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Evaluation of Analgesic and Anti-inflammatory Activities of Polyalthia simiarum (Hook. F. &Thomson)

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Abstract

Background: *Polyalthia simiarum* (Hook. F. &Thomson) exhibits different effects in human body. Objective: As a part of ongoing research on medicinal plants of Bangladesh, the present study is focused to investigate the analgesic and anti-inflammatory activities of stem bark of *Polyalthia simiarum* (Annonaceae). Methodology: The ethyl acetate (EA) and petroleum ether (PE) extracts were subjected to qualitative chemical investigation for the identification of different phytoconstituents. The analgesic activity was determined for its central and peripheral pharmacological actions using tail immersion method and acetic acid-induced writhing test. The anti-inflammatory activity was evaluated by carrageenan induced paw edema in rats. Analgesic and anti-inflammatory data were evaluated statistically analysed by Dunnett's-T test. Result: Both extracts at the dose of 50- and 100 mg/kg b.w., produced significant increase in pain threshold in tail immersion method whereas significantly reduced the writhing caused by acetic acid in a dose dependent manner. The EA and PE extracts showed anti-inflammatory activities at 50- and 100 mg/kg body weight. Among all the extracts, the EA extract showed a dose dependent and comparable analgesic activity in all the tested methods and also reduced the paw edema considerably (27.5% and 39.1% inhibition after 4h), in dose dependent manner when compared to carrageenan induced control rat. Conclusion: Therefore, the EA and PE extracts of *Polyalthia simiarum* were capable to exhibit moderate analgesic and anti-inflammatory activities. This is the first report of analgesic and anti-inflammatory potential of *Polyalthia simiarum* and can be further investigated to isolate the active compounds responsible for the biological activities. *[Journal of National Institute of Neurosciences Bangladesh, 2019;5(1):18-23]*

Keywords: Analgesic; anti-inflammatory; *Polyalthia simiarum;* Annonaceae

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Introduction

Pain has been defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage[1]. The direct and indirect action of chemical mediators, such as arachidonic acid metabolites (prostaglandins and leukotrienes), peptides, serotonin, acetylcholine, cytokines, nitric oxide, among others, which can be produced or released following tissue injury or by exogenous irritants (formalin, acetic acid), are responsible for the multiplicity of events that occur during pain transmission, in both the peripheral and central nervous systems[2].

Inflammation results in the liberation of endogenous mediators like histamine, serotonin, bradykinin, prostaglandins etc. Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue

responses involved in inflammation[3]. Most of the anti-inflammatory drugs now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism. Hence for treating inflammatory diseases analgesic and anti-inflammatory agents are required[4]. Non steroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicine[5] but severe adverse effects[6] and tolerance and dependence induced by opiates, use of these drugs have not been successful in all the cases. Therefore, new anti-inflammatory and analgesic drugs are needed as alternatives to NSAIDs and opiates. Medicinal plants are believed to be an important source of new chemical substances.

The genus Polyalthia (Family-Annonaceae) comprises about 120 species of shrubs and trees[7]. Locally it is known as Arjan, a very tall tree. Traditionally, the plants of this genous are used as a bitter tonic, abortifacient, febrifuge, cure for scorption strings, hypertension and as respiratory stimulant[8]. Biological evaluations of this genus have shown to exhibit cytotoxic, antimicrobial[9], anticancer[10], antimalarial[11] and HIV-inhibitory[12] activities. The plant *Polyalthia simiarum* is known to exhibit antimicrobial and cytotoxic[13] activities. Previous phytochemical investigations revealed that this genus contain mostly alkaloid of aporphine, oxoaporphine, bisaporphine, bisdehydroaporphine, proaporphine, benzyl isoquinoline categories, flavonoids, acetogenin, steroid, diterpenoids and pentacyclic triterpenes[14]. Very recently the pet ether extract of stem bark of *Polyalthia simiarum* led to the isolation of a bisnor-type clerodane diterpenoid and three clerodane derivatives[15].

