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Evaluation of anti-inflammatory and analgesic activity from *Vitex negundo* Linn

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Key words: Analgesic, anti-inflammatory, phytochemical, *Vitex negundo* Linn.

Abstract

Analgesic and anti-inflammatory activity along with phytochemical screening was carried out on leaf ethanolic extract of *Vitex negundo* Linn. Phytochemical screening has shown presence of various phytochemicals like carbohydrates, proteins, triterpenoids, calcium oxalate, volatile oils, starch, saponin, flavonoides and tannins. Analgesic activity was carried out by using two models (acetic acid model and tail immersion model). Acetic acid model showed significant effects whereas tail immersion model showed non-significant results which mean that the analgesic effects of *Vitex negundo* is "orally controlled". Anti-inflammatory activity shows significant results. On the basis of present study it can be suggested that this plant has great potentials as a good and cheap source for anti-inflammatory and analgesic drugs preparations. Further investigation is required to evaluate the therapeutic properties of the plant.

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Introduction

Vitex negundo is a shrub of 1-2m height and may reach the size of a tree up to 5 m tall. The plant is found all over the world, mostly in India, Afghanistan, Africa, China, Pakistan and Philippine (Padu *et al*., 1999). In Pakistan the plant is found in plain areas and is absent at very high altitudes usually found around villages and near river banks. It is also commonly present in graveyard in Swat and Malakand (Zabiullah *et al*., 2006). Locally it is known as Marvendaey in Pashto and Atlaq, Banna, Danna, in urdu at different localities of Khyberpakhtunkhuwa.

It has whitish to grayish bark. Full plant is tomentose except the upper surface of leaves and fruits. Leaves opposite-decussate, 3-5-foliolate, Petiolate, petiole 3-6 cm long; leaflets usually lanceolate, 5-10cm long, 1.5-4 cm broad, middle one largest, entire to irregularly denticulate, sub sessile to petiolulate. Inflorescence terminal with length of 10-25cm. Blue to violet colors flowers appears with 3-5mm width usually sub sessile to shortly pedicelled (pedicels up to 1 mm long). Calyx 2mm long, increasing up to 3mm in fruit, persistent, campanulate and 5-toothed. Corolla tube as long as that the calyx with 2-lipped and densely ciliate, up to 2mm long, largest one obovate-orbicular, undulated or crenulated and the others oblong and smaller. Stamens are didynamous, 4 in number protruding earlier but with anther cells divaricating later. Fruit is 5mm, drupe subglobose or somewhat ovoid usually 4-celled, with 1 seed in each cell (Web source).

Ethanobotanical survey shows that *Vitex negundo* Linn has a significant role in traditional medicines. It is used locally as anti-allergic, decoction of root act as a cooling agent on cattle and leaves are vermifuge (Zabiullah *et al*., 2006). In district Buner, Khyberpakhtoon khuwa Fresh roots are used to relieve pain , branches used as tooth brush (miswak), flowers are used as tonic , used to make shelters for tobacco seedlings whereas leaves are used as astringent, febrifuge, diuretic and anthelmentic (Humayun, 2003). Humayun *et al*. 2006 also reported that in district Swat *Vitex negundo* Linn is used locally for relieving pain of head (migraine pain). The leaves are also helpful in toothache problems and are used to relieve labor pain in women (Hussain *et al*.,(2006)

Materials and methods

Plant materials

Fresh leaves of *Vitex negundo* Linn were collected from the different localities of Peshawar Then the plant parts (leaves) were washed with water then was spread on newspapers in shade to make them completely free from moisture content. The drying period took 15-20 days. The leaves were ground with electric grinder to make them into fine powder. Then Ethanolic extract was made to perform following bioassays.

Phytochemical tests

Phytochemical screening was done by following the method of Evans, 1989.

