ABSTRACT
Background: Petersianthus macrocarpus (Lecythidaceae) is widely used in the folk medicine in Nigeria to relieve pain and fever associated with malaria. This study evaluated the analgesic and antioxidant activities of the methanol extract and fractions of the stem bark of the plant.

Materials and Methods: The analgesic activity was determined in mice using hot plate and acetic acid-induced writhing models. Morphine sulphate (5 mg/kg, i.p.) and aspirin (100 mg/ml, p.o.) were used as reference analgesic agents. The antioxidant potential was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical; reducing power, iron chelating properties and determination of total phenolic content.

Results: The extract at 200 and 500 mg/kg, produced an insignificant (P > 0.05) increase in pain threshold in hot plate but a significant (P < 0.05) increase at 1000 mg/kg. The extract significantly (P < 0.05) reduced the writhing induced by acetic acid in mice in a dose dependent manner. Fractionation increased the analgesic activities significantly (P < 0.05) in ethyl acetate and aqueous fractions (200 mg/kg). The extract demonstrated strong DPPH radical scavenging activity with IC₅₀ 0.05 mg/ml, good reducing power and weak iron chelating activities. The total phenol content was 142.32 mg/g in term of gallic acid. The antioxidant effects were more pronounced in ethyl acetate and aqueous fractions.

Conclusion: The findings of the study suggested that the extract has strong analgesic and antioxidant activities which reside mainly in the polar fractions thus confirming the traditional use of the plant to alleviate pains.

Key words: Anti-nociceptive, Lecythidaceae, pain, radical scavenging, writhing

SUMMARY
• Analgesic and antioxidant activities of extract and solvent fractions of Petersianthus macrocarpus investigated indicated that extract has analgesic and antioxidant properties that reside mainly in the polar fractions.

INTRODUCTION
Pain has been defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage.[1,2] 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.[3,4] Reactive oxygen species (ROS) including free radicals are involved in sensitization of dorsal horn neurons that plays a fundamentally important role in pain. Antioxidant supplements are known to increase the threshold of pain perception.[5] The study of plants used traditionally as analgesics is still a fruitful and logical strategy in the search for new analgesic drugs and pain mechanisms.[6] Drugs such as morphine, steroidal or non steroidal anti-inflammatory agents that are currently being used for the treatment of pain clinically have severe adverse effect thereby necessitating the search for naturally occurring agents with reduced side effects as substitutes.

Petersianthus macrocarpus belongs to the family Lecythidaceae and in Nigeria it is used in the treatment of pains, headache, “recurrent” fever and malaria.[7] The aqueous extract of the stem bark is traditionally used in the treatment of constipation, haemorrhoids, veneral diseases and as an abortifacient.[8] The methanolic extract of the stem bark was also reported to produce hypotensives effects.[9] In this study, we investigated analgesic and antioxidant potential of methanol extract P. macrocarpus stem bark and its fractions.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Orabueze CI, Adesegun SA, Coker HA. Analgesic and antioxidant activities of stem bark extract and fractions of Petersianthus macrocarpus. Phcog Res 2016;8:181-5.
MATERIALS AND METHODS

Chemicals and reagents
Deionized water, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma-Aldrich Co.), trichloroacetic acid (Sigma-Aldrich Co.), acetic acid, DMSO, anhydrous ferric chloride, potassium ferricyanide, ferrozine, ascorbic acid and other chemicals were all of analytical grade.

Experimental animals
Albino mice (20–22 g) of both sexes were obtained from Laboratory Animal House of College of Medicine, University of Lagos, Iledi-Araba, Nigeria. They were kept in cages at room temperature (30 ± 2°C) and food and water were supplied ad libitum up to the commencement of the experiment. All protocols were carried out in accordance to internationally accepted principles for laboratory animal use and care.

Plant material
The stem bark of *P. macrocarpus* was collected at Nnewi, Anambra State, Nigeria and identified by Mr. I. K. Odewo, a former curator in the Department of Botany, University of Lagos with voucher specimen number LUH 3153. The barks were dried at 40°C and milled to produce fine powder.

