



Journal of Applied Sciences

ISSN 1812-5654

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

Cytotoxicity, Analgesic and Antidiarrhoeal Activities of *Asparagus racemosus*

¹U.K. Karmakar, ¹S.K. Sadhu, ²S.K. Biswas, ²A. Chowdhury, ³M.C. Shill and ²J. Das

¹Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh

²Department of Pharmacy, BGC Trust University Bangladesh, Chittagong, Bangladesh

³IPD Pharmacy, Square Hospitals Ltd., Dhaka-1205, Bangladesh

Abstract: In Ayurveda, *Asparagus racemosus* Willd is known as the queen of herbs because it has a strong rejuvenating, nurturing and stabilizing effect on excessive air, gas, dryness and agitation in the body and mind. Ethanol extracts of *Asparagus racemosus* (EEAR) belonging to the family Liliaceae was investigated for biological action. The present study was designed to evaluate the cytotoxicity, analgesic and antidiarrhoeal properties of the ethanol extract of whole plant of *Asparagus racemosus*. The test for analgesic activity of the crude ethanol extract was performed using acetic acid induced writhing model in mice. On the other hand, antidiarrhoeal test of the EEAR was done according to the model of castor oil induced diarrhoea in mice and brine shrimp lethality bioassay was used to determine the cytotoxic activity of ethanol extract of the plant. In acetic acid induced writhing in mice, the ethanol extract exhibited significant inhibition of writhing reflex 67.47% ($p < 0.01$) at the dose of 500 mg kg⁻¹ body weight. The plant extract showed antidiarrhoeal activity in castor oil induced diarrhoea in mice. It increased mean latent period and decreased the frequency of defecation with number of stool count at the dose of 250 and 500 mg kg⁻¹ body weight, respectively comparable to the standard drug loperamide at the dose of 50 mg kg⁻¹ body weight. In addition to these, the brine shrimp lethality test showed the significant cytotoxic activity of the plant extract (LC₅₀: 10 µg mL⁻¹ and LC₉₀: 47.86 µg mL⁻¹). The obtained results support the traditional uses of the plant and require further investigation to identify the chemical constituent(s) responsible for cytotoxicity, analgesic and antidiarrhoeal activities.

Key words: *Asparagus racemosus*, phytochemical, analgesic, antidiarrhoeal, cytotoxicity

INTRODUCTION

Asparagus racemosus Willd belonging to the family Liliaceae is a climber with thin leaves commonly known as “Shatamuli”. The roots of the plant are white, long and are tapering at both ends. Small white and fragrant flowers appear on this plant in the beginning of the rainy season. Fruits in the shape of small berries appear in the autumn. It grows wild in forest and can also be planted in gardens in most of the areas (Ghani, 2003). *Asparagus* contains steroidal glycosides (asparagosides), bitter glycosides, asparagine and flavonoids. Asparagine is a strong diuretic. In addition to these, leaves contain diosgenin and other saponins such as shatavarins I and IV (Ravikumar *et al.*, 1987). It was also reported that quercetin, rutin and hyperoside were found in flowers and fruits of the plant. The fruits of *Asparagus racemosus* also contain cyanidin-3-glycoside, sitosterol, stigmasterol, sarsasapogenin and two furostanolic saponins. Tubers and roots contain saccharine matters and mucilage. An

antioxytotic compound, named racemosol (a 9, 10-dihydrophenanthrene derivative) has been isolated from this plant (Sekine *et al.*, 1997).