Analgesic Activity

The mice were purchased from animal store house at the Department of Pharmacy, University of Peshawar. The mice weights 18-22gm of albino type. Analgesic activity was conducted by following the method of Koster *et al*., 1959. The animals were kept under standard conditions (25°C and 12/2 light/dark cycles) and were provided with proper food and water. Two types of analgesic activities were carried out, which are

1. Acetic acid induced writhing test
2. Tail immersion test

Acetic acid induced writhing test

Mice were withdrawn from their food 2hours before the start of the experiment and were divided into 5 groups

Group1 was used as control and was injected with normal saline (10ml/kg) only to serve as negative control.

Group 2 was given standard analgesic drug diclofenac sodium (10mg/kg) as positive control
While Group 3, 4 and 5 were treated with plant extract doses of 100, 200 and 300mg/kg body weight.

To induce pain all the animals were injected with 1% acetic acid into peritoneal cavity of animals. After 30 min of saline diclofenac sodium and plant extract injections. After acetic acid injections, the animals started writhing (constriction of abdomen and extension of hind limbs). The numbers of writhes in 10 minutes were recorded for each group of animals and the results were calculated as percent inhibition (Akuodor *et al*., 2011).

Tail immersion test
Saline, plant extract at doses of 100, 200 and 300mg/kg and tramadol (30mg/kg) were administered intraperitoneally. The water bath was set on 55+0.5 °C. The animal tail was kept vertically reaching up to 5cm into the water bath. The time (seconds) taken by the mice to withdraw its tail was the reaction time. The reaction time for control group was also noted. The percentage was found by using formula

Percentage analgesic activity= time of extract reading-time of control/time of control x 100

Anti-inflammatory activity
The mice weights were 25-30gm and were of albino type. The anti-inflammatory activity was done by following the method of Winter *et al*., 1962.
Mice were randomly divided into 5 groups with 6 animals.

Group1 was given normal saline (10ml/kg) act as control (A)

Group 2 was given diclofenac sodium (10mg/kg) act as standard and rest of groups 3, 4 5 were treated with extract doses (100, 200 and 300mg/kg) (B). These treatments were given peritoneally. After 30minutes above doses, carrageenan (1%) was injected subcutaneously in sub-plantar tissue of the right paw of each mouse. Plethysmometer was used to measured inflammation. The readings were taken at 1, 2, 3, 4 and 5 hrs. The average foot swelling in drug treated animal as well as standard was compared with control. Percentage inhibition was determined by using formula
Percent inhibition= A-B/A x 100
Where A represent control and B represent paw edema of tested group.

Result and Discussion
Phytochemical tests
In the present study phytochemical screening of *Vitex negundo* was carried out by using three extracts namely aqueous, ethanolic and chloroform extract. The results are shown in Table 1.

The results showed that the three solvents showed almost same results like presence of carbohydrates, triterpenoids, proteins, starch, flavonoides, volatile oils, tannins, calcium oxalate. Anthraquinone were present only in chloroform extract. Alkaloids, anthraquinone, fat, catechin and mucilage were absent in all the three extracts. Thus it can be concluded that the plant possesses many therapeutic properties due to the presence of flavonoides, saponins, carbohydrates etc.

Many researchers have carried out the phytochemical screenings of medicinal plants e.g. *Vitex doniana* (Tijjani *et al*., 2011), *Vitex leucoxylon* Linn (Gopalakrishna *et al*., 2009), *Phyla nodiflora* Linn (Salve & Bhuktar, 2012) and *Lippia alba* (Saha *et al*., 2011). Our work is in line with all the above researchers.

Analgesic activity
Drugs relieving pains act on central nervous system to remove the feelings of pain from the nerve cells (Kumar *et al*., 2010).

Table 1. Preliminary phytochemical tests of *Vitex negundo* leaf.