Extraction and fractionation
About 800 g of the powdered stem bark was extracted with methanol (2.5 L) using Soxhlet apparatus for 72 h. The extract was filtered and concentrated to dry powder (7.0% w/w) using rotatory evaporator at 40°C. Extract (30.0 g) was suspended in water then partitioned between n-hexane, chloroform and ethyl acetate successively to obtain respective fractions that were concentrated and subjected to analgesic and anti-oxidant investigations.

Preliminary phytochemical screening
Phytochemical screening of the extract was carried out to determine the presence of various secondary metabolites using standard methods.

Acute toxicity studies
Sixty mice were divided into control and test groups containing ten animals each. The control group received 5% DMSO and test groups were treated orally with a solution of the extract (1-5 g/kg) in 5% DMSO. The mice were observed over a period of 48 h and up to 7 days for behavioral changes and mortality.

Analgesic studies
Acetic acid-induced writhing response in rats
The analgesic activity of the plant extract was determined in terms of its ability to inhibit writhing responses of the mice produced by intra peritoneal administration of acetic acid. Different groups of five mice each received orally 5% DMSO as negative control and acetyl salicylic acid (100 mg/kg) or plant extract (200, 400, 1000 mg/kg). Thirty minutes later, 0.7% acetic acid (10 ml/kg) solution was injected intra-peritoneally to all the animals in the different groups. The number of writhes (abdominal constrictions) occurring between 0 and 20 min after acetic acid injection was counted. A reduction of writhes in test animals compared to those in the negative control group was considered as an anti-nociceptive response. The analgesic activities of hexane, chloroform, ethyl acetate and aqueous fractions were tested at 200 mg/kg using similar procedure.

Hot plate method
The analgesic activity of the extract and its fractions were assessed using hot-plate method. About 150 Swiss albino mice of either sex were screened and those that did not respond within 15 s were left out of the experiment. Thirty (30) selected mice were divided into six groups of five animals each and received orally 5% DMSO solution, extract (200-1000 mg/kg in 5% DMSO) and morphine 5 mg/kg intra-peritoneally respectively 30 min prior to the test. Each mouse was then placed on a hot plate at 50°C and the time taken to lick the hind paw or to jump was recorded. The response times of these mice were measured 30 min prior and after the treatment. Analgesic activity was expressed as the increase in response time with respect to control. The analgesic activities of the hexane, chloroform, ethyl acetate and aqueous fractions were tested at 200 mg/kg using similar procedure. The mean reaction time for each treated group was determined and compared with the result for each group before treatment. Percentage increase in response time (1 %) was derived using the formula:

\[ I\% = \left( \frac{I_t - I_o}{I_o} \times 100 \right) \]

Where: 
- \( I_t = \) reaction time at time \( t \),
- \( I_o = \) reaction time at time zero.

Total phenolic content
The total phenolic content was determined using the Folin-Ciocalteu reagent and was measured as gallic acid equivalent. The extract (100 mg/ml, 1.0 ml) was mixed thoroughly with 5 ml Folin-Ciocalteu reagent (diluted ten-fold) and after 5 min, 4.0 ml of sodium carbonate (0.7 M) was added and the mixture was allowed to stand for 1 h with intermittent shaking. The absorbance was measured at 765 nm in a spectrophotometer. All determinations were carried out in triplicate.

Antioxidant activities
Evaluation of free radical scavenging effect
The determination of the free radical scavenging activity of the extract was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. Various concentrations of extract (0.05-1.00 mg/ml) in methanol were prepared. To 1 ml of each solution, 3 ml of methanol was added and then 1 ml of a 1mM DPPH in methanol was added to make up to 5 ml. The mixture was shaken and allowed to stand at room temperature in a dark chamber for 30 min. The change in color from deep violet to light yellow was then measured at 517 nm. The experiment was repeated with fractions (0.1 mg/ml) and ascorbic acid (0.005-0.200 mg/ml) which served as positive control. All determinations were carried out in triplicates. The same procedure was repeated without the extract for blank experiment. The decrease in absorbance was then converted to percentage scavenging activity (% SA) using the formula:

\[ \% \text{Radical scavenging activities} = \left( \frac{A_b - A_t}{A_b} \right) \times 100 \]

Where:
- \( A_b = \) Absorbance of the blank solution;
- \( A_t = \) Absorbance of the test (extract) solution or the standard (ascorbic acid).