Literature reviews indicated that no studies combining the analgesic and anti inflammatory activity of the stem bark of *Polyalthia simiarum* have so far been undertaken. Taking this in view and as a part of our ongoing research on Bangladeshi medicinal plants, the present study aimed to evaluate the analgesic and anti inflammatory activity of stem bark of *Polyalthia simiarum*.

Methodology

Plant Material: The stem bark of *P. simiarum* was collected from Mirpur, Dhaka in the month of June 2008 and identified by Mr. Sarder Nasir Uddin, Scientific Officer, Bangladesh National Herbarium, Dhaka, where a voucher specimen (DACB-34201) representing this collection has been deposited.

Chemicals: Diclofenac-Na, Nalbuphine and Indomethacin were collected from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals and reagents were of highest analytical grade.

Preparation of extract: The air dried and powdered plant material (750 g) was extracted in a Soxhlet apparatus with ethyl acetate and pet ether (60-80°C). The extract was filtered through a fresh cotton plug followed by Whatman no.1 filter paper. The filtrate was then concentrated with a Buchii rotavapor at low temperature and pressure to afford ethyl acetate extract (EA, 3.5 g) and pet ether (PE, 2.25 g).

Preliminary phytochemical investigation: The extracts were subjected to qualitative chemical investigation for the identification of different phytoconstituents like sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins, proteins, terpenoids[16].

Animal: Swiss albino mice (25-30 g) and Wister rats (175-250 g) of both sexes were used for assessing the biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of six animals which were fasted overnight prior to the experiments. Experiments with the animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Department of Pharmacy, BRAC University, Bangladesh.

Acute toxicity: The acute oral toxicity of the plant extract in Swiss albino mice was studied as per established protocol[17].

Analgesic activity

Tail flick test: The animals were divided into six groups with six mice in each group. Group I animals received vehicle (1% Tween 80 in water, 10 mL kg^{-1} body weight), animals of Group VI received nalbuphine at 5 mg kg^{-1} body weight while animals of Group II and Group IV were treated with 50 and 100 mg kg^{-1} body weight (p.o.) of the PE extract of *P. simiarum* and Group III and Group V were treated with 50 and 100 mg kg^{-1} body weight (p.o.) of the EA extract of *P. simiarum*. From 1-2 cm of the tail of mice was immersed in warm water kept at constant temperature of 60°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 second was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60 and 90 min after the oral administration of drugs[18].

Acetic acid-induced writhing test: The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-Na was administered intraperitonially 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min[19].

Anti-inflammatory activity

Carrageenan induced paw edema test in rats: Male Wister rats (175-250 g) were divided into six groups of six animals each. The test groups received 50 and 100 mg/kg, *p.o.* of each extract. The reference group received indomethacin (10 mg/kg, *p.o.*) while the control group received 3 mL/kg of distilled water. After 1 h, 0.1 mL, 1% w/v carrageenan suspension in normal saline was injected into the sub plantar tissue of the right hind paw[20]. The paw volume was measured at 1, 2, 3 and 4 h after carrageenan injection using a micrometer screw gauge. The percentage inhibition of the inflammation was calculated from the formula: % inhibition = $(1 - D_t/D_0) \times 100$. Whereas D_0 was the average inflammation (hind paw edema) of the control group of rats at a given time, D_t was the average inflammation of the drug treated (i.e. extract/fractions or reference indomethacin) rats at the same time21.

Statistical analysis: Analgesic and anti inflammatory data are expressed as mean ± S.E.M. (n = 6 mice per groups). Statistical significance (p) calculated ANOVA followed by Dunnett's-T test *P<0.01 and **P<0.001 were considered to be statistically significant.

Results

Preliminary Phytochemical Investigation

The phytoconstituents present in the EA and PE extract of P.simiarum were identified by various chemical tests which showed the presence of alkaloids, terpenoids, phenolic and flavonoid compounds and steroids (Table 1).

Acute Toxicity Study

The acute toxicity study was conducted to establish the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species. The extracts of P. simiarum were safe up to a dose of 1000 mg/kg (p.o.) body weight.

