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 2016; 5(6): 366-370
Received: 18-09-2016
Accepted: 19-10-2016

Amer Khorshed Alam
Department of Pharmacy,
The University of Asia Pacific,
Green Road, Dhaka, Bangladesh

Chand Sultana Khatun
Department of Pharmacy,
Mawlana Bhashani Science and Technology University, Santosh,
Tangail, Bangladesh

Correspondence
Amer Khorshed Alam
Department of Pharmacy,
The University of Asia Pacific,
Green Road, Dhaka, Bangladesh

Phytochemical analysis and antioxidant, analgesic and thrombolytic activity investigation of methanol extract of *Pisum sativum* seed

Amer Khorshed Alam and Chand Sultana Khatun

Abstract
The aim of the present study was to investigate the presence of major phytochemicals and the analgesic, antioxidant and thrombolytic effects of methanol extract of the seeds of a plant *Pisum sativum*. Phytochemical analysis of the extract of *Pisum sativum* seeds indicated the presence of Glycoside, Alkaloid, Flavonoids, Saponin and Tannin types of compounds. Significant analgesic effect was monitored (p<0.05) as the oral extract dose of 500 mg/kg showed 42.75% inhibition of writhing movements compared to standard drug Diclofenac which inhibited 74.20% writhing movement. Also in present study the methanol extract of *Pisum sativum* seeds have mild antioxidant activity. The IC_{50} of the extraction was 489.25 µg/ml, whereas IC_{50} of reference anti-oxidative agent Ascorbic acid was 16.28 µg/ml. *Pisum sativum* extracts showed thrombolytic activity of (18.25 ± 0.04%) comparing with standard Streptokinase (66.98 ± 0.11%).

Keywords: *Pisum sativum*, antioxidant, thrombolytic, analgesic

1. Introduction
Plants and plant-derived sources not only provide us foodstuff, shelter but also they provide remedies for many years. Different chemical constituents contained in plant exhibit different activities for alleviating abnormal health of human or animals. Therefore, traditional medicine practitioners appreciate to use different parts of plant having several chemical constituents. Considerable portion of current diseases are caused due to the 'oxidative stress' which results in enormous amount of free radicals, causing tumor, atherosclerosis and cardiovascular illnesses [1]. Cells of the human body ensure themselves against harm caused by free radicals by catalysts such as ascorbic acid, tocopherol and glutathione [2]. However, frequently these defensive systems are becoming upset by different neurotic techniques, and cell reinforcement supplements are imperative to battle oxidative harm. This is because much consideration has been controlled towards the improvement of medicine with solid cell reinforcement properties.
Thrombus formed in the circulatory system due to the loss of homeostasis leads to complications such as vascular-blockage, atherosclerosis, myocardial or cerebral localized necrosis and in many cases death. Current anticoagulants have many limitations such as high risk of bleeding due to intracranial hemorrhage, severe anaphylactic reaction and lack of specificity to gastrointestinal bleeding or hypertension that is why tremendous efforts have been directed towards the discovery and development of natural products such as the oral anticoagulants [3].
Drugs which are currently used for pain management and inflammatory conditions in most cases are either steroidal like corticosteroids or non-steroidal like NSAIDs. All of these drugs possess more or less adverse effects such as renal failure, allergic reactions, hearing loss or affecting platelet function. On the contrary many plant derived medicines had been used from centuries ago without any serious adverse effects. Thus, large scale researches should be conducted to develop new pain management medicines with plant based origin [4, 5].
Pisum sativum is an annual plant, with a life cycle of one year. It is a cool season crop grown in many parts of the world; planting can take place from winter to early summer depending on location. Seeds provide nutrients that are important for maintaining bone health. They are an excellent source of vitamin K1, which activates osteocalcin, the major non-collagen protein in bone. Osteocalcin anchors calcium molecules inside of the bone. Therefore, without enough vitamin K1, osteocalcin levels are inadequate and bone mineralization is impaired [6].
It also serves as a very good source of vitamin B6 and folic acid. These two nutrients help to reduce the buildup of a metabolic byproduct called homocysteine, a dangerous molecule can obstruct collagen cross-linking, resulting in poor bone matrix and osteoporosis.