Constituents	Tests	Aqueous extract	Ethanolic extract	Chloroform
Carbohydrate	Molisch's test	+	+	+
	Benedict's test	+	+	+
	Fehling's test	+	+	+
proteins and Amino acids	Ninhydrin test	+	+	+
Triterpenoids	Salkowski's test	+	+	+
alkaloids	Mayer's test	-	-	-
	Dragendroff's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
Flavonoides	Flavonoides	+	+	+
Tannin	Ferric chloride test	+	+	+
Anthraquinone	Anthraquinone	-	-	+
Calcium Oxalate	HCl + Conc. H2So4	+	+	+
Saponin	Frothing test	+	+	+
Starch	Starch	+	+	+
Fat and Oil	Paper test	-	-	-
Catechin	Flame test	-	-	-
Volatile oils	Volatile oils test	+	+	+
Mucilage	Mucilage	-	-	-

Vitex negundo Linn has been used as traditional remedy for curing various diseases (like used in cold, cough, fever and relieving pain. According to Shahidullah *et al*. (2009) the people of Bangladesh use the leaves of *Vitex negundo* Linn for relieving pain of head. The present work was done to check the effectiveness of plant as an analgesic

In present work, two models were used

1. Acetic acid induced writhing test
2. Tail immersion test

Acetic acid induced writhing test

Ethanolic extract was taken with concentrations of 100, 150 and 200mg/kg body weight were administered. The results are shown in Table 2., fig.1.

Table 2. Acetic acid induced writhing model for Analgesia of *Vitex negundo* .

Groups	Treatment	Writing (10 min)	% Inhibition
Normal saline	10 ml/kg	75 ± 0.19	
Plant extract	100 mg/kg	18.33 ± 2.45**	75.9
	150 mg/kg	30.00 ± 2.12*	69
	200 mg/kg	25.34 ± 1.98*	57.5
Diclofenac sodium	10 mg/kg	13.66 ± 1.02**	70.1

Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *P < 0.05, **P < 0.01.

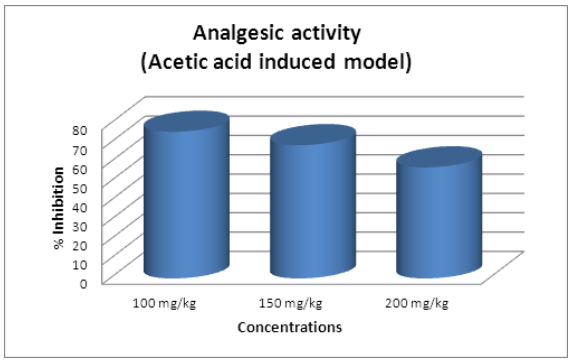

Fig. 1. Acetic acid induced writhing test of *Vitex negundo,* leaf extract for analgesia.

The data collected was analyzed by ANOVA with Dunnett's pest hoc. The 100, 150 and 200mg/kg concentrations showed mean with standard error value of 18.33 ± 2.45, 30.00 ± 2.12 and 25.34 ± 1.98 respectively with inhibition rate of 75.9%, 69% and 57.5% respectively. The results show that at low concentrations the extract has more effect as compared to higher concentrations, hence it can be said that the plant is effective at low concentration only. At low concentration the dose is highly significant more than the Standard drug (Diclofenac).

Tail immersion test

The ethanolic extract in doses of 100, 150 and 200mg/kg body weight were used. The results are shown in Table 3. and fig.2. The data collected was analyzed by ANOVA with Dunnett's pest hoc. The results show no significant effect.

Table 3. Tail immersion model for analgesia of *Vitex negundo* leaf.

Groups	Treatment	Tail withdrawal	% Inhibition
Normal saline	10 ml/kg	20.6 ± 2.3	
Plant extract	100 mg/kg	13.7± 3.1	30.4
	150 mg/kg	12.5± 4.9	59.1
	200 mg/kg	10± 10.6	71.2
Diclofenac sodium	10 mg/kg	3.6± 5.03	75.6

Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett's test. The result shows no significant values from control. *P < 0.05, **P < 0.01.