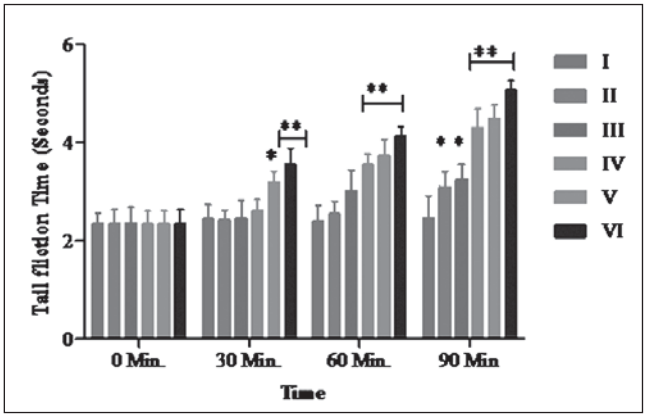

Figure 1: Effects of the PS on tail withdrawal reflex of mice induced by tail immersion method. Values are mean ±SEM, (n = 6); *p<0.01 and **p<0.001, Dunnett's-T test as compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II and Group IV were treated with 50 and 100 mg/kg body weight (p.o.) of the PS-PE extract. Group III and V were treated with 50 and 100 mg/kg body weight (p.o.) of the PS-EA extract. Group VI received Nalbuphine 5 mg/kg body weight (p.o).

Analgesic Activity

Tail Flick Method

The tail withdrawal reflex time following administration of the EA and PE were found to increase with increasing dose of the sample. The result was statistically significant (*p<0.01-0.001) and was comparable to the reference drug nalbuphine (Figure 1).

As noted, nalbuphine, the reference narcotic analgesic drug (5 mg/kg, *p.o.*) exhibited significant and paramount analgesic effects (supra spinal), the tail immersion (spinal) test; whereas, EA and PE (for both extract 50 and 100 mg/kg, *p.o.*) also produced a statistically significant but lesser in degree

Table 1: Result of chemical group tests of the EA and PE extract of *Polyalthia simiarum*

Extract	Triterpene	Diterpene	Flavonoid	Phenol	Sterol	Alkaloid
EA extract	+	+++	+++	++	+++	++
PE extract	++	++	+	+	+++	+

EA: Ethyl acetate; PE: Petroleum ether; (+): Present; (-): Absent; (+ + +): Reaction intensity is high; (+ +): Reaction intensity is medium; (+): Reaction intensity is normal.

anti-nociceptive response to that of nalbuphine in this test suggesting that the plant extracts may act as a narcotic analgesic.

Writhing Test Method

Table 2 shows the effects of the extract of on acetic acid-induced writhing in mice. The oral administration of both doses of EA and PE significantly (**p<0.001) inhibited writhing response induced by acetic acid in a dose dependent mann.

Table 2: Effects of the Polyalthia simiarum on acetic acid-induced writhing in mice

Group	Dose (Mg/kg body. Wt.)	No. of Writhing	Percentage inhibition of writhing
I		30.0 ± 2.57	
II	50	25.0±2.15	16.66
III	50	19.0±1.5	36.66
IV	100	18.0 ± 1.15 **	40.0
V	100	13.0 ± 0.57 **	58.06
VI	10	9.0 ±1.57 **	70.96

Values are mean ± SEM, (n = 6); ** p<0.001, Dunnett's-T test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group VI received Diclofenac Na 10 mg/kg body weight (p.o.), Group II and Group IV were treated with 50 and 100 mg/kg body weight (p.o.) of the PS-PE. Group III and Group IV were treated with 50 and 100 mg/kg body weight (p.o.) of the PS-EA.

Anti-inflammatory Activity

To the carrageenan induced paw edema in mice, the EA and PE extract showed dose dependent inhibition on paw edema compared to the control group (Figure 2).

