Turk J Pharm Sci 11(2), 137-144, 2014

Original article

Antinociceptive activity of methanol extract of *Spilanthes paniculata* Linn.

Biplab Kumar DAS[1,2]*, Kaysar AHMED[2], Azim UDDIN[2], Rajib BHATTACHARJEE[2], Md. Mamun AL-AMIN[2]

[1] University of Dhaka, Department of Pharmaceutical Chemistry, Dhaka-1000, BANGLADESH,
[2] North South University, School of Life Sciences, Department of Pharmacy, Bashundhara, Dhaka-1229, BANGLADESH

Spilanthes paniculata Linn. (Asteraceae) is a well-known plant for its folklore use in toothache and throat infection. The methanol extract of *S. paniculata* was investigated for it's probable antinociceptive activity by hot plate, acetic acid-induced writhing and formalin test. The extract at the doses of 250 and 500 mg/kg body weight showed significant ($p<0.001$) and dose-dependent antinociceptive activity in all three models used. This indicates the existence of both central and peripheral mechanisms of antinociceptive action of the extract. All the experimental results suggest that the methanol extract of *S. paniculata* contains constituents having antinociceptive properties and support its popular folkloric uses in the management of pain.

Key words: *Spilanthes paniculata*, Asteraceae, Antinociceptive activity, Hot plate test, Acetic acid-induced writhing test, Formalin test.

Spilanthes paniculata Linn. Bitkisinin Metanol Ekstresinin Antinosiseptif Aktivitesi

Spilanthes paniculata Linn. (Asteraceae) halk arasında diş ağrısı ve boğaz enfeksiyonunda kullanılması ile iyi bilinen bir bitkidir. *S. paniculata*'nın metanol ekstresinin muhtemel antinosiseptif aktivitesi hot plate, asetik asitle indüklenmiş kıvranma ve formalin testi ile incelenmiştir. Ekstre 250 ve 500 mg/kg vücut ağırlığı dozlarında kullanılan üç model de anlamlı ($p<0.001$) ve doz bağımlı antinosiseptif aktivite tespit edilmiştir. Bu sonuç ekstrenin antinosiseptif aktivitesinin hem santral hem de periferal mekanizma ile var olduğunu göstermiştir. Tüm deneysel sonuçlar *S. paniculata* bitkisinin metanol ekstrenin antinosiseptif özelliğe sahip bileşikler içerdiğini ve ağrı tedavisindeki yaygın folklorik kullanımının bu çalışma ile desteklendiğini göstermektedir.

Anahtar kelimeler: *Spilanthes paniculata*, Asteraceae, Antinosiseptif aktivite, Hot plate testi, Asetik asitle indüklenmiş kıvranma testi, Formalin testi.

***Correspondence:** E-mail: bkdas72@northsouth.edu; bkdas72@du.ac.bd, Tel: +88-01720051994.

INTRODUCTION

No doubt, pain is an unpleasant sensation but on the whole it is usually beneficial to human or animal. It is mainly a protective mechanism for the body that occurs whenever any tissues are being damaged, and it causes the individual to react to remove the pain stimulus (1). With many pathological conditions, tissue injury is the immediate cause of the pain, and this result in the local release of a variety of chemical agents, which are assumed to act on the nerve terminals, either activating them directly or

enhancing their sensitivity to other forms of stimulation (2). Pharmacologic management of pain requires the use of analgesic drugs. These drugs can cause serious side effects. The search for potent analgesic agents with minimal side effects remains the goal of many scientific studies (3). Medicines from indigenous plants form the basis of primary health care for a majority of people living in urban and rural or suburban of the third world countries. The reason for this dependence is the perceived low cost, easy access and ancestral experience as well as the belief that these medicines are devoid of adverse effects (4).

