academicJournals

Vol. 10(13), pp. 244-249, 8 April, 2016
DOI: 10.5897/AJPP2015.4457
Article Number: D878AFF57781
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Phytochemical Analysis and Evaluation of Analgesic and Anti-inflammatory Properties of *Xanthoxylum fraxineum*

Mansoor Ahmad[1], Faheem Ahmed Siddiqui[1], Mehjabeen[2]* and Noor Jahan[3]

[1]Research Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Karachi, Pakistan.
[2]Department of Pharmacy, Department of Pharmacology, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan.
[3]Department of Pharmacology, Dow University of Health Sciences, Karachi, Pakistan.

Received 4 October, 2015; Accepted 4 February, 2016

Purpose of this study was to standardize the drug *Xanthoxylum fraxineum* by performing chemical analysis including FTIR and to evaluate its analgesic and anti-inflammatory properties as the drug has folkloric uses as an anti- inflammatory, antispasmodic and analgesic agent. Chemical analysis revealed presence of flavonoids, alkaloids, tannins and phytosterols. Mice were employed to determine the analgesic activity by inducing writhes with acetic acid and licking by formalin injection. Both methods revealed good analgesic activity. Drug was evaluated at 300 and 500 mg/kg doses and the analgesic effects were compared with that of the standard that is, aspirin 300 mg/kg. Both doses produced significant analgesic activity at P<0.05. Analgesia caused by the drug was higher than that caused by the standard. Moderate anti-inflammatory activity was also observed during formalin test. The results were in consistence with the folkloric claims made for this drug suggesting that the drug can be used for the claimed purposes. However, identification, safety and mechanism of action of its active constituents should be established to compare it with already available analgesics.

Key words: Standardize, analgesic, anti-inflammatory, Fourier transform infra-red.

INTRODUCTION

Xanthoxylum fraxineum Mill is native to northern and eastern America and Canada. Genus *Xanthoxylum* consists of two hundred and fifty species either shrubs or trees belonging to family Rutaceae. These species are indigenous to temperate, warm and subtropical areas. Their generic name is due to their yellow heart heartwood (Grant et al., 2001). The native North Americans used *X. fraxineum* for the treatment of fever, cough, rheumatism and gonorrhoea (Erichsen-Brown, 1979). The plant is commonly known as Toothache Tree because its bark is chewed to relieve toothache (Felter and Lloyd, 1983). Bark is also used in the form of a tincture or infusion for problems like renal calculi, heart troubles, dyspepsia, dysentery, neuralgia and rheumatic conditions (Foster

*Corresponding author. E-mail: mehjbn1@gmail.com.

and Duke, 2000; Grieve, 1931). Fruit which is a berry is thought to be a good stimulant and tonic. It is used for various chest and throat problems (Erichsen-Brown, 1979). Fruits also have powerful anti-spasmodic, anti-rheumatic and diuretic properties (Foster and Duke, 2000; Grieve, 1931). Significant anti-fungal activity by extracts of different parts of the plant has also been reported. Extracts of leaves and fruits showed greater antifungal activity than that of stem and roots (Bafi-Yeboa et al., 2005). Decoction made by roots of the plant is used to cure throat inflammation and to increase sweating (Erichsen-Brown, 1979).

Present study was carried out to standardize the drug *X. fraxineum* by performing chemical analysis and also by evaluating its anti-inflammatory and analgesic properties as the drug has a good reputation as an anti-inflammatory, antispasmodic and analgesic agent (Foster and Duke, 2000; Grieve, 1931). In homoeopathy, it is used for the treatment of paralysis, rheumatic affections, painful hemorrhages, neurasthenia, neuralgia etc. (Boericke, 1906).

MATERIALS AND METHODS

The drug *X. fraxineum (*mother tincture), Lot No.1010509, manufactured by Willmar Schwabe, Germany was procured from the local market in Karachi, Pakistan. The drug was dried by rotary evaporator to obtain a dark solid residue.

Experimental animals

The experiments were conducted on Swiss albino mice (25 to 30 g) of either sex. Animals were kept on standard diet and water *ad libitum*. Animals were allowed to get used to the environment before carrying out experiments. Four groups each consisting of 6 mice, were formed. First group, used as control was administered only vehicle. Second and third groups were given the drug (300 and 500 mg/kg body weight respectively). The fourth group was treated with the standard drug that is, aspirin 300 mg/kg body weight. Prior to experiments, permission was sought from the Ethical Committee Research Institute of Pharmaceutical Sciences, (Reference number: FAM/13/XF University of Karachi and the animals were disposed of after experiments in accordance to the standard procedure.