Reducing power assay
The reducing power was determined using Yen and Chen method with little modifications, the aliquot of various concentrations of the standard and test extract (0.01- 2 mg/ml) in 1.0 ml of methanol were mixed with 2 ml of phosphate buffer (pH 7.4) and 2 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C for 20 min. after cooling, aliquots of 2 ml of 10% trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 20 min. The upper layer of solution (2 ml) was taken and diluted with 2ml of deionized water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV-visible spectrometer (Systronic double beam-UV- 2201). A blank was prepared without the extract. The experiment was repeated with fractions (0.1 mg/ml). Ascorbic acid was used as the standard and the experiment was carried out in triplicate.
Metal chelating activity assay
The chelating of ferrous ions by extract was estimated using Ebrahimzadeh et al. method, with modifications. Various concentrations of extract (0.1-2.0 mg/ml) in methanol were prepared. To 0.5 ml of each solution, 0.1 ml, 1 mM FeCl₂ was added and mixture was allowed to stand for 5 min. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine, the mixture was made up 4 ml with ethanol and shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. Increase in absorbance, indicates poor Fe²⁺ chelating activity. Ethylene diamine tetra-acetic acid (EDTA) was used as positive control. Blank containing no extract was used as the negative control. The percentage inhibition of ferrozine-Fe²⁺ complex formation was calculated as:

\[
\left(\frac{A_n - A_0}{A_n}\right) \times 100.
\]

Where: \(A_n\) = Absorbance of the control/blank; 
\(A_0\) = the absorbance of the extract or EDTA (positive control).

Statistical analysis
All data were expressed as mean ± standard deviation of mean. Analysis of variance was performed by ANOVA procedures and \(P < 0.05\) was considered significant.

RESULTS
Phytochemical screening
The preliminary phytochemical screening of the extract revealed the presence of secondary metabolites such as saponins, phenolic compounds and steroidal nucleus while anthraquinones and alkaloids were not detected.

Acute toxicity study
In this study, oral administration of graded doses of the extract to the mice did not produce any significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects. No mortality or any toxic reaction like convulsion, ataxia, diarrhea or increased diuresis was recorded in any group after observation for 72 h after administering the extract to the mice. The extract was safe at the tested doses.

Analgesic activity
Effect of extract on acetic acid-induced writhing
The results of the acetic acid writhing test in mice are shown in Table 1. At all tested doses, the extract inhibited the writhing responses of the mice caused by the intra-peritoneal administration of acetic acid in dose dependent fashion. The percentage inhibition of the writhing reflex also increased from zero in the negative control group to 70.53% in the highest dose of the extract (1000 mg/kg) treated group which was higher than the 62.63% observed in the group that received acetyl salicylic acid (100 mg/kg). The inhibitory effects of aqueous, ethyl acetate, chloroform and hexane fractions (200 mg/kg) were 77.52, 72.93, 3.87 and 41.86%, respectively.

Effect of extract in hot-plate test
The results of analgesic activity of the extract assessed using hot plate test are shown in Table 2. The extract at 200 and 500 mg/kg produced slight increase in mean post reaction time when compared to the group that received 7% DMSO (negative control group). However at 1000 mg/kg, there was a significant (\(P < 0.05\)) increase in the latency for jumping or licking when compared to DMSO group but less than morphine (10 mg/kg) used as positive control [Table 2]. The aqueous, ethyl acetate, chloroform, and hexane fractions (200 mg/kg) had 100.41, 86.35, 56.40 and 56.98% elongation of reaction time to thermal stimulus induced pain.

DPPH radical scavenging activity
The free radical scavenging ability of the extract was tested by reduction of stable radical DPPH to the yellow colored diphenyl picrylhydrazine. The in-vitro investigations revealed that with increase in concentration, the radical scavenging ability of the extract increased by preventing the formation of the DPPH radical [Figure 1]. The activity was significantly less (\(P < 0.05\)) than that of ascorbic acid used as positive control. The IC50 values were 0.05 and 0.02 mg/ml for extract and ascorbic acid respectively. The ethyl acetate and aqueous fractions (0.1 mg/mL) showed the greatest activity 93.42 and 93.72% respectively while n-hexane and chloroform had lower activity 28.98 and 40.55% respectively.