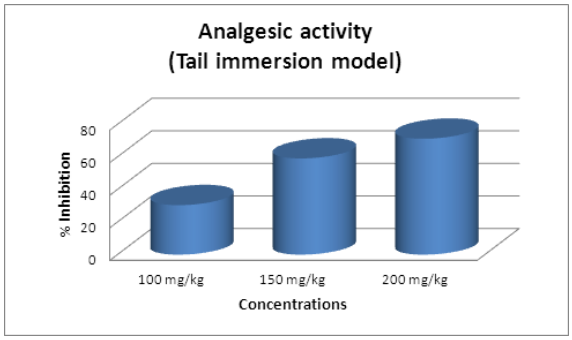

Fig. 2. Analgesic activity of *Vitex negundo* leaf extract in tail immersion model test.

This means that the *Vitex negundo* can act on central nervous system to reduce pain as shown by acetic acid induced test but failed to show any effect on peripheral nervous system as shown by tail immersion test.

When a drug acts on peripheral nervous system it blocks the generation of impulses at chemoreceptor site of pain and change the physiological response to suppress the pain (Shreedhara *et al*., 2009).

Taesotikul *et al.* (2003) explained the phenomena of analgesic activity by using acetic acid induced model. They explain that the acetic acid induced writhing occur by liberating endogenous substances. These endogenous substances excite the pain nerve endings and causes analgesia.

Kalyani *et al.* (2011) worked on leaves of *Lantana camara* to observe its analgesic activity and reported that the plant is effective at low concentrations than standard drug, Rahman *et al.* (2011) worked on analgesic activity of *Clerodendrum viscosum* vent by using leaves extract and showed that the activity is effective at low concentrations as compared to high concentrations. They use acetic acid induced method to evaluate analgesic effect of *Clerodendrum*. The present work is in accordance with the above researcher's findings.

Mutalik *et al.* (2003) Linn evaluated analgesic activity of *Solanum melangena* by using acetic acid induced method. However the results showed that the standard was 65.3% whereas 100, 250 and 500mg/kg concentrations have showed 6.1%, 19.1% and 33.6% inhibitions respectively showing that the plant has dose dependent effects. Hence present work is parallel to the work of Mutalik *et al.* (2003).

Das *et al.* (2011) showed that *Clerodendrum viscosum* has analgesic effect in tail immersion method and reported it to be non-significant. Present work is in line with the results of Das *et al.* (2011). It can be concluded that *Vitex negundo* have no effect on peripheral nervous system, whereas it has an effective analgesic effect on central nervous system.

Flavonoids are also responsible for analgesic activity along with glycosides and alkaloids (Souza *et al.*, 2002). Hossinzadeh *et al.* (2002) reported that a numbers of flavonoids are responsible for analgesic activity in plants. Flavonoids act as inhibitors of prostaglandin synthetase (Ramaswamy *et al.*, 1993). As the preliminary phytochemical test on *Vitex negundo* showed the presence of flavonoids so this analgesic activity of the plant might be due to the presence of flavonoids.

Anti-inflammatory activity

Many plants show anti-inflammatory activity. The agents responsible for this activity are may be steroidal or non-steroidal (Rosa *et al.*, 1971).

In present study anti-inflammatory activity of *Vitex negundo* Linn was evaluated by using carrageenan induced method. The results are shown in Table 4. and Fig.3.

Table 4. Anti-inflammatory activity of *Vitex negundo* Linn.

Group	Treatment /kg	Means paw edema					% Inhibition
		0	1	2	3	4	
Control	10 ml	0.23± 0.23	0.22± 0.02	0.23± 0.01	0.23± 0.22	0.22± 0.10	
Diclofenac	10 mg	0.22± 1.00	0.15± 0.00**	0.14± 0.90**	0.16± 0.12**	0.15± 1.02**	70
Plant extract	200 mg	0.23± 0.12	0.23± 2.10	0.22± 2.54	0.23± 2.11	0.23± 2.33	36.1
	400 mg	0.23± 0.99	0.23± 1.76	0.20± 1.46*	0.19± 1.76*	0.20± 1.87*	68.8
	500 mg	0.22± 1.46	0.19± 1.87**	0.18± 2.56**	0.18± 2.16**	0.18± 2.98**	78.1

Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values control. *P < 0.05, **P < 0.01.