One study showed that postmenopausal women who were not considered deficient in folic acid lowered their homocysteine levels simply by supplementing with folic acid by itself. In addition to affecting bone health, homocysteine contributes to atherosclerosis through its ability to damage the blood vessels, keeping them in a constant state of injury. Therefore the folic acid and vitamin B6 in green peas are supportive of cardiovascular health as well. In fact, folic acid is so important for cardiovascular function that a major 1995 study concluded that 400 micrograms per day of folic acid could prevent 28,000 cardiovascular deaths per year in the United States [7].

This research investigated the phytochemical presence, anti-oxidative potential, thrombolytic effect and analgesic effect of *Pisum sativum* seed's methanol extract.

2. Materials and Methods

2.1 Plant Materials

Pisum sativum seeds were collected from Mohammadpur, district Dhaka and the plant authentically were confirmed form the Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh.

2.2 Preparation of Plant Extract

The collected plant seeds were dried at room temperature (30 ± 3 °C), for 30 days to ensure the active constituents free from decomposition. The dried seeds were powdered in an electrical grinder after overnight drying in an oven below 50 °C. The powder was extracted with methanol at room temperature. The bottle were kept at room temperature and allowed to stand for 11 days with occasional shaking. When the solvent become concentrated, the liquid alcohol contents were filtered through cotton & then through filter paper (Whatman Filter Paper No. 1). Then, the solvents were allowed to evaporate using rotary evaporator at temperature 40-45 °C. Finally, a highly concentrated methanol crude extract was obtained.

2.3 Phytochemical Screening Methods

2.3.1 Test for Glycosides

2 ml solution of the extract was taken into a test tube. 1 ml mixture of Fehling solution was added into the test tube. The tube was placed in a water-bath at 60 °C. If a brick red color form that shows the presence of glycosides.

2.3.2 Test for Alkaloids

In testing for Alkaloids, about 0.5 g of each extract will be stirred with 5 ml of 1 percent aqueous hydrochloric acid on a water bath; 1 ml of the filtrate is to be treated with a few drops of mayer's reagent and a second 1 ml portion is to be treated the same way with Dragendorff's reagent. Presence of orange-red color indicates the presence of alkaloid.

2.3.3 Test for Flavonoids

A small quantity of test residue was dissolved in 5 ml of ethanol (95% v/v) and treated with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. If the pink, crimson or magenta color is developed within a minute or two that mean flavonoids are present.

2.3.4 Test for Tannins

About 5 g of each portion of plant extract will be stirred with 10 ml distilled water, filtered, and ferric chloride reagent will then be added to the filtrate. If dark green or deep blue color is obtained, tannins are present.

2.3.5 Test for Saponins

A few mg of the test residue was taken in a test tube and shaken vigorously with small amount of sodium bicarbonate and water. If stable, characteristic honeycomb like froth is obtained, saponins are present [8].

2.4 Analgesic property test

2.4.1 Drugs and Chemicals

Acetic acid was collected from laboratory of the University. The standard drug Diclofenac-Na was purchased from Square Pharmaceuticals Limited of Bangladesh.

2.4.2 Experimental Animals

Eight week-old Swiss albino mice were (50-52 g) purchased from Jahangirnagar University, Dhaka, Bangladesh and were housed in animals cages under standard environmental conditions (22-25 °C, humidity 60-70%, 12 hours light: 12 hours dark cycle). The mice were feed with standard pellet diet taken from, Jahangirnagar University Dhaka. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute.

2.4.3 Experimental Protocols

For Analgesic test 16 mice were divided into three groups-

- Negative Control (NC Group, Vehicle 0.5% MC, n = 3)
- Standard Group (ST, Diclofenac sodium 10 mg/kg, n = 3)
- Extract Group (ML Group, 500 mg/kg, n = 3)

For analgesic test all mice were divided into three groups (Control Group, Standard Group and Extract Group). Each group comprises 3 mice. Control group (received 0.5% Methyl cellulose), Standard Group (received Diclofenac sodium 10 mg/kg), and Extract Group (received 300 mg/kg extract).