Chemical screening of crude extract of *X. fraxineum*

The phytochemical screening of the extract was performed by using different chemical tests. Fourier transform infra-red (FT-IR) spectrophotometric analysis was performed by using FT-IR Spectrophotometer: Thermo Electron Corporation, Nicolet Avatar 330 FT-IR, USA. Following chemical tests were performed for the identification of main constituents present in the alcoholic extracts of crude drugs. The precipitates / colour produced in these reactions were noted.

Test for alkaloids with Mayer's reagent

Two milliliter of Mayer's reagent was added to the extract and colour of the product was recorded after comparison with blank (Brain and Turner, 1975; Purohit, 2007). Presence of alkaloids is indicated by formation of yellow cream precipitates.

Test for alkaloids, Dragendorff's reagent

2.5 ml of the extract was shaken with 2 ml of Dragendorff's reagent in a test tube. The test tubes were agitated and colour of the product was recorded after comparison with blank (Brain and Turner, 1975; Purohit, 2007). Formation of orange red precipitates indicates alkaloids.

Test for alkaloids with Wagner's reagent

The extract, approximately 10 ml was taken in a test tube and 5 ml of Wagner's reagent was added. Colour of product was recorded (Gutal, 2011; Purohit, 2007). Formation of red to reddish brown precipitates is indicative of the presence of alkaloids.

Test for alkaloids with Hager's reagent

2.5 ml of the extract was taken in a test tube and 2 ml of Hager's reagent test solution was added in it. The test tubes were shaken and colour of the product was recorded after comparison with blank (Gutal, 2011; Purohit, 2007). Yellow precipitates indicate the presence of alkaloids.

Test for reducing sugar with Fehling's reagent

2.5 ml of extract was shaken and heated in a test tube with Fehling's reagent. Colour of the product was recorded after comparison with blank (Gutal, 2011; Sharma et al., 2013). Red to brown ppt. shows the presence of reducing sugars.

Test for carbohydrates with Molisch's reagent

Take 2.5 ml of extract in a test tube and add few drops of Molisch's reagent and then few drops of concentrated H_2SO_4 along the sides of test tube (Gutal, 2011; Sharma et al., 2013). Change of colour was noted. Formation of brown ring indicates carbohydrates.

Test for amino acids/protein with Ninhydrin reagent

2.5 ml of the extract was taken in a test tube and 2 ml of Ninhydrin reagent was added in it. The mixture was then heated on a water bath (Gutal, 2011; Koster et al., 1959; Purohit, 2007). The colour of the product was noted. Blue colouration indicates presence of amino acids or proteins.

Test for lignins with Phloroglucinol reagent

5 ml of the extract was taken in a test tube and concentrated by heating it on a water bath. Few drops of concentrated hydrochloric acid were added in it. The mixture was then cooled and 5 ml of phloroglucinol test solution was added to it. Colour of the resultant product was recorded (Gutal, 2011). Red violet colour is a positive indication of lignins.

Test for lignins with Safranin reagent

2.5 ml of the extract was taken in a test tube and concentrated by

heating it on a water bath. The concentrated extract was cooled and 2 ml of safranin test solution was added to it. Colour of the resultant product was recorded (Gutal, 2011). Red colour indicates lignins.

Test for flavonoid with Lead acetate

Test solution (2 ml) was taken in a test tube and few drops of lead acetate solution were added to it and observed for yellow colored precipitate (Koster et al., 1959; Sharma et al., 2013).

Froth test for Saponins

A small quantity of the extract was shaken with water. Formation of foam indicates the presence of saponins (Shah and Seth, 2010).

Test for tannins with ferric chloride test solution

5 ml of the extract was taken in a test tube and concentrated it to about 2.5 ml by heating it on a water bath. The concentrated extract was cooled and 2 ml of ferric chloride test solution was then added to it (Gutal, 2011; Koster et al., 1959). Green or bluish black colour indicates the presence of tannins.

