* showed moderate clot lysis efficacy (27.11%) at a dose of 10 mg/ml. The presence of saponins and tannins in the plant extract may play an important role for this activity by affecting the activation of plasminogen. Plasminogen works as a fibrin-dependent mechanism similar to that of streptokinase (the standard drug). In this case, plasminogen induces extreme synthesis of plasmin, which may disrupt fibrin (the prime component of thrombi) and result in blood clots being easier more easily dissoluble (24,25).

Conclusion

Based on our experimental results, we conclude that the methanolic extract of *X. indicum* fruits possesses a high amount of phytoconstituents that might be bioactive. The crude extract possesses remarkable analgesic and membrane-stabilizing activities. In addition, it also showed moderate thrombolytic efficacy. Advanced research explorations are warranted to elucidate the exact bioactive compounds liable for the above activities for understanding the mechanism of such activities for a drug development program.

Contribution Categories	Name of Author
Development of study idea	M.M.O.R, S.A.
Methodological design of the study	S.A., M.M.O.R., R.A.
Data acquisition and processing	S.A., M.M.H., M.S.H.
Data analysis and interpretation	M.S.H., S.A., M.M.H.
Literature review	R.A., S.A., M.M.H.
Manuscript write-up	M.M.O.R, S.A., M.S.H., R.A.
Manuscript review and revision	M.M.O.R., M.M.H., R.A., M.S.H

Ethical approval and consent to participate: Authors declare that 'Principles of the laboratory animal care' (NIH publication no. 85-23, revised 1985) and 'national animal care laws' were followed strictly during the experiment. Research protocol and plan were approved by the institutional ethical committee.

Acknowledgement: Authors show gratitude to the authority of JU, Bangladesh for supplying mice and formulated feed from the animal house.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support.

References

1. Munz PA, Keck DD. A California flora and supplement. University of California Press, Berkeley, CA. 1973.
2. Rastogi RP, Mehrotra BN, Sinha S, Seth R. Compendium of Indian Medicinal Plants. Central Drug Research Institute, Lucknow and Publications and Information Directorate, New Delhi, India 1990;2.
3. Kumara NKVMR: Identification of strategies to improve research on medicinal plants used in Sri Lanka. In: WHO Symposium; University of Ruhuna, Galle, Sri Lanka 2001.
4. Davies MJ, Thomas AC. Plaque fissuring: the causes of acute myocardial infarction, sudden ischaemic death, and crescendo angina. Br Heart J 1985;53(4):363-373. [CrossRef]
5. DeWood MA, Spores J, Notske R, Mouser LT, Burroughs R, Golden MS, et al. Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. N Engl J Med 1980;303(16):897-902. [CrossRef]
6. Prasad S, Kashyap RS, Deopujari JY, Purohit HY, Taori GM, Daginawala HF. Effect of Fagonia Arabica (Dhamasa) on in-vitro thrombolysis. BMC Complement Altern Med 2007;7:36. [CrossRef]
7. Demrow HS, Slane PR, Folts JD. Administration of wine and grape juice inhibits in-vivo platelet activity and thrombosis in stenosed canine coronary arteries. Circulation 1995;91(4):1182-1188. [CrossRef]
8. Briggs WH, Folts JD, Osman HE. Administration of raw onion inhibits platelet-mediated thrombosis in dogs. J Nutr 2001;131(10):2619-2622. [CrossRef]
9. Leta GC, Mourao PAS, Tovar AMF. Human venous and arterial glycosaminoglycans have similar affinity for plasma low-density lipoproteins. BBA Mol Basis Dis 2002;1586(3):243-253. [CrossRef]
10. Li Z, Wang H, Li J, Zhang G, Gao C. Basic and clinical study on the antithrombotic mechanism of glycosaminoglycan extracted from sea cucumber. Chin Med J 2000;113(8):706-711.
11. Rajapakse V, Jung WK, Mendis E, Moon SH, Kim SK. A novel anticoagulant purified from fish protein hydrolysate inhibits factor XIIa and platelet aggregation. Life Sci 2005;76(22):2607-2619. [CrossRef]
12. Yamamoto J, Yamada K, Naemura A, Yamashita T, Arai R. Testing various herbs for antithrombotic effect. Nutrition 2005;21:580-587. [CrossRef]
13. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Development of an in-vitro model to study clot lysis activity of thrombolytic drugs. Thromb J 2006;4:14. [CrossRef]
14. Anwar AK, Ashfaq M, Nasveen MA. Pharmacognostic studies of selected indigenous plants of Pakistan. Pakistan Forest Institute, Peshawar NWFP, Pakistan 1979:15-35.
15. Gillman MW, Cupples LA, Gagnon D, Posner BM, Ellison RC, Castelli WP, et al. Protective effect of fruits and vegetables on development of stroke in men. JAMA 1995;273(14):1113-1117. [CrossRef]
16. Perianayagam JB, Sharma SK, Pillai KK. Anti-inflammatory activity of Trichodesma indicum root extract in experimental animals. J Ethnopharmacol 2006;104(3):410-414. [CrossRef]
17. Anosike CA, Obidoa O, Ezeanyika LUS. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (Solanum aethiopicum). DARU J Pharm Sci 2012;20:76. [CrossRef]
18. Munro JM, Cotran RS. The pathogenesis of atherosclerosis: atherogenesis and inflammation. Lab Invest 1988;58:249-261.
19. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801-809. [CrossRef]
20. Alexander RW. Inflammation and coronary artery disease. N Engl J Med 1994;331(7):468-469. [CrossRef]
21. Omale J, Okafor PN. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of Cissus multistriata, Afr J Biotechnol 2008;7(7):3129-3133.
22. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Membrane stabilizing activity — a possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil. Fitoterapia 1999;70:251-257. [CrossRef]
23. Howlader MSI, Sayeed MSB, Ahmed MU, Mohiuddin AK, Labu ZK, Bellah SF, et al. Characterization of chemical groups and study of anti-oxidant, antidiarrhoeal, antimicrobial and cytotoxic activities of ethanolic extract of Diospyros blancoi (Family: Ebenaceae) leaves. J Pharm Res 2012;5:3050-3052.
24. Rashid MM, Hussain MS, Rashid MMO, Halim MA, Sen N, Millat MS, et al. In-vivo analgesic potential in Swiss albino mice and in-vitro thrombolytic and membrane stabilizing activities of the methanolic extract from Suaeda maritime whole plant. Bagcilar Medical Bulletin 2017;2(1):13-18. [CrossRef]
25. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Effect of Fagonia arabica (Dhamasa) on in-vitro thrombolysis. BMC Complement Altern Med 2007;7:36. [CrossRef]
26. Ahmed F, Hossain MH, Rahman AA, Shahid IZ. Antinociceptive and sedative effects of the bark of Cerbera odollam Gaertn. Orient Pharm Exp Med 2006;6:344-348. [CrossRef]
27. Duarte IDG, Nakamura M, Ferreira SH. Participation of the sympathetic system in acetic acid-induced writhing in mice. Braz J Med Biol Res 1988;21:341-343.
28. Zakaria ZA, Ghani ZD, Nor RN, Gopalan HK, Sulaiman MR, Jais AM, et al. Antinociceptive, anti-inflammatory, and antipyretic properties of an aqueous extract of Dicranopteris linearis leaves in experimental animal models. J Nat Med 2008;62(2):179-187. [CrossRef]
29. Das BK, Al-amin MM, Russel SM, Kabir S, Bhattacherjee R, Hannan JMA. Phytochemical screening and evaluation of analgesic activity of Oroxylum indicum. Indian J Pharm Sci 2014; 76(6):571-575.