Reducing power assay
The reducing power of the extract was determined using ascorbic acid as the positive control. The reducing ability increased with concentration [Figure 2]. The reducing power showed good linear relationship in the extract (\(R^2 =0.7616\)) as well as ascorbic acid (\(R^2 =0.9133\)). The maximum absorbance for crude extract was 2.890 at 1.0 mg/mL compared to 3.228 of ascorbic acid used as positive control. The reducing ability of fractions however, increased from hexane < chloroform < aqueous < ethylacetate with absorbance 0.577, 0.759, 2.409 and 2.921 respectively.

Iron chelating power
Ferrous ion chelating ability of the extract was shown in Figure 3. The extract showed 43.49% iron chelating ability at 0.2 mg/ml whereas EDTA used as positive control showed 99.36% at the same concentration. The chelating ability of the extract was significantly lower (\(P < 0.05\)) than EDTA a known metal chelator. The IC50 of extract and EDTA were 0.05 and 0.02 mg/ml respectively.

DISCUSSION
There is an increasing interest in the development of analgesics from natural products due to side effects of commercially available drugs (opioids and non steroidal anti-inflammatory drugs). In this study, stem bark extract and fractions of \(P. macrocarpus\) stem bark were investigated for central and peripheral analgesic activities. Acetic acid induced nociception is highly sensitive and commonly used in the evaluation of mild peripheral

---

**Table 1:** Effect of methanol extract of \(Petersianthus macrocarpus\) stem bark on acetic acid induced writhing in mice

| Sample   | Dose (mg/kg) | Number of writhes in 20 min | Percentage of inhibition |
|----------|--------------|----------------------------|-------------------------|
| 5% DMSO  | 47.5±0.18    | -                          |                         |
| Aspirin  | 100          | 17.75±0.21                 | 62.63                   |
| Extract  | 200          | 27.01±0.95                 | 43.16                   |
|          | 500          | 20.25±1.02                 | 57.37                   |
|          | 1000         | 14.00±0.47                 | 70.53                   |

DMSO: Dimethyl sulfoxide; Values are expressed as mean ± SDM; n=5

**Table 2:** Effect of methanol extract of \(Petersianthus macrocarpus\) stem bark on thermal stimulus induced pain (hot test) in mice

| Sample   | Dose mg/kg | Reaction time in sec (mean±SDM) | Basal (s) | 30 min after | Percentage of elongation |
|----------|------------|--------------------------------|-----------|--------------|-------------------------|
| 5% DMSO  |            |                                |           |              |                         |
| Morphine | 10         | 5.0±1.42                       | 5.48±1.31 | 9.6          |                         |
| Extract  | 100        | 5.79±1.31                      | 11.83±2.08| 105.74       |                         |
|          | 500        | 7.41±0.98                      | 8.35±1.11 | 12.69        |                         |
|          | 1000       | 7.29±0.46                      | 13.45±1.61| 84.5         |                         |

DMSO: Dimethyl sulfoxide; SEM: Standard deviation of mean; Values are expressed as mean ± SDM; n=5
Radical scavenging effects of *Petersianthus macrocarpus* demonstrated analgesic action in mice by inhibiting acetic acid induced analgesic compounds.

The results of the study indicated that extract and its fractions might not be unconnected with the activities of these triterpenoid saponins, petersaponins III and IV.

Preliminary phytochemical screening showed the presence of polyphenolic compounds and saponins which were reported to be poor capacity for iron binding suggesting that iron chelation has little or no role in its anti-oxidant activity. It has been reported that chelating agents are effective secondary anti-oxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion.

The metal chelating power of the extract and fractions were measured by ferrous ion ferrozine complex. Ferrozine combines with ferrous ion to form a red coloured complex that absorbs at 562 nm. In the presence of chelating agent, the ferrozine complex formation is disrupted leading to reduction in red color of the complex. The decrease in absorbance of this complex is an indication of increased activity. The result of this study indicated that the extract has poor capacity for iron binding suggesting that iron chelation has little or no role in its anti-oxidant activity.