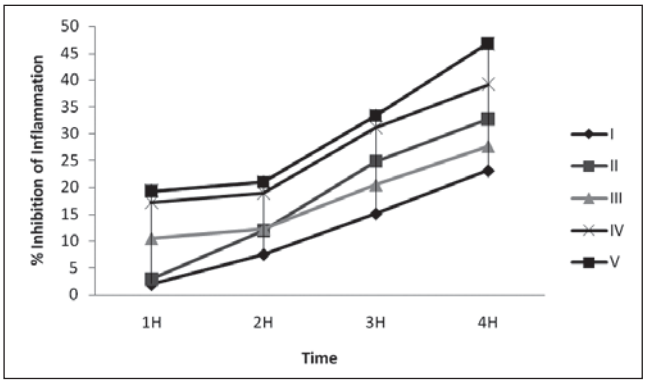

Figure 2: Inhibition of inflammation by different (Group-I and Group-II animal treated with 50 and 100 mg/kg body weight of PS-PE extract (p.o), Group-III and Group-IV animal treated with 50 and 100 mg/kg body weight of PS-EA extract (p.o) and Group-V received indomethacin 10 mg/kg body weight (p.o.)

Discussion

The tail flick method is commonly used for assessing central antinociceptive response. The method is further distinguished by their tendency to respond to the pain stimuli conducting through neuronal pathways as tail immersion mediates a spinal reflex to nociceptive stimuli[22]. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain[23-24]. As noted, nalbuphine, the reference narcotic analgesic drug (5 mg/kg, p.o.) exhibited significant and paramount analgesic effects in both the hot plate (supra spinal) as well as the tail flick (spinal) test; whereas, EA (50 and 100 mg/kg, p.o.) and PE (50 and 100 mg/kg, p.o.) also produced a statistically significant but lesser in degree antinociceptive response to that of nalbuphine in both test suggesting that the plant extract may act as a narcotic analgesic. However, the mechanism(s) behind the central analgesic response of EA and PE in both tested methods is not completely understood and may need further investigation.

On the otherhand, acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics and represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipid[25]. The response is thought to be mediated by peritoneal mast cells[26], acid sensing ion channels[27] and the prostaglandin pathways[28]. The organic acid has also been postulated to act indirectly by inducing the release of endogenous mediators, which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics[29]. It is well known that non-steroidal anti-inflammatory and analgesic drugs mitigate the inflammatory pain by inhibiting the formation of pain mediators at the peripheral target sites where prostaglandins and bradykinin are proposed to play a significant role in the pain process[30].

In addition, it was suggested that non narcotic analgesics produce their action by interfering with the local reaction to peritoneal irritation thereby reducing the intensity of afferent nervous stimulation in the acetic acid induced writhing test, a model of visceral pain[31]. Therefore, it is likely that EA and PE might have exerted its peripheral antinociceptive action by interfering with the local reaction caused by the irritant or by inhibiting the synthesis, release and/or antagonizing the action of pain mediators at the target sites and this response in agreement with the previous studies of D. indica, leaves[32]. The above findings clearly demonstrated that both central and peripheral mechanisms are involved in the antinociceptive action of EA and PE.

Carrageenan induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1 to 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages[33]. Since the extracts significantly inhibited paw edema induced by carrageenan in the second phase and this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme.

Phytochemical analysis showed that the extract contained alkaloids, phenolic compounds, sterols, diterpenes, triterpenes and flavonoids. Flavonoids and phenolic compounds have all been associated with various degrees of anti-inflammatory[34]. Therefore, the anti-inflammatory effects observed in this study are perhaps due to the activity of one or more of the identified classes of compounds. Because an essential database on the chemical profile of *P. simiarum* extract is established in which flavonoids and triterpenoids are the major constituents, this information should be considered for future purification of analgesic and anti-inflammatory active compounds from this natural source. These results indicate that *P. simiarum* extracts have both effective analgesic and anti-inflammatory activity.