Test for tannins with gelatin test solution

5 ml of the extract was taken in a test tube and concentrated it to about 2.5 ml by heating it on a water bath. The concentrated extract was cooled and 2 ml of 1% gelatin test solution was then added to it (Gutal, 2011; Koster et al., 1959; Sharma et al., 2013). White precipitates are formed in case of tannins.

Libermann Burchard's test for phytosterols

Alcoholic extract was dried and extracted with chloroform. The filtrate was treated with few drops of acetic anhydride followed by concentrated H_2SO_4 from side walls of the test tube. Formation of brown ring at the junction indicates the presence of phytosterols (Brain and Turner, 1975).

Analgesic activity

By writhing method

The tests were performed according to the modified method of Hunskaar and Hole (1987). Mice were used as the test animals in this method. According to this method writhes were induced by intra-peritoneal administration of 1% acetic acid solution (10 ml/kg body weight). Thirty minutes prior to administration of acetic acid, the animals were treated orally with the test substance. Number of writhes was counted for 30 min immediately after acetic acid administration. A reduction in the number of writhes as compared to the control animals was considered as evidence for the presence of analgesia and expressed as percent inhibition of writhing. Mice were divided into 4 groups of 6 animals each (Group-A for control, Group-B and Group-C for 300 and 500 mg/kg oral doses of crude extract respectively, and Group-D for standard). Each group comprised 6 animals, weighing 25 to 30 g. Acetyl salicylic acid (aspirin) as 300 mg/kg orally was used as the reference compound. The crude drug and the acetyl salicylic acid were diluted in distilled water and administered orally. The control animals were treated orally with the same volume of saline as the crude extract.

Formalin test

Swiss albino mice (25 to 30 g) were divided into 4 groups of 6 animals each. 20 µl of 2% formalin was injected in the right hind paw and the left hind paw was injected with an equal volume of normal saline. Two distinct phases of intensive licking and biting of right hind paw were observed during 0 to 5 min (early phase) and 15 to 30 min (late phase) after formalin injection. These phases were scored separately for studying drug effect. Vehicle and the drug were administered orally 30 minutes before formalin injection (Hunskaar and Hole, 1987; Rathi, 2003).

Anti-inflammatory activity

Formalin test in mice (Vernier caliper method)

Mice were divided into four groups each consisting of 6 mice. First group served as control and received only vehicle. Second and third groups were given crude extracts (300 and 500 mg/kg body weight). The fourth group was treated with the standard drug that is, aspirin 300 mg/kg body weight. Inflammation was induced in the left hind paw by injecting 20 µl of 2% formalin into the left hind paw, 30 to 40 min after administering the oral doses of crude extracts and aspirin. The induced edema due to inflammation in the plantar tissue was measured as increase in the size of the paw after 30 min of formalin injection by using a Vernier Caliper in millimeters. This increase in the paw volume was noted in the control, treated and the standard groups up to 4 h. Percentage inhibition in the edema was calculated as:

% inhibition of edema= (Vc-Vt)/Vc X 100

Where Vc and Vt are the mean paw volumes of the control and the treated mice respectively (Rana, 2008). The experimental data were calculated as ± S.E.M., evaluated by student t-Test. Values of $P \leq 0.05$ were considered statistically significant (Posten 1978).

RESULTS

The drug was standardized using FTIR and performing phytochemical analysis. The results obtained are shown in Table 1 and Figure 1. The standardization data can be used in the future for the identification of this plant. Phytochemical screening using different reagents revealed presence of different compounds present in the drug as mentioned in Table 2.

The drug produced very significant dose dependent analgesic activity during acetic acid induced writhing method. Drug was evaluated at 300 and 500 mg/kg doses and the analgesic effects were compared with that of standard (aspirin 300 mg). Both doses produced very significant activity in comparison to the standard. In the 1st phase (0 to15 min), the lower dose (300 mg/kg) produced 50% analgesia in comparison to 36.6% analgesia produced by the standard. In the 2nd phase (15-30 minutes), it caused even greater inhibition of pain that is, 72.7% in comparison to 38.6% by the standard (Table 3). The higher dose (500 mg/kg) produced 64 and 80% analgesia in the 1st and 2nd phase respectively.