The plant also possesses diuretic properties and is effective in the treatment of cystitis. Ethanol extract of aerial parts possesses anti-cancer properties whereas, bark shows antibacterial and antifungal activities. Tuberos roots are used as aphrodisiac, alterative, tonic, demulcent, diuretic and are commonly prescribed in gastrointestinal disorders like bilious dyspepsia, flatulence, diarrhea and dysentery. Moreover, the roots play an important role in lactation of mother and appetite and nourishment in children. It is also used in treating acidity and as hair tonic. Juice of the roots taken with milk is useful in gonorrhoea. The plant is also used in diabetes, jaundice and other urinary disorders. Aqueous and ethanol extracts of the plant possess strong molluscicidal property (Ghani, 2003). It is also reported that the ethanol extract of the plant possesses anti-oxidant activities (Karmakar *et al.*, 2012). The methanolic root extract of the

plant is also used in the treatment of ischemia and shows cerebroprotective potentials (Nandagopal *et al.*, 2011). In addition to these, the aqueous root extract of the plant shows glucose homeostasis in aged rats (Velavan and Begum, 2007a) and acts to alleviate the indices of oxidative stress associated with stress (Velavan and Begum, 2007b). The objective of the present study was to identify the cytotoxicity, analgesic and antidiarrhoeal activities with phytochemical analysis of ethanol extract of *Asparagus racemosus*.

MATERIALS AND METHODS

Collection of plant materials and preparation of plant extract:

The whole plant of *Asparagus racemosus* was collected from Natore, Bangladesh in the month of May, 2009 and the plant was identified by the taxonomists of Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. DACB 34216). The voucher specimen was deposited at Pharmacy Discipline of Khulna University Bangladesh. The collected plant was dried under shade. After complete drying, the sample was cut into small pieces and then slashed to coarse powder with the help of mechanical grinder. The powder materials were stored in a suitable container. About 500 g of powder was extracted by maceration over 20 days with 1200 mL of 80% ethanol. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material and then it was filtered through filter paper. The filtrate thus obtained was evaporated by using a rotary evaporator to get a viscous mass which was dried to get a dried ethanol extract (approx. yield value 16%). The extract thus obtained was used for experimental purposes.

Animals: Swiss-Albino mice of either sex (20-25 g body weight) were collected from animal resources branch of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) and were used for the experiments. The animals were kept in the standard polypropylene cages and provided with standard diets formulated by ICDDR, B. The animals were acclimatized in animal house under standard Laboratory conditions (relative humidity 55-60%, room temperature $25\pm 2^\circ\text{C}$ and 12 h light: dark cycle) for period of 14 days prior to performing the experiments (Chatterjee, 1993).

Drugs: The standard drugs diclofenac sodium, Loperamide and Chloramphenicol were collected from Beximco Pharmaceuticals Ltd. Dhaka, Bangladesh.

Preliminary phytochemical screening: The crude extract was subjected to preliminary phytochemical screening for the detection of major functional groups according to the standard procedures (Trease and Evans, 1989).

Determination of analgesic activity: The analgesic activity of EEAR was studied using acetic acid induced writhing model in mice. Experimental animals were randomly selected and divided into four groups denoted as control group, Positive control group and Test group-I and Test group-II consisting of five mice in each group. Control group received 1% Tween-80 orally at the dose of 10 mg kg^{-1} body weight whereas, Positive control group received diclofenac sodium orally at the dose of 25 mg kg^{-1} body weight. Test group I and Test group II were given the test sample orally at the dose of 250 and 500 mg kg^{-1} body weight. A 30 min interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intra-peritoneally to each of the animals of a group. Five min was also given for absorption of acetic acid and number writhing was counted for 15 min. The animals did not always perform full writhing and the incomplete writhing was taken as half-writhing, so two half-writhing were taken as one full writhing. This is why total writhing was halved to convert all writhing to full writhing or real writhing (Whittle, 1964; Ahmed *et al.*, 2004).

Antidiarrhoeal activity: Antidiarrhoeal activity of EEAR was tested using the model of castor oil induced diarrhoea in mice (Chatterjee, 1993). All the mice were screened initially by giving 0.5 mL of castor oil and only those showing diarrhea were selected for the experiment. The test animals were randomly chosen and divided into four groups having five mice in each group. Control group received 1% Tween-80 at the dose of 10 mg kg^{-1} body weight whereas, positive control group received the standard antiperistalsis drug, loperamide at the dose of 50 mg kg^{-1} body weight as oral suspension. Group I and Group II were considered as test groups treated with ethanol extract of *Asparagus racemosus* at the oral dose of 250 and 500 mg kg^{-1} body weight. In this study, the control vehicle and extract were administered orally 30 min prior to oral administration of castor oil at the dose of 0.5 mL. Individual animal of each group was placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour in 5 h study after the castor oil administration. Number of stool

on any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment. The latent period of each mouse was also counted. At the beginning of each hour, new papers were placed for old ones.