Conclusion

Based on the results of the present study, we conclude that the plant extracts possess moderate analgesic and anti inflammatory potential. However, further studies are necessary to examine underlying mechanisms of analgesic and anti inflammatory effects and to isolate the active compound(s) responsible for these pharmacological activities.

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References

1. Hossain MS, Alam MB, Chowdhury NS, Asadujjaman M, Zahan R, Islam MM, et al. Antioxidant, analgesic and anti-inflammatory activities of the herb Eclipta prostrata. J Pharmacol Toxicol 2011;6(5):468-480
2. Alam MB, Rahman MS, Hasan M, Khan MM, Nahar K, Sultana S. Anti-nociceptive and antioxidant activities of the Dillenia indica bark. International Journal of Pharmacology. 2012;8(4):243-51
3. Ravikiran G, Elumalai A, Eswaraiah MC, Naresh V. An annual review on antiinflammatory medicinal plants. Int J Phyto 2011;5(5):508-206
4. Anilkumar M. Ethnomedicinal plants as Anti-inflammatory and Analgesic agents. Ethnomedicine: A Source of Complementary Therapeutics. Research Signpost, Kerala, India. 2010;267-293
5. Conforti F, Sosa S, Marrelli M, Menichini F, Statti GA, Uzunov D, et al. The protective ability of Mediterranean dietary plants against the oxidative damage: the role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents. Food Chem 2009;112: 587-597
6. Chowdhury MA, Abdellatif KRA, Don Y, Das D, Suresh MR, Knaus EE. Synthesis of celecoxib analogues possessing a N-Difluoromethyl-1,2-dihydropyrid-2-one s-Lipoxygenase pharmacophore: Biological evaluation as dual inhibitors of cyclooxygenases and 5-Lipoxygenase with anti-inflammatory activity. J Med Chem 2009;52:1525-1529.
7. Ghani NA, Ahmat N, Ismail NH, Zakaria I, Zawawi NKNA. Chemical constituents and cytotoxic activity of Polyalthia cauliflora var. cauliflora. Res J Med Plant 2012;6(1):74-82.
8. Kirtikar KR, Basu BD. Indian Medicinal Plants, Singh B, Singh MP (Eds), Dehra Dun: India, 1980
9. Sashidhara KV, Singh SP, Shukla PK. Antimicrobial Evaluation of Clerodane Diterpenes from Polyalthia longifolia var. pendula. Nat Prod Commun 2009;4(3): 327-30.
10. Sashidhara KV, Singh SP, Sarkar J, Sinha S. Cytotoxic clerodane diterpenoids from the leaves of Polyalthia longifolia. Nat Prod Res 2010;24(18): 1687-94.
11. Kanokmedhakul S, Kanokmedhakul K, Yodbuddee D. New antimalarial bis-dehydroaporphine alkaloids from Polyalthia debilis. J Nat Prod 2003;66: 616-619.
12. Li HY, Sun NJ, Kashivada Y. Anti-AIDS agents, Suberosol, a new C31 lanostane-type triterpene and anti-HIV principle from Polyalthia suberosa. J Nat Prod 1993;56: 1130-1133.
13. Kabir S, Chowdhury AMS, Rashid MA. Antimicrobial and cytotoxic activities of the crude extracts of Polyalthia simiarum. Dhaka Univ J Sci 2011;59:199-200.
14. Paarakh PM, Khosa RL. Phytoconstituents from the genus Polyalthia: A Review. J Phar Res 2009;2(4): 594-605.
15. Kabir S, Rahman MS, Chowdhury AMS, Hasan CM, Rashid MA. An unusual bisnor-clerodane diterpenoid from Polyalthia simiarum. Nat Prod Commun 2010;5:1543-6.
16. Patel J, Kumar GS, Deviprasad SP, Deepika S, Qureshi MS. Phytochemical and anthelmintic evaluation of Lantana camara (L.) var. aculeate leaves against Pheretima posthuma. J of Global Trends in Pharm Sci 2011;2(1): 11-20.
17. Riaz A, Khan RA, Ahmed S, Afroz S. Assessment of acute toxicity and reproductive capability of a herbal combination. Pak J Pharm Sci 2010;23(3): 291-294.
18. Toma W, Graciosa JS, Hiruma-Lima CA, Andrade FDP, Vilegas W, Souza-Brita ARM. Evaluations of the analgesic and antiedematogenic activities of Quassia amara bark extract. J Ethnopharmacol 2003;85:19-23.
19. Ghule RS, Venkatnarayanan R, Thakare SP, Jain H, Ghule PR. 2011. Analgesic activity of Cuscuta campestris Yuncker a parasitic plant grown on Nerium indicum Mill. J of Adv Phar Educ Res