2.4.4 Acetic acid-induced writhing test for Analgesic activity

The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. Acetic acid was administered intraperitoneally to NC group. Test samples and vehicle was administered orally 30 min before administration of 1% acetic acid to extract group. Diclofenac-Na was administered 30 min before administration of 1% acetic acid to standard group. The mice were observed for specific contraction of body referred to as "writhing" for the next 30 min [9, 10].

2.4.5 Statistical Analysis

All values were expressed as mean ± Standard Deviation (SD). Statistical comparison were performed by One-way analysis of variance (ANOVA), followed by using Dunnet test. Results were considered as significant when p values less than 0.05 ($p<0.05$).

2.5 Antioxidant Property Test

2.5.1 Reagents

Methanol, DPPH (1, 1 - diphenyl - 2 – picrylhydrazyl - hydrate)

2.5.2 Procedure

Stock solution of the plant extract was prepared in methanol from which a serial dilution was carried out to obtain concentration of 1 µg/ml, 5 µg/ml, 10 µg/ml, 50 µg/ml, 100 µg/ml, 500 µg/ml and 1000 µg/ml. Diluted solutions (2 ml)

were added to 2 ml of solution of DPPH then mixed and allowed to stand for 30 minutes for reaction to occur. The absorbance was determined at 517 nm and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against log concentration and from the graph IC_{50} was calculated. The experiment was performed 3 times and average absorption was noted for each concentration [11-13].

2.6 Thrombolytic activity test

The blood was drawn from healthy volunteers without a history of oral contraceptive or anticoagulant therapy and 1.0 ml of venous blood was transferred to the previously weighed micro centrifuge tubes and was allowed to clot.

The thrombolytic activity of all extractives was evaluated by the method developed by Daginawala using streptokinase (SK) as the standard substance. The extractive (100 mg) from each plant was suspended in 10 ml of distilled water and it was kept overnight. Then the soluble supernatant was decanted and filtered through a 0.22 micron syringe filter. For clot lysis venous blood drawn from healthy volunteers was distributed in different pre-weighed sterile micro centrifuge tube (1 ml/tube) and incubated at 37 °C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each tube containing the clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube–weight of tube alone). The ethical clearance for the experiment was obtained from the institutional ethical review committee and was performed by following the safe animal handling protocol [14].

To each micro centrifuge tube with the pre-weighed clot, 100 µl aqueous solution of different participants and crude extract was added separately. Then, 100 µl of streptokinase and 100 µl of distilled water were separately added to the positive and negative control tubes,

Respectively all tubes were then incubated at 37 °C for 90 minutes and observed for lysis of clot, if any. After incubation, the released fluid was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

% of clot lysis, = (wt of clot after release of fluid/clot wt) × 100

2.6.1 Streptokinase (SK)

Commercially available lyophilize Streptokinase vial (Beacon Pharmaceuticals Ltd.) was collected and 5 ml sterile distilled water was added to it and mixed properly. This suspension was used as a stock from which 100 µl (30,000 IU) was used for in vitro thrombolysis studies.

2.6.2 Statistical Analysis

Three replicates of each sample were used for each assay to facilitate statistical analysis and the values are reported as mean ± SD [14].

3. Results and Discussion

3.1 Result of Phytochemical Screening

Table-1 shows the results of the phytochemical screening of the Methanolic Extract of *Pisum sativum*

Table 1: Results of chemical group tests

Tested groups	Methanolic Extract of *Pisum sativum*
Glycoside	+
Alkaloid	+
Saponin	+
Flavonoid	+
Tannin	+

Note: + = Indicates the presence of the tested group, − = Indicates the absence of the tested group. The tests identify the presence of Alkaloids, Glycoside, Flavonoid, Saponin and Tannins in methanol extract of *Pisum sativum*

3.2 Result of Analgesic Test

Table 2 shows Analgesic effect of *Pisum sativum* extract on Acetic acid-induced writhing in mice

Table 2: Effects of the methanol extract of *Pisum sativum* on Acetic acid-induced writhing in mice

Animal Group	Writhing Counting (Mean)	Percentage of Writhing Inhibition
Negative Control Group	41.33	-
Standard Group	10.66	74.20
MC Group (500 mg/kg)	23.66	42.75

Values were expressed in mean value. Each group comprised 3 animals (n=3); $p<0.05$ Dunet test as compared to Control Group. Control Group animal received vehicle (1% Tween 80 in water), Standard Group received Diclofenac 10 mg/ kg body weight, Extract Group was treated with 500 mg/kg crude extract of *Pisum sativum*.