We also evaluated the antioxidant activities of the extract and its fractions. Reactive oxygen species produced by living organisms has been implicated in various diseases such as cancer, ageing, diabetes, cardiovascular diseases and inflammation. Free radicals are also known as contributing factor in modulation of pain and tissue injury. Therefore, antioxidants from medicinal plants that may reduce the risk of various chronic diseases are beneficial. DPPH is a stable free radical scavenger which converts unpaired electrons to paired ones through proton donation. The results also showed that extract and its fractions exhibited strong radical scavenging power suggesting that their constituents are capable of donating protons thus quenching radicals. Total phenolic content of the extract measured using Folin-Ciocalteu reagent was calculated as 142 mg/g in terms of gallic acid. Reports have implicated phenolic compounds in antioxidant and analgesic activities due their interactions with prostaglandins and superoxides.

The conversion of Fe$^{3+}$ to Fe$^{2+}$ in the presence of extract and fractions was measured to determine the reducing power. The reducing properties are generally associated with the presence of reductones (anti-oxidants), which have been shown to exert anti-oxidant action by breaking the free radical chain.

The anti-oxidant constituents of the extract or fractions caused reduction of ferric-cyanide complex to the ferrous form due to hydrogen donation from phenolic compounds.

It has been reported that chelating agents are effective secondary anti-oxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion. The metal chelating power of the extract and fractions were measured by ferrous ion ferrozine complex. Ferrozine combines with ferrous ion to form a red coloured complex that absorbs at 562 nm. In the presence of chelating agent, the ferrozine complex formation is disrupted leading to reduction in red color of the complex. The decrease in absorbance of this complex is an indication of increased activity. The result of this study indicated that the extract has poor capacity for iron binding suggesting that iron chelation has little or no role in its anti-oxidant activity.
CONCLUSION
In conclusion, the present study demonstrated that extract of *P. macrocarpus* stem bark possesses strong analgesic and antioxidant activities which reside mainly in ethylacetate and aqueous fractions thus justifies its use in folklore medicine in treating pain related diseases. However, there is need to isolate compound(s) responsible for the observed pharmacological activities which may lead to development of novel compound for drug discovery.

Acknowledgements
The authors would like to express their thanks to Mr. D. Ota of Department of Physiology, College of Medicine, University of Lagos, Nigeria for his assistance in animal studies.