Almost similar analgesic activity was observed in the formalin test (Table 4). Reduction in licking time indicated dose dependent anti-nociceptive effects in both

Table 1. FTIR Peak cm^{-1}, *X. fraxineum.*

No. of peak	cm^{-1}	Functional group
1	2921	CH of aldehyde
2	2843	C-H stretch
3	1695	C=O
4	1638	C=C (aromatic)
5	1437	C=C (aromatic)
6	1245	C=O stretch
7	972, 910, 804, 702	C-H (aromatic) out of plane bend.

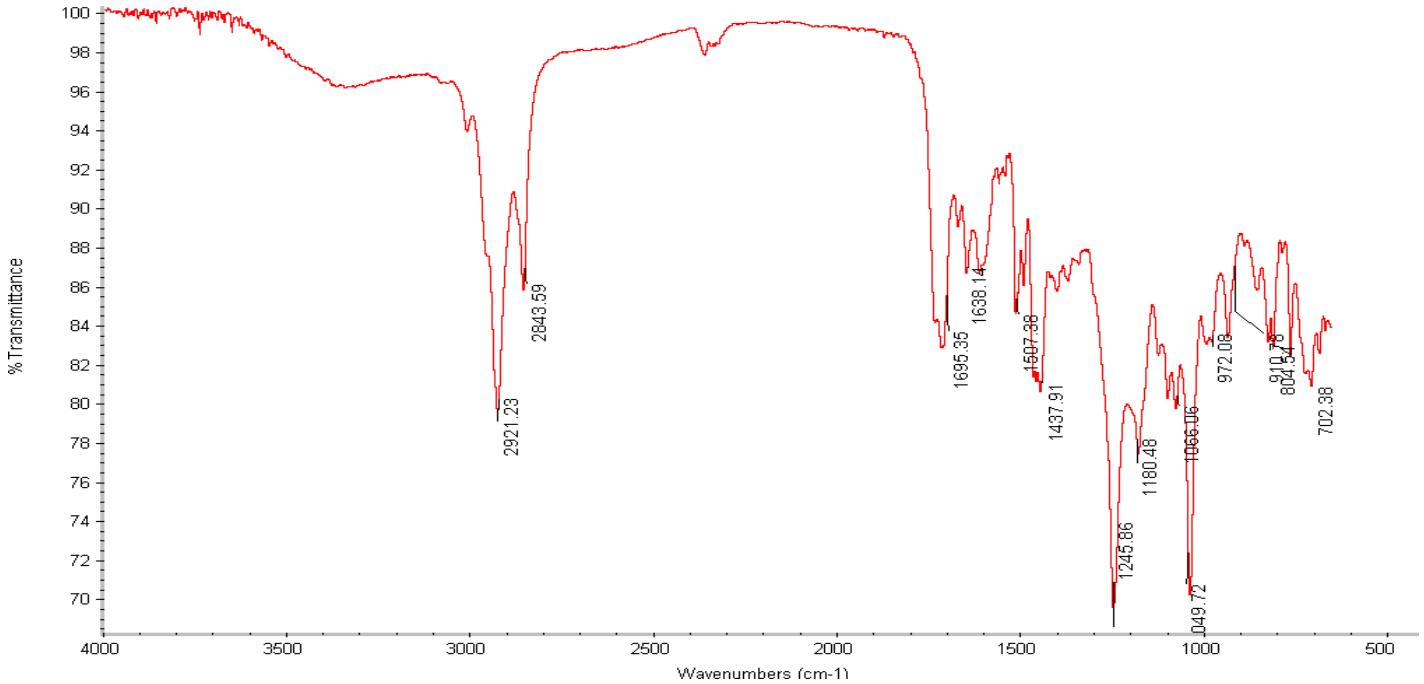

Figure 1. FTIR of *X. fraxineum.*

Table 2. Phytochemical Analysis of *X. fraxineum.*

Phyto-constituents	Result
Alkaloids	+
Carbohydrates	-
Amino acids	-
Protein	-
Flavonoids	+
Tannins	+
Phenolic compounds	+
Phytosterols	+

neurogenic and inflammatory phases. Anti -nociceptive effects were more pronounced in the 2nd phase (15 to 30 min). The drug also showed moderate dose-dependent anti-inflammatory effects during formalin induced edema test on the hind paw of mice. Results are shown in Table 5.

Table 3. Analgesic activity of *X. fraxineum* in mice by acetic acid induced writhing method.