3.3 Result of Anti-oxidant Test

Table 3 shows antioxidant activities of standard antioxidant ascorbic acid.

Table 3: Antioxidant activity of Ascorbic Acid

Conc. (µg/ml)	Absorbance (nm)	% of DPPH remaining	% Inhibition
5	0.538	67.08	32.91
10	0.403	50.24	49.75
50	0.322	40.14	59.85
100	0.220	27.43	72.56
500	0.121	15.08	84.91
1000	0.081	10.09	89.90

Table 4 shows antioxidant activities of *Pisum sativum* extract.

Table 4: Antioxidant activity of Extract Solution

Conc. (µg/ml)	Absorbance (nm)	% of DPPH remaining	% Inhibition
5	0.802	98.53	1.47
10	0.701	86.11	13.89
50	0.614	75.42	24.58
100	0.478	58.73	41.27
500	0.314	38.58	61.42
1000	0.197	24.21	75.79

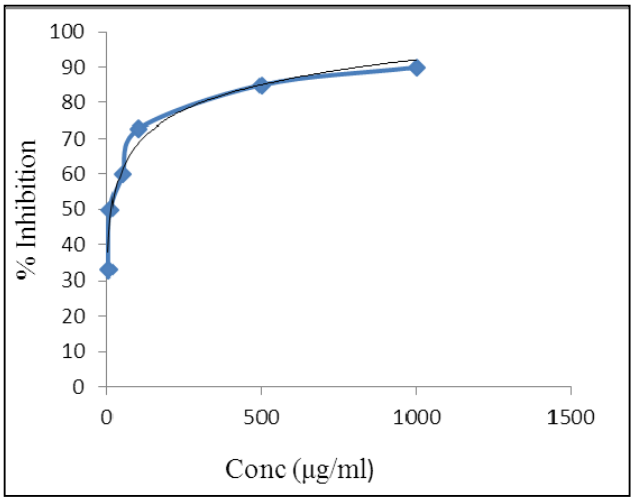

Fig 1: Anti-oxidative activities of ascorbic acid

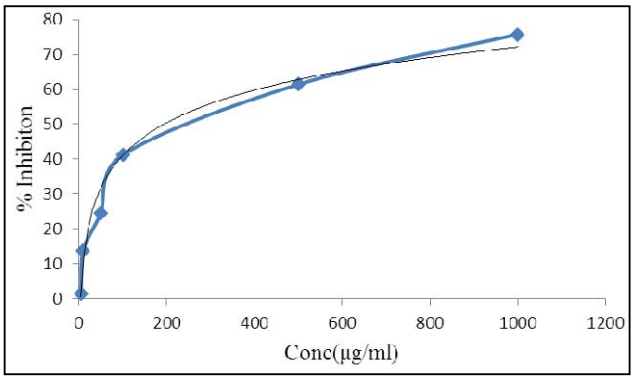

Fig 2: Anti-oxidative activities of Extract

The antioxidant activity of the methanol extract of *Pisum sativum* is evaluated using DPPH free radical scavenging activity method. The methanol extract of *Pisum sativum* has minor antioxidant activity. The IC_{50} of the extraction is 489.25 µg/ ml, whereas IC_{50} of Ascorbic Acid is 16.28 µg/ ml.

3.4 Thrombolytic activity test

Table 5: Thrombolytic activity (in terms of % clot lysis) of. *Pisum sativum*

Sample	% of clot lysis
Blank	3.14 ± 0.31
Streptokinase	66.98 ± 0.11
Extract	18.25 ± 0.04

SK = Streptokinase (positive control), ME= Methanol extract, Blank= Water as negative control.

Addition of SK, a positive control (30,000 IU), to the clots and subsequent incubation for 90 minutes at 37 °C, showed 66.98±0.11% lysis of clot. On the other hand, distilled water was treated as negative control which exhibited a negligible percentage of lysis of clot 3.14±0.31%. The mean difference of in percentage of clot lysis between positive and negative control was found to be statistically significant. In this study *Pisum sativum* displayed mild thrombolytic activity (18.25 ± 0.04 %).