Spilanthes paniculata Linn. (Asteraceae), locally known as '*Shormoni*' (Bengali name: Marhati-tiga) is an annual or short-lived perennial herb found in the southern areas in Bangladesh. The genus *Spilanthes* comprises 30 species and 9 additional infraspecific taxa that are mainly distributed in the tropical and subtropical regions around the world (5). In particular, this species is well known for its folkloric use in toothache and throat infection, for which it has been recognized as the "toothache plant." All species of *Spilanthes* showed larvicidal activity against *Anopheles* mosquitoes suggesting a possible role for *Spilanthes* in the treatment as well as prevention of malaria (6). *Spilanthes* contains a number of biologically active compounds (7), of which the most studied have been the alkylamides, which this plant possesses in abundance (8). Isolated alkylamides from *Spilanthes* have showed activity against mosquito larvae. Although there are no published reports of antiplasmodial activity of alkylamides isolated from *Spilanthes*, but alkylamides from other plants have shown such activity (9). It is evident from the existing information that *S. paniculata* is rich in biologically active compounds but the basis for the traditional uses of this plant in painful condition has not yet been significantly reported.

The present study is, therefore, intended to investigate the antinociceptive activity of the methanol extract of *S. paniculata.* Accordingly, we herein disclose the results of antinociceptive activity of the *S. paniculata* to establish the scientific basis of the traditional uses of this plant.

MATERIALS AND METHODS

Plant material collection and extraction

The plants were collected from Noakhali, Bangladesh on 2nd of January, 2012 and were identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (Accession no DACB-35019). A voucher specimen is deposited in the Department of Pharmacy, North South University, Dhaka, Bangladesh. *S. paniculata* leaves has been used as a traditional folk remedy for toothache and pain. The whole plant was selected, fully dried in the sun and then ground into coarse powder with the help of a mechanical grinder. About 500 g of coarse powdered material was taken in a clean, flat-bottomed glass container and soaked in 1000 ml of 80% MeOH for 5 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through filter paper and the filtrate was evaporated by Rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) to render a gummy concentrate of chocolate black color. The gummy concentrate was then dried to get the crude extract which was used for phytochemical and pharmacological screening.

Animals

The experimental animals, cross breed Swiss-albino mice, of both gender (21-35 g) were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The mice were acclimatized for a month in temperature controlled animal house in the Department of Pharmacy, North South University with a 12 h light/dark cycle and fed with standard laboratory food and tap water. The mice had no access to food during the whole day of experiment. The influence of circadian rhythms was avoided by starting all experiments at 8.30 a.m. Ethical Committee of Department of Pharmacy, North South

University approved the animal study (No: NSU/DP/2012/846).

Preliminary phytochemical screening

The freshly prepared crude extract was qualitatively tested for the identification of chemical constituents, such as, alkaloids, flavonoids, steroids, glycosides, saponins, terpenoids, gums and tannins. In each test 10 % (w/v) solution of the extract was taken unless otherwise mentioned in individual test (10, 11). *Test for alkaloids:* Dragendroff's test: 2 mL solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. After adding 1 ml of Dragendroff's reagent, orange brown precipitate indicated the presence of alkaloids. *Test for flavonoids*: A few drops of concentrated hydrochloric acid were added to a small amount of extract solution. Immediate appearance of a red color indicated the presence of flavonoids. *Test for saponins*: 1 mL solution of the extract was diluted to 20 mL with distilled water and shaken in a graduated cylinder for 15 minutes. 1 cm layer of foam indicated the presence of saponins. *Test for gums*: Molisch test was performed for the existence of gum in the sample. 5 mL solution of the extract was taken and then Molisch's reagent and sulphuric acid were added. Appearance of red violet ring at the junction of two liquids indicated the presence of gums. *Test for tannins*: Ferric chloride test: About 0.5 g of extract was dissolved in 5 to 10 mL of distilled water and filtered. A few drops of 5 % ferric chloride solution were added to the filtrate. A greenish black precipitate was formed which confirmed the presence of tannins. *Test for terpenoids*: Salkowski test: 5 mL of the extract solution was mixed in 2 mL of chloroform, and concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids. *Test for reducing sugars*: Fehling's test: In a test tube 1mL of Fehling's A and 1mL of Fehlings's B solution were added. These mixed solutions were boiled for a minute. Then equal amount (2 mL) of test solution was added. Brick red precipitate was observed which confirmed the presence of carbohydrates.