Three concentrations (200, 400 and 500mg/kg) by weight were used. The data collected was analyzed by ANOVA with Dunnett's pest hoc. The % inhibition of three concentrations was 36.1%, 68.8% and 78.1% respectively. The result show that after 3 hours of duration the extract showed more effects of anti-inflammation. The results also show significance

value as compared to the standard drug (Diclofenac). Higher doses show more effects than the lower doses. Similarly after 4 hours results show greater effects as compared to early hours.

Carrageen induced inflammation is useful to detect anti-inflammatory agents. Vinegar *et al.* 1969 described the formation of edema in paw as a biphasic event. In the first phase histamine and serotonine are released and in second phase prostaglandin like substance are released which causes swelling of paw. Second phase is considered to be sensitive for clinically useful non-steroidal and steroidal anti-inflammatory agents (Damas *et al.*, 1986).

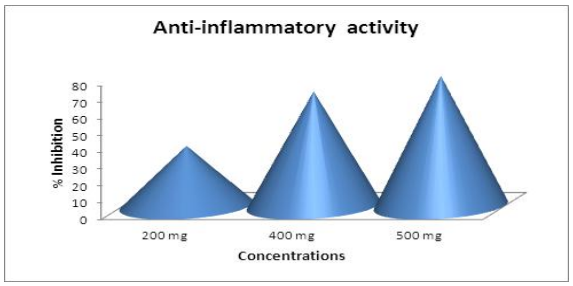

Fig. 3. Antiinflammatory activity of *Vitex negundo* leaf.

Many researchers have given various reasons for anti-inflammatory activity. According to Narayana *et al.* (2001) flavonoids are known to target prostaglandins which are involved in late phase of acute inflammation and pain perception. Argal & Pathak (2006) reported that steroids and saponin are responsible for central nervous system activities. Similarly Viljoen *et al.* (2012) have shown that secondary metabolite namely iridoides are considered to be responsible for this activity as this secondary metabolite was found from various plants showing anti-inflammatory activity.

Gopalakrishna *et al.* (2009) worked on *Vitex leucoxylon* Linn and explained the phenomenon of anti-inflammatory activity by pointing out towards the flavonoids and tannins responsible for this activity.

The preliminary phytochemical screenings have shown that the flavonoids and tannins are strongly present in *Vitex negundo* Linn as shown by all the three extracts. Hence it can be concluded that *Vitex negundo* has anti-inflammatory effects.

Many researchers have done work on the anti-inflammatory activity of various plants showing that with increase in dose the activity become stronger e.g. Das *et al.* (2011) worked on *Clerodendrum infortunatum* with dose of 250 and 500mg/kg body weight showing 49.6 and 65.6 % inhibition. Thus the activity was dose dependent. Deepak & Handa (2000) and Calvo *et al.* (1998) who worked on *Verbena officinalis,* showed that the activity was dose dependent and with increase concentration of extracts the plant showed more effects even from the standard dose. Similarly leaves of *Bouchea fluminensis* was used to evaluate the potential of anti-inflammation by Costa *et al.* (2003) and was found to have effect with high concentrations of doses, showing that the activity was dose dependent. Uzcategu *et al.* (2004) work on *Lanta trifolia* Linn had reported that at higher concentrations (300mg/kg) significant value (4.96± 0.47) whereas at low concentration (100mg/kg) the extract showed less significant value (5.06± 0.96).

Zheng *et al.* (2009) have evaluated the activity of *Vitex negundo* Linn by using its seeds extracts. They used dichloromethane extract and followed the same method but they showed that the seeds extracts were not that much effective in anti-inflammatory activity. Mathur *et al.* (2011) work on *Murraya koenigii* and show that the activity was dose dependent.

In present work our findings are in line with the work of these researchers showing that *Vitex negundo* Linn has good potential of anti-inflammation.