Financial support and sponsorship
The authors are grateful to University of Lagos and Tertiary Education Tax Fund (TETFUND), Nigeria for financial support.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Hossain MS, Alam MB, Chowdhury NS, Asadujaman M, Zahan R. Antioxidant, analgesic and anti-inflammatory activities of the herb *Eclipta prostrata*. J Pharmocol Toxicol 2011;6:1-13.
2. Belfrage M, Sollevi A, Segerdahl M, Sjölund KF, Hansson P. Systemic adenosine infusion alleviates spontaneous and stimulus evoked pain in patients with peripheral neuropathic pain. Anesth Analg 1999;88:713-7.
3. Sawaynok J. Adenosine receptor activation and nociception. Eur J Pharmacol 1998;347:1-11.
4. Besson JM. The neurobiology of pain. Lancet 1999;353:1610-5.
5. Ali DW, Saltner MW. NMDA receptor regulation by Src kinase signalling in excitatory synaptic transmission and plasticity. Curr Opin Neurol Biol 2001;11:336-42.
6. Zhang X, Wu J, Fang L, Willis WD. The effects of protein phosphatase inhibitors on nociceptive behavioral responses of rats following intradermal injection of capsaicin. Pain 2003;106:443-51.
7. Caixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz J Med Biol Res 2000;33:179-89.
8. Burkhill HM. The Useful Plants of West Tropical Africa. Fam. J-L. Vol. 3. Kew: Royal Botanical Garden; 1995. p. 46-7.
9. Sandberg F, Cronlund A. An ethnopharmacological inventory of medicinal and toxic plants from equatorial Africa. J Ethnopharmacol 1982;5:187-204.
10. Ogundaini AO. From Greens into Medicine: Taking a Lead from Nature. An Inaugural Lecture Delivered at Obafemi Awolowo University. Ser. 176. Nigeria: OAU Press Limited, 2005. p. 12-6.
11. Sofowora A, editor. Screening plants for bioactive agents. In: Medicinal Plants and Traditional Medicine in Africa. 2nd ed. Ibadan, Nigeria: Spectrum Books Ltd.; 1993. p. 134-56.
12. Parasuraman S. Toxicological screening. J Pharmacol Pharmacother 2011;2:74-9.
13. Owoyele BV, Olaleye SB, Oke JM, Elegbe RA. Anti-inflammatory and Analgesic activities of leaf extract of *Lanobapha owariensis*. Afr J Biomed Res 2001;4:131-3.
14. Zakaria ZA, Sulaiman MR, Morsid NA, Aris A, Zainal H, Pojan NH, et al. Antinociceptive, anti-inflammatory and antipyretic effects of *Solanum nigrum* aqueous extract in animal models. Methods Find Exp Clin Pharmacol 2009;31:81-8.
15. Adesegun SA, Fajana A, Orabueze CI, Coker HA. Evaluation of antioxidant properties of *Phalodium fasciopila* C.B.CI. (Anacardiaceae). Evid Based Complement Alternat Med 2009;6:277-31.
16. Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 2001;15:127-30.
17. Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their anti-mutagenicity. J Agric Food Chem 1995;43:27-32.
18. Ebrahimzadeh MA, Nabavi SM, Nabavi SF. Correlation between the in vitro iron chelating activity and poly phenol and flavonoid contents of some medicinal plants. Pak J Biol Sci 2009;12:934-8.
19. Lima V, Silva CB, Mafezoli J, Bezerra MM, Moraes MO, Mourão GS, et al. Antinociceptive activity of the pyranocoumarin seselin in mice. Fitoterapia 2006;77:574-8.
20. Del-Velchio-VG, Sousa OV, Miranda MA, Senna-Vallie L, Kaplan MA. Analgesic and anti-inflammatory properties of essential oil from *Ageratum farigiatum*. Braz Arch Biol Tech 2009;52:1115-21.
21. Hassan H, Ahmadu AA, Hassan AS. Antinociceptive and anti-inflammatory activities of *Asparagus africanus* root extract. Afr J Tradit Complement Altern Med 2007;5:27-31.
22. Ahmed M, Shikha HA, Sadhu SK, Rahaman MT, Datta BK. Analgesic, diuretic, and anti-inflammatory principle from *Scoparia dulcis*. Pharmazie 2001;56:657-60.
23. Paviya US, Kumar P, Wanjadi MM, Thennmozhi S, Balakrishnan BR. Antinociceptive effect of *Khaya senegalensis* root bark of *Grewia asiatica* Linn. In rodents. Anc Sci Life 2013;32:195-6.
24. Yim JH, Lee OH, Choi UK, Kim YC. Antinociceptive and anti-inflammatory effects of ethanol extracts of *Glycine max* (L.) Merr and *Rhyynchosia nulubulis* seeds. Int J Mol Sci 2009;10:442-53.
25. Nemirovsky A, Chen L, Zelman V, Jurna I. The antinociceptive effect of the combination of spinal morphine with systemic morphine or buprenorphine. Anesth Analg 2001;93:197-203.
26. Lompo M, Dubois J, Guissou IP. In vitro preliminary study of free radical scavenging activity of extracts from *Khaya senegalensis* A. Juss. (Meliaceae). J Boil Sci 2007;7:677-80.
27. Khalil Z, Lu T, Helme RD. Free radicals contribute to the reduction in peripheral vascular responses and the maintenance of thermal hyperalgesia in rats with chronic constriction injury. Pain 1999;79:31-7.
28. Narayana KR, Reddy MS, Chaluvadi MR, Krishna DR. Bio-flavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J Pharmacol 2001;33:2-17.
29. Meir S, Kanner J, Akiri B, Philosoph-Hadas S. Determination and involvement of aqueous reducing compounds in oxidative systems of various senescing leaves. J Agric Food Chem 1996;43:1813-9.
30. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 1999;269:337-41.
31. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 1999;269:337-41.
32. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 1999;269:337-41.
33. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 1999;269:337-41.
34. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 1999;269:337-41.