Antinociceptive Activity Tests

Hot plate test

The hot plate test method was employed to assess the analgesic activity in accordance with the method described previously with minor modification (12, 13). The experimental animals were divided into control, positive control and test groups with six mice in each group. The animals of test groups received test samples at the doses of 250 and 500 mg/kg body weight, positive control group was administered ketorolac at the dose of 10 mg/kg body weight and vehicle control group was treated with 1% Tween 80 solution in distilled water at the dose of 10 mL/kg body weight orally. In this test, the animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5 ºC. The test samples and the standard drug were administered 30 minutes before the beginning of the experiment. Mice were observed before and at 30, 60, 120, 180 and 240 min after administration. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60, 120,180 and 240 min after oral administration of the samples. A cut-off period of 20 seconds was observed to avoid the damage of the paw. The antinociceptive response latency was recorded from the time between placement and licking of fore or hind paws or jumping movements of the animals.

Preparation of test samples for bioassays

Test samples were prepared after suspending in tween-80 solution and distilled water. The test samples were administered at two doses (250 mg/kg; 250 mg/kg; p.o.). The control group animals received dosing vehicle that is normal saline (10 ml/kg). Ketorolac (10 mg/kg) were used as reference drug.

Acetic acid- induced writhing test

To evaluate the analgesic effect of the plant extract, the method described by (14) was used with minor modifications. Different groups of

six mice each received orally normal saline solution (10 mL/kg) (i.e. control), ketorolac (10 mg/kg), or plant extract (250 and 500 mg/kg). Thirty minutes later, 0.6 % acetic acid (10 mL/kg) solution was injected intraperitoneally to all the animals in the different groups. The number of writhes (abdominal constructions) occurring between 5 to 15 min after acetic acid injection was counted. A significant reduction of writhes in tested animals compared to those in the control group was considered as an antinociceptic response. The percentage inhibition of writhing was calculated using the following formula:

Percent Inhibition = $(1\text{-}W_t/\ W_c) \times 100$. Where, W_c and W_t represent the average number of writhing produced by the control and the test group, respectively.

Formalin test

The formalin test was carried out as described by previous workers (15). Four groups of mice were treated orally with the methanol extract of *Spilanthes paniculata* (250 and 500 mg/kg), ketorolac (10 mg/kg) and normal saline (10 mL/kg). Formalin solution (0.5 % v/v) was injected subcutaneously into the right hind paw of mice, 30 min after administration of the extract, ketorolac and control (vehicle). The time (in seconds) spent in licking, biting and scratching responses of injected paw was considered as an indicator of pain response. Responses of the first 5 min were considered as early phase (neurogenic phase) and the period of 15-30 min as the late phase (inflammatory phase). The percentage inhibition was calculated by the following formula: Percent Inhibition = $(1\text{-}T_t/\ T_c) \times 100$. Where, T_c and T_t represent the average time (in seconds) spent for licking, biting and scratching by the control and the test group, respectively.

Statistical analysis

Data from the experiments were analyzed using the Statistical Package for Social Science (SPSS) software for windows version 17 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD as appropriate. Statistical analysis of the results was performed by using the one way ANOVA (analysis of variance) followed by Dennett's test. P values of 0.05 or less were considered statistically significant.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening of the extract of *S. paniculata* revealed the presence of various bioactive components of which alkaloids, gums and carbohydrates, reducing sugars, terpenoids and tannins were the most prominent and the results of phytochemical test has been summarized in the Table 1.

Table 1. Phytochemical investigation of MeOH extract of *S. paniculata.*

Test	Tannins	Flavonoids	Steroids	Saponins	Gums and Carbohydrates	Alkaloids	Reducing sugars
Observations	+	+	+	+	−	+	−

+: indicates presence, −: indicates absence of phytochemicals, SP = *S. paniculata*

Hot plate test

Results of hot plate test are presented in Table 2. The MeOH extract in both doses (250 and 500 mg/kg body weight) was found to exhibit a significant prolongation of latency time. The effect begun early at 30 min after administration of MeOH extract and persist until the following fourth hour that was comparable to the standard drug ketorolac. The results were found to be statistically significant ($p<0.001$).

Table 2. Effect of MeOH extract of *S. paniculata* on hot plate test in mice.