Reference

Akuodor G C, Usman M I , Ibrahim J A, Chilaka K C, Akpan J L, Dzarma S, Muazzam I and Osunkwo U A. 2011. Antinociceptive, anti-inflammatory and antipyretic effects of the methanolic extract of *Bombax buonopozense* leaves in rats and mice. African Journal of Biotechnology **10(16)**, 3191-3196.

Argal A, Pathak A K. 2006. CNS activity of *Calotropis gigantean* roots. Journal of Ethnopharmacology **106**, 142-145.

Calvo M I, Vilalta N, Julian A S and Fernandez M. 1998. Anti-inflammatory activity of leaf of extract of *verbena officinalis* L. Phytomedicine **5(6)**, 465-467.

Costa V B, Coube C S, Marinho B G, Matheus M E and Leitace S G. 2003. Anti-inflammatory and analgesic activity of Bouchea fluminensis. Fitoterapia **74(4)**, 364-371.

Damas B, Volon GR, Deflandre E. 1986. Further studies of the mechanism of counter irritation by Turpentine. Archives of Pharmacal Research **33(2)**, 196-200.

Das JK, Kandar CC, Dey SP, Mandal SC. 2011. Evaluation of analgesic activity of *Clerodendrum viscosum* Linn (Verbenaceae) leaves on experimental animal model. International Journal of Pharma and Bio Sciences **2(2)**, 345-349.

Deepak M, Handa SS. 2000. Anti-inflammatory activity and chemical composition of extracts of *Verbena* officinalis. Phytotherapy Research **14(6)**, 463-465.

Evans WC. 1989. Trease and Evan's Pharmacognosy. 13th Edn, Baillier Tindal, London.
Gopalakrishna, B., S. Prabodh and S. Padmini. 2009. Preliminary phytochemical screening and evaluation of anti-inflammatory potential of *Vitex leucoxylon* Linn. Ethnobotanical Leaflets **13**,956-961.

Hossinzadeh H, Ramezani RM, Fadishei M, Mahmoudi M. 2002. Antinociceptive, anti-inflammatory and acute toxicity effects of *Zhumeria majdae* extracts in mice and rats. Phytomedicine **9**, 135-141.

Hussain F, Badshah L, Dastagir G. 2006. Folk medicinal uses of some plants of South Waziristan, Pakistan. Pakistan Journal of Plant Science **12**, 27–39.

Humayun M. 2003. Ethanobotanical studies of some useful shrubs and trees of District buner, N.W.F.P, Pakistan. Ethanobotanical Leaflets **1**, 1-12.

Humayun M, Khan S A, Sohn E Y and Lee I J. 2006. Folk medicinal knowlrdge and conservation status of some economically value medicinal plants of district Swat, Pakistan. Journal of Ecology and Application **11(2)**, 101-113.

Kalyani L, Rao A L and Mishra U S. 2011. Antibacterial and analgesic activity of Lantana camara. International Journal of Phytomedicine **3**, 381-385.

Kumar M, A Shete, Z Akbar. 2010. A review on analgesic: from Natural sources. IJPBA **1(2)**, 95-100.

Mathur A, Verma SK, Singh SK, Prasad GB, Dua VK. 2011. Investigation of the antimicrobial, antioxidant and anti-inflammatory activities of compound isolated from Murraya koenigii. Int. J. Applied Biology and Pharmaceutical Technology **2(1)**, 470-477.

Mutalik S, Paridhavi K, Rao C M A and Udupa N. 2003. Antipyretic and analgesic activity of leaves of *Solanum melongena* Linn in Rodents. Indian Journal of Pharmacology **35**, 312-315.

Narayana KR, Reddy MS, Chaluvadi MR, Krishna DR. 2001. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian Journal of Pharmacology **33**, 2-16.

Padu DLS, Bunyapraphatsar N, Lemmens RHM. 1999. Medicinal and Poisnous plants, plant Resources of South East Asia. Backhuys publishers, Leiden. p. 22-35.