Treatment	Dose mg/kg orally	Mean no. of writhes ± SEM 0 to 15 min	% Inhibition	Mean no. of writhes ± SEM 15 to 30 min	% Inhibition
Control	0.5 ml saline	30±0.77	-	36.66±0.71	-
Xanthoxylum fraxineum	300 mg/kg orally	15±1.2	50*	10±1	72.7**
	500 mg/kg orally	10.83±1.8	64**	7.33±1.3	80**
Aspirin	300 mg/kg orally	19±2.03	36.66	22.5±0.76	38.6

Results are shown as Mean±SEM, *Significant at P<0.05; **Highly significant at p<0.01.

Table 4. Analgesic activity of *X. fraxineum,* formalin test.

Treatment	Dose mg/kg orally	Licking time seconds ±SEM 1st Phase (0 to 5 min)	% Inhibition	Licking time seconds ±SEM 2nd Phase (15 to 30 min)	% Inhibition
Control	0.5 ml saline.	47±0.427	_	30±1.8	-
Xanthoxylum fraxineum	300 mg/kg orally	26±1.32	44.5*	5±1.4	83.3**
	500 mg/kg orally	11±1.75	76.5**	2±0.41	93.3**
Aspirin	300 mg/kg orally	35±0.76	25.5	18±0.8	40*

Results are shown as Mean ± SEM, *Significant at p<0.05; **Highly significant at p<0.01.

Table 5. Anti-inflammatory activity of *X. fraxineum* by formalin edema test.

Treatment	Dose mg/kg	Mean paw volume increase after 1 h ±S.E.M (mm)	% Inhibition	Mean Paw volume increase after 2 h ±S.E.M (mm)	% Inhibition	Mean Paw volume increase after 3 h ±S.E.M.(mm)	% Inhibition	Mean Paw volume increase after 4 h ±S.E.M (mm)	% inhibition
Control	0.5 ml saline	4.04±0.05	-	4±0.05		3.89±0.05	-	3.82±0.05	-
Xanthoxylum fraxineum	300 mg/kg orally	3.23±0.1	20	3±0.04	25	2.84±0.19	27	2.87±0.19	25
	500 mg/kg orally	3.11±0.07	23	2.6±0.1	35	2.72±0.09	30	2.79±0.1	27
Aspirin	300 mg/kg orally	2.83±0.03	30	2.6±0.17	35	2.41±0.3	38	2.3±0.03	40*

Results are shown as Mean±SEM, *Significant at p<0.05; **Highly significant at p<0.01.

DISCUSSION

Inflammation causes redness, swelling and pain. Although inflammation is an important part of body's defense against infective organisms but excessive and prolonged inflammation may damage different tissues and organs resulting in great pain and discomfort. During inflammation, activated macrophages and monocytes produce large quantities of cytokines like TNF-α, IL-6, IL-1β, PGs and reactive oxygen species (Janero, 1990). Different plant drugs have been used traditionally to treat pain and inflammatory conditions. Based on folkloric uses, we investigated the analgesic and anti-inflammatory properties of *X. fraxineum* (Mother Tincture) in animal model by using two methods, acetic acid induced writhing method and formalin method.

Acetic acid causes release of inflammatory substances like PGs, serotonin and cytokines which results in painful sensation (Manjavachi et al., 2010). NSAIDs and centrally acting analgesics like morphine can block this nociceptive effect. In the present study we studied and compared the analgesic and anti-inflammatory effects of *X. fraxineum* (300 and 500 mg/kg) with control and the standard, aspirin (300 mg/kg). Both doses produced very significant analgesic effects during acetic acid induced writhing test and formalin test.

Formalin test consists of two phases, neurogenic nociceptive phase and inflammatory nociceptive phase. Centrally acting drugs inhibit both these phases while NSAIDs block only the second phase which occurs from 15 to 30 min after injecting formalin (Reeve and Dickenson, 1995). According to results, *X. fraxineum* inhibited both phases, predominantly the second phase. Aspirin, being a non-steroidal anti-inflammatory drug inhibited the second phase mainly. Good peripheral analgesic activity was noted during acetic acid induced writhing method also.

The drug showed moderate anti-inflammatory effects in formalin induced edema which could be attributed to the flavonoid content of the drug detected during phytochemical analysis. Flavonoids present in plants have been found to have anti-inflammatory activity by decreasing reactive oxygen species and inflammatory cytokines (Jin et al., 2010; Serafini, Peluso and Raguzzini, 2010).