Determination of cytotoxic activity: The brine shrimp eggs were hatched in a conical flask containing brine shrimp medium (300 mL). The flask were well aerated with the aid of an air pump and kept in a water bath at 29-30°C. A bright light was left on it. The nauplii hatched within 48 h. The extract was dissolved in brine shrimp medium with addition of few drops of 5% dimethyl sulfoxide (DMSO) to obtain a concentration of 5, 10, 20, 40, 60, 80, 160 and 320 µg mL⁻¹. Each preparation was dispensed into clean test tube in 10 mL volume. For control, same procedure was followed except test samples. A series of same concentration as of sample was prepared for positive control, chloramphenicol. After making the test tube properly, 10 living shrimps were added to each of the test tubes with the help of a Pasteur pipette. The test tubes containing the sample, control and positive control were then incubated at 29°C for 24 h in a water bath, after which each test tube was examined and the surviving brine shrimp counted and recorded. From this, the percentage of mortality was calculated at each concentration to determine the LC₅₀ and LC₉₀ (Myers, 1982).

Statistical analysis: Student's t-test was used to determine significant differences between the control group and test groups. p-values less than or equal to 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening: In the preliminary phytochemical screening, EEAR showed the

presence of alkaloids, tannins, saponins, glycosides, flavonoids and carbohydrates which are shown in Table 1.

Analgesic activity: Analgesic activity of EEAR was tested using acetic acid induced writhing model in mice. The extract produced 52.39% (p<0.05) and 67.47% (p<0.01) writhing inhibition in test mice at the dose of 250 mg kg⁻¹ and 500 mg kg⁻¹ body weight which were comparable to the standard drug diclofenac sodium showing 70.65% (p<0.01) writhing inhibition at the dose of 25 mg kg⁻¹ body weight (Table 2).

Antidiarrhoeal activity: Antidiarrhoeal activity of the EEAR was tested by castor oil induced diarrhoea in mice. The extract caused an increase in latent period (0.326 and 0.574 h) i.e., delayed the onset of diarrhoeal episode at the dose of 250 and 500 mg kg⁻¹ body weight, respectively as compared to the standard antidiarrhoeal agent Loperamide where the mean latent period was 1.57 h (Table 3). The extract also decreased the frequency of defecation at the dose of 250 and 500 mg kg⁻¹ b.wt., respectively where the mean number of stool at the 1st, 2nd, 3rd, 4th and 5th h of study were 2.3, 1.9, 1.7, 1.6, 1.5 and 2.1, 1.8, 1.5, 1.4, 1.3, respectively which were comparable to the standard drug loperamide where the mean number of stool at the 1st, 2nd, 3rd, 4th and 5th h of study were 3.1, 2.2, 1.6, 1.3 and 1.1, respectively (Table 3).

Cytotoxic activity: Brine shrimp lethality bioassay indicates cytotoxicity of the ethanol extract. The extract was found to show lethal activity against brine shrimp nauplii where LC₅₀ and LC₉₀ values were 10 µg mL⁻¹ and 47.86 µg mL⁻¹ (Table 4).