Rahman MM, Rumzhum NN, Zinna KK. 2011. Evaluation of antioxidant and anticiceptive properties of methanolic extract of *Clerodendrum viscosum* vent. Stamford Journal of Pharmaceutical Sciences **4(1)**, 74-78.

Ramaswamy S, Krishnamurthy V, C and Mythirayee, Kameswaran L. 1993. Analgesic activity of certain flavones derivatives: A structure-

Activity study. Clinical and Experimental Pharmacology and Physiology **20(1)**, 54-63.

Rosa MD, Papadimitriou JM, Willoughby DA. 1971. A histopathological and pharmacological analysis of the mode of action of non- steroidal anti-inflammatory drugs. Pathology **105(4)**, 239-250.

Saha D, Pahari SK, Moity T, Sur D, Kayal S, Ghindora GL, Dhirehe UK. 2011. Pharmacognostic studies of leaf of Lippia alba. Asian Journal of Pharmaceutical Research **1 (1)**, 17-18.

Salve SD, Bhuktar AS. 2012. Pharmacognostic study of Phyla nodiflora Linn. International Journal of Pharmacy **3(3)**, 255-260.

Shahidullah M, Mujahidee M A, Uddin S M N, Hossan M S, Hanif A, Bari S and Rahmatullah M. 2009. Medicinal plants of the Santal Tribe Residing in Rajshahi district, Bangladesh. American-Eusrasian Journal of Sustainable Agriculture **3(2)**, 220-226.

Shreedhara CS, Vaidya VP, Vagdevi HM, Latha KP, Muralikrishna KS, Krupanidhi AM. 2009. Screening of *Bauhinia purpurea* Linn for analgesic and anti-inflammatory activities. Indian Journal of Pharmacology **4(2)**, 75-79.

Souza AE, Silva TMS, Fernandes GC, Carvalho MG, Echevarria A. 2002. Cytotoxic activities against *Ehrlich carcinoma* and human K562 Leukaemia of alkaloids and flavonoid from Solanum species. Journal of the Brazilian Chemical Society **13(6)**, 838-842.

Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer J J C. 2003. Anti-inflammatory, antipyretic and antinociceptive activities of Tabernaemontana pandacaqui Poir. Journal of Ethnopharmacology **84**, 31-35.

Tijjani MA, Abdurahman FI, Khan IZ, Sandabe UK. 2011. An investigation of the phytochemical and elemental content of stem bark of *Vitex doniana* sweet (Black plum). International Journal of Basic and Applied Chemical Sciences **1(1)**, 99-106.

Uzcategu B, Avila D, Roca HS, Quintero L, Ortega J, Gonzaez YB. 2004. Anti-inflammatory, antinociceptive and antipyretic effects of *Lantana trifolia* Linn in experimental animals. Journal of Clinical Investigation **45(4)**, 110-115.

Viljoen A, Mncwangi N and Vermaak I. 2012. Anti-inflammatory iridoids of botanical origins. Current Medicinal Chemistry **19(14)**, 2104-2127.

Vinegar R, Schreiber W, Hugo R. 1969. Biphasic development of carrageenan edema in rats. Journal of Pharmacology and Experimental Therapeutics **166**, 96-103.

Winter CA, Risely EA, Nuss OW. 1962. Carrageenan induced oedema in hind paw of the rat as an assay for anti-inflammatory activity drugs. Experimental Biology and Medicine **111**, 544-547.

Zabihullah Q, Rashid A, Akhtar N. 2006. Ethnobotanical survey in Kot manzaray baba valley Malakand agency, Pakistan. Pakistan Journal of Plant Science **12(2)**, 115-121.

Zheng CJ, Huang BK, Wang Y, Q Ye, Han T, Zheng QY, Zheng H, Qin LA. 2009. Anti-inflammatory diterpenes from seeds of Vitex negundo Linn. Bioorganic and Medicinal Chemistry **18(1)**, 175-181.