2011;1: 45-51.
20. Winter CA, Risley EA, Nuss GW. Carrageenan induced oedema in hind paw of the rats as an assay for anti-inflammatory drugs. Pro Soc Exp Biol Med 1962;111: 544-547.
21. Gupta M, Mazunder UK, Sambath KR, Gomath P, Rajeshwar Y, Kakoti BB, et al. Anti-inflammatory, analgesic and antipyretic effects of methanol extract from Bauhina racemosa stem bark in animal models. J Ethnopharmacol 2005;98: 267-273.
22. Chapman CR, Casey KI, Dubner R, Foley KM, Gracely RH, Reading AE. Pain measurement: an overview. Pain 1985;22: 1-31.
23. Elisabetsky E, Amador TA, Albuquerque RR., Nunes DS, Cavalho ACT. 1995. Analgesic activity of Psychotria colorata (Willd. Ex R. and S.) Muell. Arg. Alkaloids. J Ethnopharmacol 1995;48:77-83.
24. Pal S, Sen T, Chaudhuri AK. Neuropsychopharmacological profile of the methanolic fraction of Bryophyllum pinnatum leaf extract. J Pharm Pharmacol 1999;51: 313-318
25. Ahmed F, Hossain MH, Rahman AA, Shahid IZ. Antinociceptive and sedative effects of the bark of Cerbera odollam Gaertn. J Ori Pharm Exp Med 2006;6: 344-348.
26. Ribeiro RA, Vale ML, Thomazzi SM, Paschoalato AB, Poole S, Ferreira SH, et al. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol 2000;387:111-118.
27. Voilley N. Acid-sensing ion channels (ASICs): New targets for the analgesic effects of Non-Steroid Anti-Inflammatory Drugs (NSAIDs). Curr Drug Targets Inflamm Allergy 2004;3: 71-79.
28. Hossain MM, Ali MS, Saha A, Alimuzzaman M. Antinociceptive activity of whole plant extracts of Paederia foetida. Dhaka Univ J Pharm Sci 2006;5:67-69.
29. Adzu B, Amos S, Kapu SD, Gamaniel KS. Anti-inflammatory and anti-nociceptive effects of Sphaeranthus senegalensis. J Ethnopharmacol 2003;84: 169-174.
30. Hirose K, Jyoyama H, Kojima Y. Pharmacological properties of 2-[44-(2-thiazolyloxy)-phenyl]-propionic acid (480156-5), a new non-steroidal anti-inflammatory agent. Drug Res 1984;34: 280-286.
31. Vogel HG, Vogel WH. Drug Discovery and Evaluation – Pharmacological Assays. Springer-Verlag, Berlin Heidelberg, 1997;1231
32. Bose U, Gunasekaran K, Bala V, Rahman AA. 2010. Evaluation of Phytochemical and Pharmacological properties of Dillenia indica Linn. Leaves. J Pharmacol Toxicol 2010;5:222-228
33. Kaushik D, Kumar A, Kaushik P, Rana AC. Analgesic and Anti-Inflammatory Activity of Pinus roxburghii Sarg. Advances in Pharmacological Sciences. 2012;2012
34. Wang JR. Zhou H, Jiang ZH, Wong YF, Liu L. In vivo anti-inflammatory and analgesic activities of a purified saponin fraction derived from the root of Ilex pubescens. Bio Pharmaceu Bull 2008;31: 643-650