Treatment group	Dose (mg/kg) p.o.	Latency time(S)[a]					
		0 min	30 min	60 min	120 min	180 min	240 min
Control (vehicle)	10.	12.3 ± 1.2	10.7 ± 0.8	9.2 ± 0.6	8.1 ± 0.6	6.6 ± 0.5	6.1 ± 0.5
MeOH extract	250	9.2 ± 0.54	11.2 ± 1.2	12.1 ± 1.3	13.4 ± 1.4**	14.2 ± 0.8***	10.8 ± 0.6*
MeOH extract	500	8.2 ± 1.1	10.3 ± 0.7	12.8 ± 1.4	14.9 ± 0.8***	15.6 ± 1.6***	11.7 ± 0.6**
Ketorolac	10	9.4 ± 0.65	12.2± 0.4	13.7 ± 0.4	15.2 ± 0.5***	17.1 ± 0.1***	14.4 ±0.2***

[a] Values are expressed as mean ± SEM; (Number of animals, n = 6); vehicle = 1% tween-80 in distilled water p.o. = per oral; One way Analysis of Variance (ANOVA) followed by Dennett's test was performed as the test of significance. The minimum value of $p < 0.05$ was considered significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ vs. control.

Acetic acid-induced writhing test

In the acetic acid-induced writhing assays the extract induced a significant decrease in the number of writhes and produced 42.5 % ($P<0.01$) and 60 % ($P<0.001$) writhing inhibition at the doses of 250 and 500 mg/kg body weight respectively, which was comparable to the standard drug ketorolac where the inhibition was 58.4 % at the dose of 10 mg/kg body weight (Table 3). The result at the dose 500 mg/kg was more significant than 250 mg/kg body weight.

Table 3. Effect of MeOH extract of *S. paniculata* on acetic acid-induced writhing in mice.

Treatment group	Dose (mg/kg) p.o.	No. of writhings[a]	Inhibition (%)
Control (vehicle)	10	45.2 ± 2.9	-----
MeOH extract	250	26.0 ± 1.5**	42.5
MeOH extract	500	18.2 ± 1.3***	60.0
Ketorolac	10	18.8 ± 0.3***	58.4

[a] Values are expressed as mean ± SEM (Number of animals, n=6); *P< 0.05, **P< 0.01, ***P< 0.001 vs. control.

Formalin Test

In this model, the extract demonstrated significant and dose-dependent inhibition in both early (22.77% and 41.58%) and late (46.15% and 67.7%) phases of the formalin induced pain as manifested by the licking responses at the doses of 250 and 500 mg/kg respectively (Table 4). The treatment of animals with ketorolac showed significant inhibition of the late phase (75.38%) but not in the early phase (49.5%).

Table 4. Effect of the MeOH extract of *S. paniculata* on formalin-induced pain in mice.

Treatment group	Dose (mg/kg) p.o.	Total time spent in licking (s)[a]			
		0-5 min	Inhibition (%)	15-30 min.	Inhibition (%)
Control (vehicle)	10	25.2 ± 0.4	------	16.2 ± 0.7	-----
MeOH extract	250	19.5 ± 1.5**	22.7%	8.7 ± 0.8***	46.1
MeOH extract	500	14.7 ± 1.1***	41.5%	5.2 ± 0.8***	67.7
Ketorolac	10	12.7 ± 0.7***	49.5%	4.0 ± 0.4***	75.4

[a]Data are represented as the mean ± SEM (Number of animals = 6); p.o. = per oral; *p < 0.05, **p < 0.01, ***p < 0.01 vs. control.

DISCUSSION

Three models (hot plate test, acetic-acid induced writhing and formalin test) were employed for studying the antinocicpetive activity of MeOH extract of *S. paniculata* so that both the centrally and peripherally mediated effects could be investigated. The hot plate test involves the central mechanism whereas the acetic acid-induced writhing test involves the peripheral mechanism. The formalin test is believed to show the involvement of both peripheral and central mechanisms.

The hot plate test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the well-validated models used for studying central antinociceptive activity (16, 17). The extracts of the plants and ketorolac (10 mg/kg) also presented a longer latency time than the control group in the hot plate test in a dose related manner. Nociceptive pain inhibition was noticed higher at 180 minutes after administration of the extracts and the response was increased from 8.2 seconds to 15.8 seconds that was comparable to standard drug ketorolac where the response time was 17.1 seconds at 180 minutes of study. As the hot plate method is considered to be selective for the drugs acting centrally analgesics, the effect of the extract on this pain model indicates that it must have a centrally acting antinociceptive activity.