Conclusion

During this study the preliminary phytochemical screening indicated the presence of alkaloids, flavonoids, tannins and phytosterols. Results of the present study validated the folkloric use of *X. fraxineum* as an analgesic to treat various types of pain like toothache, nerve pain, rheumatic conditions, renal colic etc. Moderate anti-inflammatory activity was also noted during the experiments.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Bafi-Yeboa N, Arnason J, Baker J, Smith M (2005). Antifungal constituents of Northern prickly ash, Zanthoxylum americanum Mill. Phytomedicine 12(5):370-377.

Boericke W (1906). Pocket manual of homoeopathic materia medica, comprising the characteristic and guiding symptoms of all remedies. Third Edition. New York, Boericke and Runyon Publication. pp. 669-670.

Brain KR, Turner TD (1975). The practical evaluation of phytopharmaceuticals (Vol. 1). Bristol: Wright-Scientechnica. pp. 187-189.

Erichsen-Brown C (1979). Use of Plants for the past 500 years. Aurora, ON: Breezy Creeks Press. " Aurora: Ont., Breezy Creeks Press xxii,.-illus.. En Icones. General (KR, 198100065). 512p

Felter HW, Lloyd JU (1983). King's American dispensatory. Eclectic Medical Publications.

Foster S, Duke J (2000). Field Guide to Medicinal Plants Eastern and Central N. America. Boston New york: Houghton Mifflin Company.

Grant R, Thomas V, Van G (2001). Sappi tree spotting: Lowveld, including Kruger National Park: tree identification made easy, Jacana.

Grieve M (1931). A Modern Herbal. The Medicinal culinary Cosmetic and Economic Properties Cultivation and Folk-Lore of Herbs, Grases Fungi, Shrubs and Trees With All Their Modern Uses. CF Leyel (London, 1992).

Gutal V (2011). Phytochemical analysis of the Indian medicinal plant Argyreia involucrate. IJRPBS 2(4):1778-1782.

Hunskaar S, Hole K (1987). The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30(1):103-114.

Janero DR (1990). Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic. Biol. Med. 9(6):515-540.

Jin JH, Kim JS, Kang SS, Son KH, Chang HW, Kim HP (2010). Anti-inflammatory and anti-arthritic activity of total flavonoids of the roots of Sophora flavescens. J. Ethnopharmacol. 127(3):589-595.

Purohit AP, Kokate CK, Gokhle SB (2007). A Text Book of Pharmacognosy." Publication: Nirali Prakashan. pp. 213-358.

Koster R, Anderson M, De Beer E (1959). Acetic acid for analgesic screening. Federation Proc. 18:412-417.

Manjavachi MN, Quintão NL, Campos MM, Deschamps IK, Yunes RA, Nunes RJ, Leal PC, Calixto JB (2010). The effects of the selective and non-peptide CXCR2 receptor antagonist SB225002 on acute and long-lasting models of nociception in mice. Eur. J. Pain 14(1):23-31.

Posten HO (1978). The robustness of the two-sample t-test over the Pearson system. J. Stat. Comput. Simul. 6(3-4):195-311.

Rana A (2008). Melia azedarach: A phytopharmacological review. Pharmacog. Rev. 2(3):173.

Rathi NRK, Mediratta PK, Jain H, Cough C, Mahajan P, Sharma KK (2003). Effect of oxytocin in formalin induced pain response in mice. Ind. J. Pharmacol. 35:128-136.

Reeve AJ, Dickenson AH (1995). The roles of spinal adenosine receptors in the control of acute and more persistent nociceptive responses of dorsal horn neurones in the anaesthetized rat. Br. J. Pharmacol. 116(4):2221-2228.

Serafini M, Peluso I, Raguzzini A (2010). Session 1: Antioxidants and the immune system flavonoids as anti-inflammatory agents. Proc. Nutr. Soc. 69:273-278.

Shah BN (2009). Textbook of pharmacognosy and phytochemistry. Elsevier India.

Sharma V, Agarwal A, Chaudhary U, Singh M (2013). Phytochemical Investigation of various extracts of leaves and stems of achyranthes aspera linn. Int. J. Pharm. Pharm. Sci. 5:317-320.