3.5 Discussion

Phytochemical screening of *Pisum sativum* plant extract displayed that it contains Carbohydrate, Alkaloids, Glycoside, Flavonoid, Saponin and Tannins.

The analgesic tests displayed that it has good analgesic properties because the standard analgesic drug Diclofenac- Na inhibited 74.20% acetic acid induced writhing while the oral dose prepared by plant extract inhibited 42.75% acetic acid induced writhing.

The antioxidant test showed that the plant extract has mild antioxidant activity because the standard antioxidant agent ascorbic acid was found to have IC_{50} of 16.28 µg/ ml while the IC_{50} of the plant extract is 489.25 µg/ ml.

The thrombolytic test displayed that it has mild thrombolytic activity because the standard thrombolytic agent Streptokinase was found to have caused 66.98±0.11% lysis of the clot and the plant extract caused 18.25 ± 0.04 %.

4. Conclusion

The results stated above showed that the methanol extract of *Pisum sativum* possessed good analgesic effects. Among other activities it showed mild antioxidant and thrombolytic activities. It is evident that *Pisum sativum* has potential for further research due to its numerous beneficial properties.

5. Acknowledgement

Authors are grateful to Square pharmaceuticals for providing Diclofenac-Na, and Beacon pharmaceuticals for providing Streptokinase for this research,

6. Conflict of interest

The authors declare that there is no conflict of interest about this article with any institution.

7. References

1. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Antioxidant activity of flavonoids from *Licania licaniaeflora*. Journal of Ethnopharmacology. 2002; 79(3):379-381.
2. Valko M, Jomova K, Rhodes CJ, Kuca K, Musilek K. Redox-and non-redox metal-induced formation of free radicals and their role in human disease. Archives of Toxicology. 2016; 90(1):1-37.
3. Lee HS. How safe is the re administration of streptokinase. Drug Safety. 1995; 13(2):76-80.
4. Klawe C, Maschke M. Flupirtine: pharmacology and clinical applications of a nonopioid analgesic and potentially neuroprotective compound. Expert opinion on pharmacotherapy. 2009; 10(9):1495-500.
5. Conaghan PG. A turbulent decade for NSAIDs: update on current concepts of classification, epidemiology, comparative efficacy, and toxicity. Rheumatology International. 2012; 32(6):1491-1502.
6. Medicinal plants database of Bangladesh. http://www.mpbd.info/plants/pisum-sativum.php
7. Traditional Chinese medicine online. http://tcmdiscovery.com/2008/1-31/200813117519.html
8. Nayak BS, Pinto Pereira LM. *Catharanthus roseus* flowers extract has wound-heating activity in Sprague Dawley rats. BMC Complementary and Alternative Medicine. 2006; 6(41):1-6.
9. Siddiqua SA, Majumder MM, Hossain MM, Alam MA, Haque S, Ghani A. Evaluation of analgesic and antioxidant potential of the leaves of *Curcuma alismatifolia* Gagnep. Stamford Journal of Pharmaceutical Sciences. 2008; 1(2):3-9.

10. Sheikh NW, Patel RD, Upwar NI, Mahobia NK, Seth MV, Panchal UR. Analgesic study of methyl alcohol extract of *Cassia fistula* Pod, Journal of Pharmacy Research. 2010; 3(9):2218-2219.
11. Raquibul SM, Jamila M, Majumder MM, Akter R, Hossain MM, Ehsanul HM *et al*. Analgesic and Antioxidant Activity of the Hydromethanolic Extract of *Mikania scandens* (L.) Wild Leaves. American Journal of Pharmacology and Toxicology. 2009; 4(1):1-7.
12. Ilavarasan R, Mallika M, Venkataraman S. Anti-inflammatory and antioxidant activities of *Cassia fistula* Linn. Bark extracts. African Journal of Traditional, Complementary and Alternative medicines. 2005; 2(1):70-85.
13. Benzie FF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology. 1999; 299:15-23.
14. Jeffrey A, Xinli L, Monica S, Francis JC, Xuejun CZ, Jordan T. Domain Interactions between Streptokinase and Human Plasminogen Biochemistry. 2001; 40(48):14686-14695.