Table 1: Results of preliminary phytochemical analysis

Alkaloid	Glycoside	Steroid	Gum	Tannin	Saponin	Flavonoid	Carbohydrate
+	+	-	-	+	+	+	+

+: Present, -: Absent

Table 2: Effect of EEAR on acetic acid induced writhing in mice

Animal group (n = 5)	Treatment	Writhing count (Mean±SEM) (%Writhing)	Writhing inhibition (%)
Control	1% tween-80 solution in water (10 mL kg ⁻¹)	15.6±2.6 (100)	0
Positive control	Diclofenac sodium (25 mg kg ⁻¹)	5.4±0.60* (29.35)	70.65
Test group I	EEAR (250 mg kg ⁻¹)	7.2±0.61** (47.61)	52.39
Test group II	EEAR (500 mg kg ⁻¹)	4.8±0.74* (32.53)	67.47

Values are expressed as Mean±SEM, SEM = Standard error of mean, n = No. of mice, *: p < 0.01; **: p < 0.05 vs. Control

Table 3: Effect of EEAR on castor oil induced diarrhea in mice

Animal group (n = 5)	Treatment	Latent period (h)	Period of study (h)	Total number of stool(s)
Control	1% tween-80 solution in water (10 mL kg ⁻¹)	0.178±0.10	1	4.7±0.80
			2	5.6±0.41
			3	2.9±0.26
			4	3.2±0.62
			5	3.1±0.25
Positive control	Loperamide (50 mg kg ⁻¹)	1.57±0.352*	1	3.1±0.16**
			2	2.2±0.42
			3	1.6±0.13*
			4	1.3±0.29**
			5	1.1±0.21*
Test group I	EEAR (250 mg kg ⁻¹)	0.326±0.958*	1	2.3±0.39*
			2	1.9±0.22
			3	1.7±0.35*
			4	1.6±0.19
			5	1.5±0.11
Test group II	EEAR (500 mg kg ⁻¹)	0.574±0.51*	1	2.1±0.25*
			2	1.8±0.26*
			3	1.5±0.20
			4	1.4±0.25
			5	1.3±0.24*

Values are expressed as Mean±SEM (n = 5); *: p<0.01; **: p<0.2 vs. control

Table 4: Results of brine shrimp lethality bioassay of EEAR

Conc. (µg mL ⁻¹)	Log (conc.)	No. of alive shrimp(s)				Avg.	Mortality (%)	LC ₅₀ (µg mL ⁻¹)	LC ₂₀ (µg mL ⁻¹)
		Test 1	Test 2	Test 3					
0.5	0.301	10	9	10	9.67	3	10	47.86	
10	1.000	5	5	6	5.33	52			
20	1.301	4	3	2	3.00	75			
40	1.602	2	2	1	1.67	87			
60	1.778	1	1	0	0.67	95			
80	1.903	0	0	0	0.00	100			
160	2.204	0	0	0	0.00	100			
320	2.505	0	0	0	0.00	100			

DISCUSSION

To get preliminary idea about the active constituents present in the ethanol extract of the plant, different chemical tests were performed and the study results showed the presence of alkaloids, tannins, saponins, glycosides, flavonoids and carbohydrates. Flavonoids were reported to have a role in analgesic (Zakaria *et al.*, 2006) and antioxidant activity (Rajnarayana *et al.*, 2001; Ramesh *et al.*, 1998; Okwu and Orji, 2007). There are also reports on the role of tannins in anti-nociceptive activity (Ramprasath *et al.*, 2006; Rahman *et al.*, 2011). Besides, alkaloids are well known for their ability to inhibit pain perception (Uche and Aprioku, 2008). Tannins are also useful in bacterial infections (Agbafor *et al.*, 2011) and the antibacterial activities of the ethanol extract of the plant were reported by Karmakar *et al.* (2012). Therefore, it is assumed that these compounds may be responsible for the observed analgesic, antidiarrheal, antibacterial, antioxidant and cytotoxic activity.

Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid which is used to induce writhing, causes analgesia by liberation of endogenous substances which

in turn excite the pain nerve endings (Taesotikul *et al.*, 2003). Increased levels of PGE₂ and PGF_{2α} in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Deraedt *et al.*, 1980). Nevertheless, it was found that the intraperitoneal administration of acetic acid induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators (Hokanson, 1978). The EEAR produced significant writhing inhibition comparable to the standard drug diclofenac sodium. Results of the study suggest that the extract might possess the capability to inhibit prostaglandin synthesis.