The acetic acid-induced writhing test is normally used to evaluate the peripheral analgesic effect of drugs and chemicals. The possible mechanism is thought to be mediated by inhibition of lipooxygenase and/or cyclooxygenase in peripheral tissues induced by acetic acid, thereby reducing PGE2 synthesis and interfering with the mechanism of transduction in primary afferent nociceptor (19, 20, 21). The MeOH extract significantly inhibited acetic acid-induced writhing in mice at both doses (250 and 500 mg/kg). But more significant response was found with 500 mg/kg dose level and the response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway (18, 19). Therefore, it may be inferred that the analgesic effect of the extract could be due to the inhibition of prostaglandin pathway which is peripherally mediated.

The current studies showed that the early phase of formalin induced pain which starts immediately after injection seems to be caused predominantly by activation of C-fibers, which is responsible for the sensation of a sharp first pain and a combined process generated by peripheral inflammatory tissue. Then, there is a period (about 10 min) of reduced nociceptive activity. The late phase of moderate pain, which starts about 20 min after formalin injection, appears to be caused by tissue and functional changes in the dorsal horn of the spinal cord (20, 21). The drugs that show their activity through central mechanism, such as narcotics, inhibited both phases almost equally, while peripherally acting drugs only inhibited the second phase. Administration of the extract demonstrated significant inhibition in both phases indicate that the extract contain active

analgesic principles acting both centrally and peripherally, but the more significant of late phases of pain as observed with the extract in both doses suggests that they contain such active analgesic principles which act predominantly by peripheral mechanism.

Hossain et al. reported the significant antinociceptive activity of the ethanol extract of the leaves by using acetic acid induced-writhing method (22). Similarly, the present study demonstrated the antinociceptive activity potential of the methanol extract of the whole plant by using three different experimental methods. The results of the present study were in accord with the previous findings.

Preliminary phytochemical screening exhibits the presence of various classes of constituents such as alkaloids, flavonoids, saponins, tannins, terpenoids and steroids in the plant extract. So, the observed analgesic activity may be attributed to these compounds. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins and there are also reports on the role of tannins in antinociceptive activity (23, 24). Besides, alkaloids are well known for their ability to inhibit pain perception (25). Flavonoids and other phenolic compounds of plant origin have been reported as antioxidants and as scavengers of free radicals can also act exert analgesic activity (26). Therefore the results of phytochemical analysis strongly support the observed antinociceptive activity of the extract.

CONCLUSION

This study indicates that the MeOH extract of *S. paniculata* possesses significant antinociceptive activity at the doses investigated on the experimental laboratory animals. This could provide rationale for its traditional uses in the management of pain and suggests for further investigation and isolation of biologically active constituents responsible for the activity. The fact that the extract showed positive results in all three tests brings out the idea that the extract has been acting through both central and peripheral mechanisms. The isolation and characterization of the active principles are on progress.

REFERENCES

1. Kanodia L, Das S, A comparative study of analgesic property of whole plant and fruit extracts of *Fragaria vesca* in experimental animal models, Bangladesh J Pharmacol 4, 35-38, 2008.
2. Rang HP, Dale MM, Pharmacology, 5th edition, p 562, Published by Churchill, Livingstone, London, 1993.
3. Mukherjee KP, Quality control of herbal drugs: An approach to evaluation of botanicals, 106, p 34, 2000.
4. Ernst E, The efficacy of herbal medicine-an overview, Fundam Clin Pharmacol 19, 405-409, 2005.
5. Jansen RK, The systematics of *Acmella* (Asteraceae- Helianthaceeae), Syst Bot Monogr 8, 1-115, 1985.
6. Pandey V, Agrawal V, Raghavendra K, Dash AP, Strong larvicidal activity of three species of *Spilanthes* (Akarkara) against malaria (*Anopheles stephensi* Liston, *Anopheles culicifacies*, Species C) and filaria vector (*Culex quinquefasciatus* Say), Parasitol Res 102, 171-174, 2007.
7. Prachayasittikul S, Suphapong S, Worachartcheewan A, Lawung R, Ruchirawat S, Prachayasittikul V. Bioactive Metabolites from *Spilanthes acmella* Murr Molecules, 14, 850-867, 2009.
8. Nakatani N, Nagashima M. Pungent alkamides from *Spilanthes acmella* L. var. *oleracea* Clarke, Biosci Biotechnol Biochem 56, 759-762, 1992.
9. Sittie AA, Lemmich E, Olsen CE, Hviid L, Broegger Christensen S, Alkylamides from *Phyllanthus fraternus*, Planta Med 64, 192-193, 1998.
10. Harborne IB, Phytochemical Methods: A guide to modern techniques of plant analysis, 3rd edition, p 302, Published by Chapman and Hall, London, 1998.
11. Sazada S, Verma A, Rather AA, Jabeen F, Meghvansi MK, Preliminary phytochemical analysis of some important medicinal and aromatic plants, Adv in Biol Res 3, 188-195, 2009.
12. Lanhers MC, Fleurentin J, Mortier F, Vinche A, Younos C, Anti-inflammatory and analgesic