Antidiarrhoeal activity of the EEAR was tested by castor oil induced diarrhea in mice. Castor oil mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces anti-absorptive or anti-secretory effect (Tripathi, 1999). The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Most agreed view is that ricinoleate salts stimulate the intestinal

epithelial cell's adenylyl cyclase (Racusen and Binder, 1979) or release prostaglandins which results in an increase in the net secretion of water and electrolytes in the small intestine (Beubler and Juan, 1979). The EEAR moderately inhibited and delayed the onset of diarrhea in mice. The maximum effect was found at 500 mg kg⁻¹ of body weight. On the basis of these results, it was concluded that the EEAR possessed moderate antidiarrhoeal activity. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc. of the compound (Myers, 1982; Anderson *et al.*, 1988; Alam *et al.*, 2011). The EEAR was found to show significant activity against the brine shrimp nauplii; LC₅₀ was found 10 µg mL⁻¹ and LC₉₀ was found 47.86 µg mL⁻¹. However, further investigations using carcinoma cell line are necessary to isolate the active compound(s) responsible for the activity.

CONCLUSION

According to above discussion EEAR contains important chemical constituents that confer upon it as a medicinal agent. It was revealed that the extract containing alkaloids, tannins, saponins, glycosides, flavonoids and carbohydrates might have potential roles in its analgesic, antidiarrhoeal, antimicrobial, cytotoxic and antioxidant activity. This could provide a rationale for traditional uses of this plant and further research is necessary for elucidating the active principles.

ACKNOWLEDGMENTS

The authors are grateful to the authority of International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) for providing the experimental mice and bacterial strains. The authors are also grateful to the authority of Beximco Pharmaceuticals Ltd. for providing Diclofenac sodium, Loperamide and Chloramphenicol.

REFERENCES

- Agbafor, K.N., E.I. Akubugwo, M.E. Ogbashi, P.M. Ajah and C.C. Ukwandu, 2011. Chemical and antimicrobial properties of leaf extracts of *Zapoteca portoricensis*. Res. J. Med. Plant, 5: 605-612.
- Ahmed, F., M.S.T. Selim, A.K. Das and M.S.K. Choudhuri, 2004. Anti-inflammatory and antinociceptive activities of *Lippia nodiflora* Linn. Pharmazie, 59: 329-330.
- Alam, M.B., M.S. Hossain, N.S. Chowdhury, M.E.H. Mazumder, M.E. Haque and A. Islam, 2011. *In vitro* and *in vivo* antioxidant and toxicity evaluation of different fractions of *Oxalis corniculata* Linn. J. Pharmacol. Toxicol., 6: 337-348.
- Anderson, J.E., C.J. Chang and J.L. McLaughlin, 1988. Bioactive components of *Allamanda schottii*. J. Nat. Prod., 51: 307-308.
- Beubler, E. and H. Juan, 1979. Effect of ricinoleic acid and other laxatives in net water flux and prostaglandin E release by the rat colon. J. Pharm. Pharmacol., 31: 681-685.
- Chatterjee, T.K., 1993. Handbook of Laboratory Mice and Rats. 1st Edn., Jadavpur University Press, India, pp: 133-139.
- Deraedt, R., S. Jouquey, F. Devallee and M. Flahaut, 1980. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol., 61: 17-24.
- Ghani, A., 2003. Medicinal Plants of Bangladesh. 2nd Edn., The Asiatic Society of Bangladesh, Dhaka, pp: 114.
- Hokanson, G.C., 1978. Acetic acid for analgesic screening. J. Natl. Prod., 41: 497-498.
- Karmakar, U.K., S.K. Biswas, A. Chowdhury, S.Z. Raihan, M.A. Akbar, M.A. Muhit and R. Mowla, 2012. Phytochemical investigation and evaluation of antibacterial and antioxidant potentials of *Asparagus racemosus*. Int. J. Pharmacol., 8: 53-57.
- Myers, 1982. Phytochemical Methods (A Guide to Modern Techniques to Plant Analysis). 3rd Edn., Chapman and Hall, London, pp: 335-337.
- Nandagopal, M., P. Muralidharan and G. Thirumurugan, 2011. Cerebroprotective effect of root extract of *Asparagus racemosus* Willd. in global cerebral ischemia in rats. J. Pharmacol. Toxicol., 6: 49-61.
- Okwu, D.E. and B.O. Orji, 2007. Phytochemical composition and nutritional quality of *Glycine max* and *Vigna unguiculata* (L.) Walp. Am. J. Food Technol., 2: 512-520.
- Racusen, L.C. and H.J. Binder, 1979. Ricinoleic acid stimulation of active anion secretion in colonic mucosa of the rat. J. Clin. Invest., 63: 743-749.
- Rahman, M.A., E. Haque, M. Hasanuzzaman and I.Z. Shahid, 2011. Antinociceptive, antiinflammatory and antibacterial properties of *Tamarix indica* roots. Int. J. Pharmacol., 7: 527-531.
- Rajnarayana, K., M.S. Reddy, M.R. Chaluvadi and D.R. Krishna, 2001. Biflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Ind. J. Pharmacol., 33: 2-16.

- Ramesh, M., Y.N. Rao, A.V.N.A. Rao, M.C. Prabhakar, C.S. Rao, N. Muralidhar and B.M. Reddy, 1998. Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attenuata*. *J. Ethnopharmacol.*, 62: 63-66.
- Ramprasath, V.R., P. Shanthi and P. Sachdanandam, 2006. Immunomodulatory and anti-inflammatory effects of *Semecarpus anacardium* Linn. Nut milk extract in experimental inflammatory conditions. *Biol. Pharm. Bull.*, 29: 693-700.
- Ravikumar, P.R., R. Soman, G.L. Chetty, R.C. Pandey and D. Sukh, 1987. Chemistry of Ayurvedic crude drugs: Part 6-(shatavari-1): Structure of Shatavarin-4. *Indian J. Chem.*, 26: 1012-1017.
- Sekine, T., N. Fukasawa, I. Murakoshi and N. Ruangrunsi, 1997. A 9,10 dihydrophenanthrene from *Asparagus racemosus*. *Phytochemistry*, 44: 763-764.
- Taesotikul, T., A. Panthong, D. Kanjanapothi, R. Verpoorte and J.J.C. Scheffer, 2003. Anti-inflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. *J. Ethnopharmacol.*, 84: 31-35.
- Trease, G.E. and W.C. Evans, 1989. *Trease and Evan's Textbook of Pharmacognosy*. 13th Edn., Cambridge University Press, London, pp: 546.
- Tripathi, K.D., 1999. *Essentials of Medical Pharmacology*. 4th Edn., Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, India, ISBN: 81-7179-633-8, pp: 432.
- Uche, F.I. and J.S. Aprioku, 2008. The phytochemical constituents, analgesic and anti-inflammatory effects of methanol extract of *Jatropha curcas* sp. leaves in mice and Wister albino rats. *J. Applied Sci. Environ. Manage.*, 12: 99-102.
- Velavan, S. and V.M.H. Begum, 2007a. Modulatory role of *Asparagus racemosus* on glucose homeostasis in aged rats. *Int. J. Pharmacol.*, 3: 149-154.
- Velavan, S. and V.M.H. Begum, 2007b. Restorative effect of *Asparagus racemosus* on age related oxidative damage in heart lysosome of aged rats. *Int. J. Pharmacol.*, 3: 48-54.
- Whittle, B.A., 1964. The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesics. *Br. J. Pharmacol. Chemother.*, 22: 246-253.
- Zakaria, Z.A., R.N.S.R.M. Nor, M.R. Sulaiman, Z.D.F.A. Ghani, G.H. Kumar and C.A. Fatimah, 2006. Antinociceptive and anti-inflammatory properties of *Melastoma malabathricum* leaves chloroform extract in experimental animals. *J. Pharmacol. Toxicol.*, 1: 337-345.