effects of an aqueous extract of *Harpagophytum procumbens*, Planta Med 58, 117-23, 1992.
13. Mahomed IM, Ojewole JAO, Analgesic, anti-inflammatory and Antidiabetic properties of *Harpagophytum procumbens* DC (Pedaliaceae) secondary root aqueous extract, Phytother Res 18(12), 982-989, 2004.
14. Dharmasiri JR, Ayakody AC, Galhena G, Liyanage SSP, Ratnasooriya WD, Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*, J Ethnopharmacol 87, 199-206, 2003.
15. Hunskaar S, Fasmar OB, Hole K, Formalin test in mice: a useful technique for evaluating mild analgesics, J Neurosci Meth 14, 69-76, 1985.
16. Sabina EP, Chandel S, Rasool MK, Evaluation of analgesic, antipyretic and ulcerogenic effect of Withaferin A, Int J Integ Biol 6 (2), 52-56, 2009.
17. Ibironke GF, Ajiboye KI. Studies on the anti-inflammatory and analgesic properties of *Chenopodium ambrosioides* leaf extract in rats, Int J Pharmacol 3, 111-115, 2007.
18. Duarte IDG, Nakamura M, Ferreira SH, Participation of the sympathetic system in acetic acid-induced writhing in mice, Brazilian J Med Biol Res 21, 341–343, 1988.
19. Derardt R, Jougney S, Delevalccee F, Falhout M, Release of prostaglandins E and F in an algogenic reaction and its inhibition, Eur J Pharmacol 51, 17-24, 1980
20. Dickenson AH, Sillivan AF, Suncutaneous formalin-induced activity of dorsal horn neurons in the rat: differential response to an intrathecal opiate administered pre or post formalin, Pain 30(3), 349-360, 1987.
21. Dalal A, Tata M, Allegre G, Gekiere F, Bons N, Albe-Fessard D, Spontaneous activity of rat dorsal hron cells in spinal segments of sciatic projection following transaction of sciatic nerve or of corresponding dorsal roots, Neurosci 94(1), 217-228, 1999.
22. Hossain H, Shahid-Ud-Daula AFM, Jahan IA, Nimmi I, Hasan K, Haq MH, Evaluation of antinociceptive and antioxidant potential from the leaves of *Spilanthes paniculata* growing in Bangladesh, Int J Pharm Phytopharmacol Res 1(4), 178-186, 2012.
23. Vanu MR, Palanivelu S, Panchanatham S, Immunomodulatory and anti-inflammatory effects of *Semecarpus anacardium* Linn. Nut milk extract in experimental inflammatory conditions, Biol Pharm Bull 29, 693-700, 2006.
24. Pathak D, Pathak K, Singla AK, Flavonoids as medicinal agents, recent advances, Fitoterapia 62(5), 371-389, 1991.
25. Uche FI, Aprioku JS, The phytochemical constituents, analgesic and anti-inflammatory effects of methanol extract of *Jatropha curcas* leaves in mice and Wister albino rats, J App Sci Environ Manag, 12(4), 99–102, 2008.
26. Geronikaki AA, Gavalas AM, Antioxidant and anti-inflammatory disease: synthetic and natural antioxidants with anti-inflammatory activity. Comb Chem High Throughput Screen, 9, 425-442, 2006.

Received: 14.03.2013
Accepted: 30.05.2013